Promoters Used to Regulate Gene Expression

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Except where a more recent date is noted on a page, text below was published in October 2003. Technology landscapes, by their very nature, become outdated. Some patents listed in 2003 have lapsed, and new ones have issued. Accordingly, sections of this landscape are gradually being updated by Dr. Wei Yang and Dr. Marie Connett Porceddu, and dates of searches that obtained new information since 2003 are shown on each page containing such information. We are grateful for legal input by Dr. Dianne Rees and technical assistance from Dr Nick dos Remedios, Steve Irwin, and Annet Maurer for the web version. We welcome updates and inputs by others through the comments interface available on every page of this version of the technology landscape.

Chapter 1

Introduction

When the first draft of the human genome sequence became available in February 2001 there was some surprise that instead of 100,000 genes, only about 30,000 genes were counted. Whole genome sequencing has contributed to the demise of the paradigm that a single gene or at most a few of them encode each character or attribute. On the other hand, there’s ever more evidence that gene regulation is one of the major sources of diversity in the phenotypes seen in nature. Although all cells of one organism contain more or less the same genetic information, genes are turned on and others are turned off at different locations and times during the life cycle of an organism.

Cis- and trans-acting factors regulate gene expression

The intricate pattern of gene regulation involves molecular signals that act on DNA sequences encoding protein products. Cis-acting molecules act upon and modulate the expression of physically adjacent, operably linked polypeptide-encoding sequences. Trans-acting factors affect the expression of genes that may be physically located very far away, even on different chromosomes. The expression of a particular gene may be regulated by the concerted action of both cis and trans-acting elements.

What is the role of promoters in the regulation network?

The promoters discussed throughout the paper are those that bind the RNA polymerase II enzyme, which is responsible for the generation of RNA. The promoter region is usually assumed to be the key cis-acting regulatory region that controls the transcription of adjacent coding region(s) into messenger ribonucleic acid (mRNA), which is then directly translated into proteins. DNA sequences within promoters can be identified as binding sites for trans-acting factors, "transcription factors", which may cause activation or repression of transcription.

Typical gene organization

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**Types of promoters used to regulate gene expression**

Promoters used in biotechnology are of different types according to the intended type of control of gene expression. They can be generally divided into:

1. **Constitutive promoters**. These promoters direct expression in virtually all tissues and are largely, if not entirely, independent of environmental and developmental factors. As their expression is normally not conditioned by endogenous factors, constitutive promoters are usually active across species and even across kingdoms.

2. **Tissue-specific or development-stage-specific promoters**. These direct the expression of a gene in specific tissue(s) or at certain stages of development. For plants, promoter elements that are expressed or affect the expression of genes in the vascular system, photosynthetic tissues, tubers, roots and other vegetative organs, or seeds and other reproductive organs can be found in heterologous systems (e.g. distantly related species or even other kingdoms) but the most specificity is generally achieved with homologous promoters (i.e. from the same species, genus or family). This is probably because the coordinate expression of transcription factors is necessary for regulation of the promoter's activity.

3. **Inducible promoters**. Their performance is not conditioned to endogenous factors but to environmental conditions and external stimuli that can be artificially controlled. Within this group, there are promoters modulated by abiotic factors such as light, oxygen levels, heat, cold and wounding. Since some of these factors are difficult to control outside an experimental setting, promoters that respond to chemical compounds, not found naturally in the organism of interest, are of particular interest. Along those lines, promoters that respond to antibiotics, copper, alcohol, steroids, and herbicides, among
other compounds, have been adapted and refined to allow the induction of gene activity at will and independently of other biotic or abiotic factors.

4. **Synthetic promoters**. Promoters made by bringing together the primary elements of a promoter region from diverse origins.

Apart from the promoter types mentioned above, there are regulatory expression systems based on transactivating proteins. These systems regulate the expression of genes of interest irrespective of their physical position to the target genes. In fact, several chemical–inducible promoters incorporate transactivating proteins and constitutive promoters as part of the regulatory system. Transactivating proteins constitute a whole realm of molecules in the field of gene regulation and deserve a separate analysis. We deal with some of them in this paper, but only to the extent that they overlap with the types of promoters aforementioned.

**Why the interest in promoters?**

The interest in promoters stems from the myriad opportunities for controlling gene expression. The study and understanding of the function of their multiple components and the factors associated with their performance have opened up the possibility of modulation of the expression of genes in homologous organisms as well as in heterologous organisms, where foreign promoters together with genes of interest are inserted. Promoters are regarded as molecular biological tools crucial for the regulation of the expression of genes of interest. As such, they have a huge influence in follow–on research and development in biotechnology.

**What sort of information do we provide on promoters?**

There is virtually an endless number of promoters, potentially as many as there are genes (e.g. a diploid flowering plant has an estimated 25 000 genes). As full genome sequences of different organisms are becoming available (e.g. Arabidopsis), a great number of promoters are being identified, isolated and evaluated, and many more are likely to pop up in the near future. If we attempted to present them all, it would be a daunting and almost never–ending task. Our aim instead is to present scientific and patent information on the most widely used promoters.

The information included in this document comprises:

- **Primary information about promoters**. Over the last two decades, the concept of a promoter has changed from that of a string of a few functional elements to that of a very complex region of interacting structural functional elements. We present a consensus view of a promoter, its common elements and functions and the basic differences between eukaryotic promoters and prokaryotic promoters. The information has been compiled from a variety of scientific books and journal articles.

- **Types of promoters**. This section describes different categories of promoters: constitutive promoters, tissue–specific promoters, inducible promoters, and synthetic promoters, and their modes of operation, properties, applications and drawbacks. A selection of widely used promoters within these categories is presented in detail. The scientific information has been compiled from scientific books and printed and on–line journals, and information provided by researchers.

- **Intellectual property aspects of promoters**. As in many burgeoning areas of science with potential far–reaching commercial applications, promoters and their users are being patented. The analyses of every promoter subject to patent protection would be overly time consuming and probably ultimately not very useful, because almost every gene could be a source of a promoter. Instead, our analyses focus on intellectual property aspects of the categories of promoters aforementioned, and on details of selected individual promoters in some cases. The information deals with:
  - Patents on selected widely used promoters and general promoter types. An assessment is presented of the possibilities for and limitations on further development of regulation of gene expression. The analyses include general patent information such as patent numbers, total number of patents on a particular promoter, applicant names, dates of filing and grant. Additionally, we analyze claims of relevant patents, including aspects of the prosecution history of the patents where appropriate.
  - Overlapping patent protection. Although the inventions protected by individual patents cannot be exactly the same, in certain cases there are patents that due to the breadth of their scope may encompass other protected inventions or there may be patents sharing common features. Where
that is the case, the document points out the juxtaposition of the different inventions and the possible room left to maneuver around the different entities in the field. Take into account that there are patents that while not totally directed to promoters may have an effect on gene expression control. This is the case for the restrictive reproductive technologies, dubbed as "Terminator" and "Verminator" technologies, which may have a great impact on the use and development of methods to regulate the expression of genes related to plant reproduction and seed generation.

- IP rights on commercialization of technology based on promoters. Although many people regard promoters as being confined to the research circles, media press releases and information provided by agrobiotech companies illustrate how patent rights are used as commercial assets in the agrobiotechnology industry. Patents on plant regulatory regions play a role in the subsequent development and innovation in promoters and areas that rely heavily on mechanisms for controlling gene expression.

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**Primary information about promoters**

**Eukaryotic promoters**

To turn a gene into a protein product, at least two general steps are required:

1. the gene is transcribed, spliced and processed to form mRNA, and
2. the mRNA is translated into a polypeptide.

Transcription is a controlled process. While multiple DNA regions are involved, the promoter is the main determinant for the initiation of transcription and modulation of levels and timing of gene expression.

![Structure of a typical eukaryotic gene](Image)

Promoters in eukaryotic organisms—e.g. plants, animals—comprise multiple elements, some of which are found in nearly all promoters. These include:

- **CAAT box.** A consensus sequence close to -80 bp from the start point (+1). It plays an important role in promoter efficiency, by increasing its strength, and it seems to function in either orientation. This box is replaced in plants by a consensus sequence called the **AGGA box**;

- **TATA box.** A sequence usually located around 25 bp upstream of the start point. The TATA box tends to be surrounded by GC rich sequences. The TATA box binds RNA polymerase II and a series of transcription factors (TFIIX, being X a letter that identifies an individual transcription factor) to form an initiation complex;

- **GC box.** A sequence rich in guanidine (G) and cytidine (C) nucleotides, is usually found in multiple copies in the promoter region, normally surrounding the TATA box; and

- **CAP site.** A transcription initiation sequence or start point defined as +1, at which the transcription process actually starts.
Conserved eukaryotic promoter elements | Consensus sequence
--- | ---
CAAT box | GGCCAATCT
TATA box | TATAAA
GC box | GGGCGG
CAP site | TAC

RNA polymerase II is the enzyme that transcribes a gene into RNA. It works in conjunction with other transcription factors that recognize signals embodied in the promoter region. RNA polymerase II starts its "journey" at the TATA region where it binds and travels along the DNA until it reaches the CAP site where the actual synthesis of RNA starts. The transcription process only takes place in the downstream direction, from 5' (left) to 3' (right).

These elements are normally regarded as constituents of the promoter region itself, but depending on the scope of definition of a promoter in a patent or patent application, and whether the definition is expressed in functional or structural terms, other elements may be included as part of a promoter region.

Enhancers, for example, are elements located at variable distances from the promoter "itself" and contain several closely arranged sequence elements that bind to transcription factors. These elements enhance the activity of a promoter and are orientation-independent with respect to the promoter and can be upstream or downstream of a promoter (e.g., such as within intron sequences of a gene). There is currently a high interest in studying and isolating enhancers, which can be successfully attached to heterologous promoter regions to increase transcriptional activity and in some cases to provide additional levels of control (e.g., to confer tissue-specific or stage-specific expression of a gene).

Prokaryotic promoters

Promoters of prokaryotic organisms, e.g., such as bacteria, have similar elements as the eukaryotic promoters although there are a few basic differences. Prokaryotic promoters contain at least three conserved features defining the region where the RNA polymerase binds:

- the start point, defined as +1;
- the TATA box
  is located at −10 position to the start point; in contrast to the −35 bp in eukaryotic promoters; and
- the TTGACA sequence, also called the −35 sequence, located around 35 bp upstream of the start point.

An additional feature, much more common in prokaryotic organisms, is that a promoter serves to initiate the transcription of multiple structural genes that are immediately adjacent to it. This arrangement is called an operon. A single transcribed mRNA is translated into several proteins whose functions are interrelated. In operons, promoters have adjacent, juxtaposed or interspersed regulatory sites to which regulatory proteins bind. In eukaryotic promoters, the regulatory sites are spread out over a longer distance.

There are two modes of regulation of the initiation of transcription in operons:

1. **Positive control mode**, where the interaction between the regulatory protein and regulatory region on the DNA turn the transcription on. The genes are off by default and are turned on by the activators. Transcription factors interact with the RNA polymerase and assist the enzyme in initiating transcription at the promoter. This positive fashion of controlling gene expression is more common in eukaryotes than in prokaryotes.

2. **Negative control mode**, where the interaction turns the genes off. In this case, a repressor protein binds the operator, a DNA sequence of approximately 20 to 25 nucleotides, which is next to the promoter or juxtaposed, and prevents the RNA polymerase from initiating transcription. To switch on the system, small molecules called inducers trigger the production of proteins by binding to the repressor protein and changing its conformation. This change alters the operator–repressor interaction, so that the repressor can no longer remain attached to the operator. Negative control is widely used among prokaryotes, which need to respond swiftly to changes in the environment.
One of the best-studied operon systems is the lac operon from *Escherichia coli* (*E. coli*). Since its discovery in the 1960’s, other operon systems have been extensively studied in other organisms and are currently being adapted to plant systems with the aim of tightly regulating the expression of genes in transgenic plants.

**Promoter types**

**Summary**

As mentioned in the introduction, promoters can be categorized according to the type or degree of control of gene expression: control in all or virtually all tissues or control depending on the tissue and the developmental stage of the plant. Additionally, promoters may operate in response to external and, in some cases, controllable stimuli. Thus, they can be classified as follows:

- **Constitutive promoters**, which induce the expression of the downstream-located coding region in all tissues irrespective of environmental or developmental factors.

- **Synthetic promoters**, which comprise consensus DNA sequences of common elements of natural promoter regions.

- **Inducible promoters**, which are only expressed under the presence of factors/compounds. Because their expression is normally restricted to certain plant tissues, they can also be considered as tissue-specific. Based on the nature of the factors that trigger their expression, they are divided into two groups:
  - **Chemically-regulated**, where chemical compounds, usually not naturally found within plants, switch on promoter activity. Several of the types of promoters discussed in this paper involve chimeric components gathered from human, animal, fungal and bacterial sources.
  - **Physically-regulated**, where abiotic and external factors such as light, heat, mechanical injury induce promoter activity.

- **Tissue-specific promoters**, which operate in particular tissues and at certain developmental stages of a plant. They may be induced by endogenous and exogenous factors, so they may be also classified as inducible.
Chapter 2 – Constitutive promoters

Summary

There are several advantages to using constitutive promoters in expression vectors used in plant biotechnology, such as:

- High level of production of proteins used to select transgenic cells or plants;
- High level of expression of reporter proteins or scorable markers, allowing easy detection and quantification;
- High level of production of a transcription factor that is part of a regulatory transcription system;
- Production of compounds that requires ubiquitous activity in the plant; and
- Production of compounds that are required during all stages of plant development

The first constitutive promoters used for the expression of transgenes in plants were isolated from plant pathogens.

The search for other constitutive promoters has continued, especially to identify control regions that are able to drive expression of transgenes in monocots. In some monocots such as cereals, it has been found that sequences present in 5' untranslated transcribed regions (e.g., introns) of certain structural genes are essential for efficient gene expression. Thus, promoters that work well in dicots, which lack introns, do not generally work well in monocots. For this reason, regulatory sequences from monocots have also been identified and cloned into vectors for control of transgene expression. The following constitutive promoters are among the most widely used nowadays and are the ones analyzed in this report.

- **Plant pathogen promoters**
  - Opine promoters
  - CaMV 35S promoter
- **Monocot promoters**
  - Plant ubiquitin promoter (Ubi)
  - Rice actin 1 promoter (Act-1)
  - Maize alcohol dehydrogenase 1 promoter (Adh-1)

The CaMV 35S promoter

Analysis on the CaMV 35 promoter is divided into a discussion of:

- the promoter itself
- sequences identified in patents as "35S enhancer regions"
- the "minimal" promoter

The promoter itself

Scientific aspects

At the beginning of the 1980's, Chua and collaborators at the Rockefeller University isolated the promoter responsible for the transcription of the whole genome of a Cauliflower mosaic virus (CaMV) infecting turnips. The promoter was named CaMV 35S promoter ("35S promoter") because the coefficient of sedimentation of the viral transcript whose expression is naturally driven by this promoter is 35S. It is one of the most widely used, general-purpose constitutive promoters.

The 35S promoter is a very strong constitutive promoter, causing high levels of gene expression in dicot plants. However, it is less effective in monocots, especially in cereals. The differences in behavior are probably due to differences in quality and/or quantity of regulatory factors.
The promoter responsible for the transcription of another part of the genome of CaMV, the CaMV 19S promoter, is also used as a constitutive promoter, but is not as widely used as the 35S promoter.

CaMV 35S promoter IP issues

Monsanto Company and The Rockefeller University are the owners of patents on the CaMV 35S promoter. The geographical range of their patents, according to publicly available information sources, is limited to:

- The United States and Japan in the case of Monsanto (a patent may still be pending in Brazil), and
- Only the United States in the case of the Rockefeller University.

If you are aware of additional or more up to date information about patent coverage, please add it to what is available to users through our comments interface.

How is promoter defined?

Before entering the discussion about claims granted, it is important to know how a promoter is defined in the patents.

Monsanto defines promoter in a functional manner. In the Monsanto patents a promoter is the region at the 5' end of a gene that initiates transcription of the gene to produce a mRNA transcript. The 35S promoter in particular is referred to as the promoter for the full-length mRNA of the CaMV genome.

The Rockefeller University does not explicitly define the word "promoter." Nevertheless, in the specifications of the US patents, a promoter sequence is described as necessary in order to obtain "adequate expression" of a gene inserted into a plant. So, in that sense, the concept of a promoter is also functional. With respect to the 35S promoter, the specifications disclose the use of CaMV 35S promoter as a promoter for general use. They define the 35S promoter in terms of the sequences of its subdomains.

Approximate scope of claims

Both entities have claims directed to methods and products, but they address different aspects of the 35S promoter. In general terms,

- Monsanto’s patents are directed to chimeric genes containing the 35S or the 19S promoter controlling a heterologous gene, and
- The Rockefeller University’s patents are directed to the DNA sequences of the individual subdomains of the 35S promoter, combinations of them, and the use of B subdomains in particular to form tissue-specific promoters.

In the U.S., there are currently four patents granted to Monsanto and two patents granted to The Rockefeller University. This situation may change over time as additional applications may be pending and some may lapse; if you are aware of more current information, please add comments on this page or on the page.
giving details on the patents and claims.

The U.S. claims cover:

- Monsanto
  - "Chimeric genes" having the CaMV 35S promoter or the CaMV 19S promoter, a heterologous gene and a poly(A) signal;
  - In particular, such genes in which the heterologous gene confers antibiotic resistance to a transformed plant;
  - Plant transformation vectors, both intermediates and vectors having a chimeric gene as described;
  - Differentiated dicot plants containing these chimeric genes; and
  - Methods for transforming plant cells with these chimeric genes.

- The Rockefeller University
  - Isolated DNA sequences of four of the five subdomains of domain B of the CaMV 35S promoter;
  - The same sequences of the B subdomains linked to domain A of the 35S promoter and to the minimal promoter region of the 35S promoter;
  - Sequence of a tissue-specific promoter corresponding to domain B of the 35S promoter;
  - Method for the tissue-specific expression of a chimeric gene by using domain B coupled to the domain A of the 35S promoter.

Claims in the European patent granted to Monsanto covered a "chimeric gene" very similar to the one claimed in the U.S., except that the promoter is limited to the 35S promoter and the chimeric gene also contains a 5' non-translated region. This patent has now lapsed, but it is worth mentioning because the European patent as first granted was opposed by several institutions. The claim, in the application as originally filed, was directed to a promoter from a plant virus, which could be from any plant virus, not only from CaMV. After the opposition, the promoter was restricted to the CaMV 35S promoter.

The specific patent information and a summary of the independent claims of each patent are presented in the following tables.

<table>
<thead>
<tr>
<th>Patents on 35S promoter</th>
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<td><strong>Title, Independent Claims and Summary of Claims</strong></td>
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<tr>
<td>Title – Chimeric genes for transforming plant cells using viral promoters</td>
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<tr>
<td><strong>Claim 1</strong></td>
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<tr>
<td>A chimeric gene which is expressed in plant cells comprising a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV (35S) promoter isolated from CaMV protein-encoding DNA sequences and a CaMV (19S) promoter isolated from CaMV protein-encoding DNA sequences, and a structural sequence which is heterologous with respect to the promoter.</td>
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<td><strong>Claim 4</strong></td>
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<td>A plant cell which comprises a chimeric gene that contains a promoter from cauliflower mosaic virus, said promoter selected from the group</td>
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<td>Claim 7</td>
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<td>An intermediate plant transformation plasmid which comprises a region of homology to an <em>Agrobacterium tumefaciens</em> vector, a T–DNA border region from <em>Agrobacterium tumefaciens</em> and a chimeric gene, wherein the chimeric gene is located between the T–DNA border and the region of homology, said chimeric gene comprising a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and a structural sequence which is heterologous with respect to the promoter.</td>
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<th>Claim 8</th>
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<td>A plant transformation vector which comprises a disarmed plant tumor inducing plasmid of <em>Agrobacterium tumefaciens</em> and a chimeric gene, wherein the chimeric gene contains a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and a structural sequence which is heterologous with respect to the promoter.</td>
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<th>Claim 13</th>
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<td>A DNA construct comprising: (A) a CaMV promoter selected from the group consisting of (1) a CaMV 35S promoter isolated from CaMV protein–encoding DNA sequences and (2) a CaMV 19S promoter isolated from CaMV protein–encoding DNA sequences, and (B) a DNA sequence of interest heterologous to (A), wherein (B) is under the regulatory control of (A) when said construct is transcribed in a plant cell.</td>
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<th>Claim 14</th>
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<td>A chimeric gene which is transcribed and translated in plant cells, said chimeric gene comprising a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of: a) a CaMV 35S promoter region free of CaMV protein–encoding DNA sequences and b) a CaMV 19S promoter region free of CaMV protein–encoding DNA sequences, and a DNA sequence which is heterologous with respect to the promoter.</td>
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<th>Claim 15</th>
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<td>A chimeric gene which is expressed in plants cells comprising a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter free of CaMV protein–encoding DNA sequences and a CaMV(19S) promoter free of CaMV protein–encoding DNA sequences, and a DNA sequence which is heterologous with respect to the promoter.</td>
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<th>Claim 16</th>
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<td>A chimeric gene which is transcribed in plants cells comprising a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter free of CaMV protein–encoding DNA sequences and a CaMV(19S) promoter free of CaMV protein–encoding DNA sequences, a DNA sequence which is heterologous with respect to the promoter and a 3′ non–translated polyadenylation signal sequence.</td>
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<th>Claim 17</th>
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<td>A plant cell which comprises a chimeric gene where said chimeric gene comprises a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a...</td>
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CaMV(19S) promoter, wherein said promoter is free of CaMV protein–encoding DNA sequences, and a DNA sequence which is heterologous with respect to the promoter and a 3′ non–translated polyadenylation signal sequence.

Claim elements:

- 35S and 19S promoters, as defined in the specification, are claimed as part of a chimeric construct, also containing a heterologous signal and a poly(A) signal, expressed in a plant
- There are also claims to intermediate plant transformation vectors and plant transformation vectors containing the chimeric gene for Agrobacterium–mediated plant transformation, and to plant cells having the chimeric gene.

**US 5530196**

**Title** – Chimeric genes for transforming plant cells using viral promoters

**Claim 1**

A differentiated dicotyledonous plant comprising plant cells containing a chimeric gene which comprises a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter free of CaMV protein–encoding DNA sequences and a CaMV(19S) promoter free of protein–encoding DNA sequences, and a structural sequence which is heterologous with respect to the promoter.

**Claim 4**

A differentiated dicotyledonous plant comprising plant cells containing in the plant genome a chimeric gene which comprises a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and a DNA sequence which is heterologous with respect to the promoter.

**Claim 5**

A differentiated dicotyledonous plant regenerated from plant cells, said plant cells containing a chimeric gene which comprises a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and a DNA sequence which is heterologous with respect to the promoter.

This patent is a Continuation of US 5352605.

- Differentiated dicot plants having plant cells with a chimeric gene similar as described above. The poly(A) signal is not expressly claimed as a component of the chimeric gene.

**US 5858742**

**Title** – Chimeric genes for transforming plant cells using viral promoters

**Claim 1**

A method for transforming a plant cell which comprises transforming a plant cell with a chimeric DNA construct containing a promoter isolated from cauliflower mosaic virus (CaMV), said promoter selected from the group consisting of a CaMV(19S) promoter derived from the CaMV(19S) gene and a CaMV(35S) promoter derived from the CaMV(35S) gene, and a DNA sequence which is heterologous with respect to the promoter; wherein the promoter regulates the transcription of the DNA sequence.

This patent is a Continuation of US 5530196.
Method for transforming plant cells with a chimeric DNA construct as generally described in US 5530196.

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<th>US 6255560</th>
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**Title** – Chimeric genes for transforming plant cells using viral promoters

**Claim 1**
A chimeric gene which is expressed in plant cells comprising a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to a DNA sequence which is heterologous with respect to the promoter, wherein: the promoter regulates the transcription of the DNA sequence, and the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to a plant or plant cell containing the DNA sequence relative to a wild-type plant or plant cell.

**Claim 3**
A plant cell comprising a chimeric gene which comprises a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to a DNA sequence which is heterologous with respect to the promoter, wherein: the promoter regulates the transcription of the DNA sequence, and the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to the plant cell relative to a wild-type plant cell.

**Claim 6**
An intermediate plant transformation plasmid which comprises a region of homology to an *Agrobacterium tumefaciens* vector, a T–DNA border from *Agrobacterium tumefaciens*, and a chimeric gene, wherein the chimeric gene is located between the T–DNA border and the region of homology, said chimeric gene comprising a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to a DNA sequence which is heterologous with respect to the promoter, wherein: the promoter regulates the transcription of the DNA sequence, and the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to a plant or plant cell containing the DNA sequence relative to a wild–type plant or plant cell.

**Claim 9**
A plant transformation vector which comprises a modified plant tumor inducing plasmid of *Agrobacterium tumefaciens* which is capable of inserting a chimeric gene into susceptible plant cells, wherein the chimeric gene comprises a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to a DNA sequence which is heterologous with respect to the promoter, wherein: the promoter regulates the transcription of the DNA sequence, and the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to a plant or plant cell containing the DNA sequence relative to a wild–type plant or plant cell.

**Claim 12**
A differentiated dicotyledonous plant comprising plant cells containing a chimeric gene which comprises a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to a DNA sequence encoding said polypeptide which is heterologous with respect to the promoter, wherein: the promoter regulates the transcription of the DNA sequence, and the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to the plant relative to a wild–type plant.

This patent is a Continuation of US 5858742.

- A chimeric gene where the 35S or the 19S promoter regulates the
transcription of a gene conferring antibiotic resistance to a plant.

- An intermediate plant transformation vector with the mentioned chimeric gene between a T–DNA border and a region of homology to an Agrobacterium tumefaciens vector.
- A plant transformation vector capable of inserting the chimeric gene into a plant cells. Differentiated dicot plants having plant cells with the chimeric gene.

**Claim 1**
A chimeric gene capable of expressing a neomycin phosphotransferase polypeptide in plant cells conferring antibiotic resistance to the plant when inserted into the plant genome, comprising in sequence:

a) a promoter region from a ribulose–1,5–bis–phosphate carboxyase small subunit gene;
b) a 5' non–translated region
c) a structural coding sequence encoding neomycin phosphotransferase I or II; and
d) a 3' non–translated region of a gene naturally expressed in plant cells, said region encoding a signal sequence for polyadenylation of mRNA, said promoter region being heterologous with respect to the structural coding sequence.

**Claim 4**
A chimeric gene capable of expressing a polypeptide in plant cells comprising in sequence:

a) a full–length transcript promoter region isolated from cauliflower mosaic virus
b) a 5' non–translated region
c) a structural coding sequence
d) a 3' non–translated region of a gene naturally expressed in plants, said region encoding a signal sequence for polyadenylation of mRNA, said structural coding sequence being heterologous with respect to said promoter region.

**Claim 6**
A culture of microorganisms identified by ATCC accession number 39265.

The claims are to:

- A chimeric gene capable of expressing a polypeptide in plant cells having a 35S promoter, an intron, a **structural coding sequence** and a poly(A) signal.

**Remarks**
Granted Japanese patent JP 2645217 was not analyzed. There are also a pending Japanese application (JP 7014349 B4) and a pending Brazilian application (BR 1101069).

Note: Patent information on this page was last updated on 15 March 2006.

### Patents on subdomains of the 35S promoter

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Summary of Claims and Independent Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US 5097025</strong></td>
<td>Title – Plant Promoters</td>
<td>The Rockefeller University</td>
</tr>
<tr>
<td></td>
<td>Claim elements:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Isolated DNA sequences of four of the five subdomains of domain B</td>
<td></td>
</tr>
</tbody>
</table>
of the 35S promoter (see [diagram](#)).

- DNA sequences of the combinations of each of the four subdomains B and the domain A of the 35S promoter.
- DNA sequences of the combinations of each of the four subdomains B and the minimal promoter of the 35S promoter.

### Claim 1
An isolated DNA segment consisting of the nucleotide sequence:

5’-CGACCAGCAT CGTGAAAAAA GAAGACGTTCA ACCACGTC TTCAAGGC-3’.

*sequence of subdomain B2 of 35S CaMV promoter.

### Claim 2
A DNA sequence consisting of:
- a first nucleotide sequence 5’-CGAGGAGCAT CGTGAAAAAA GAAGACGTTCA ACCACGTC TTCAAGGC-3’
- a second nucleotide sequence corresponding to domain A of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B2 of 35S CaMV promoter.

### Claim 3
A DNA sequence consisting of:
- a first nucleotide sequence 5’-CATCGTTGAAG ATGCCTCTGC CGACAGTGGT CCCAAAGATG GACCCCCACC CAC-3’
- a second nucleotide sequence corresponding to domain A of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B3 of 35S CaMV promoter.

### Claim 4
A DNA sequence consisting of:
- a first nucleotide sequence 5’-ATTCC ATTGCC ACGCTATCTGT CACTCTATTG TGAAGATAGT GGAAAAGGAA GGTGGCTCCT ACAAATGCCA TCAATGCGAT AAGGAAAGG CC-3’
- a second nucleotide sequence corresponding to domain A of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B4 of 35S CaMV promoter.

### Claim 5
A DNA sequence consisting of:
- a first nucleotide sequence 5’-TGAGACTTTT CAACAAAGGG TAATATCCGG AAACCTCTCC GGAT-3’
- a second nucleotide sequence corresponding to domain A of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B5 of 35S CaMV promoter.

### Claim 6
An isolated DNA segment consisting of the nucleotide sequence:

5’-CATCGTTGAAG ATGCCTCTGC CGACAGTGGT CCCAAAGATG GACCCCCACC CAC-3’.

*sequence of subdomain B3 of 35S CaMV promoter.

### Claim 7
An isolated DNA segment consisting of the nucleotide sequence:

5’-ATTCC ATTGCC ACGCTATCTGT CACTCTATTG TGAAGATAGT...
**Claim 8**
An isolated DNA segment consisting of the nucleotide sequence:
5’-TGAGACTTTT CAACAAAGGG TAATATCCGG AAACCTCCTC GGATT-3’.

*sequence of subdomain B5 of 35S CaMV promoter.

**Claim 9**
A DNA sequence consisting of:
a) a first nucleotide sequence 5’-CGAGGAGCAT CGTGGAAAAA GAAGACGTTC CAACCACGTC TTCAAAGGC-3’ and
b) a second nucleotide sequence corresponding to the minimal promoter region of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B2 of 35S CaMV promoter.

**Claim 10**
A DNA sequence consisting of:
a) a first nucleotide sequence 5’-CATCGTTGAAG ATGCCTCTGC CGACAGTGGT CCCAAAGATG GACCCCCACC CAC-3’

and
b) a second nucleotide sequence corresponding to the minimal promoter region of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B3 of 35S CaMV promoter

**Claim 11**
A DNA sequence consisting of:
a) a first nucleotide sequence 5’-ATTCC ATTGCCC AGCTATCTGT CACTTTATTG TGAAGATAGT GGAAAAGGAA GGTGGCTCCT ACAAATGCCA TCATTGCGAT AAAGGAAAGG CC-3’

and
b) a second nucleotide sequence corresponding to the minimal promoter region of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B4 of 35S CaMV promoter

**Claim 12**
A DNA sequence consisting of:
a) a first nucleotide sequence 5’-TGAGACTTTT CAACAAAGGG TAATATCCGG AAACCTCCTC GGATT-3’

and
b) a second nucleotide sequence corresponding to the minimal promoter region of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B5 of 35S CaMV promoter

---

**Title** – Selective gene expression in plants

**Claim elements:**

- a tissue-specific promoter fragment which causes tissue-specific expression in leaves, stems, cotyledons, and vascular tissue of the hypocotyl while causing detectable levels of expression in root vascular tissue when operably coupled directly to a DNA segment corresponding to the −72 to +8 promoter fragment of the Cauliflower Mosaic Virus 35S gene
- Use of the promoter as generally described above
Plants cells and plants containing a chimeric construct in which the promoter as generally described above is **operably coupled** to a structural gene.

**Claim 1**

In a method for the expression of a chimeric plant gene, the improvement which comprises the use of a tissue-specific promoter fragment which causes tissue-specific expression in leaves, stems, cotyledons, and vascular tissue of the hypocotyl while causing detectable levels of expression in root vascular tissue when operably coupled directly to a DNA segment corresponding to the −72 to +8 promoter fragment of the Cauliflower Mosaic Virus 35S gene, said tissue-specific promoter fragment having the sequence:

```
5′-TGAGACTTTT CAACAAAGGG TAATATCCGG AAACCTCCTC
GGATTCCATT GCCCAGCTAT CTGTCACTTT
ATTGTGAAGA TAGTGGAAAA GGAAGGTGCG TCCTACAAAT
GCCATCATTG CGATAAAGGA AAGGCCATCG
TTGAAGATGC CTCTGCCGAC AGTGGTCCCA AAGATGGACC
CCCACCCCAAC GAGGAGCATC GTGGAAAAAG
AAGACGTTCG AACCACGTCT TCAAAGCAAG TGGATTGATG TGATA-3"
```

*sequence corresponds to the complete domain B from −343 to −90 nucleotides of the 35S CaMV promoter

**Claim 2**

A chimeric plant gene comprising in sequence in the 5′ to 3′ direction a tissue-specific promoter fragment consisting essentially of the sequence:

```
5′-TGAGACTTTT CAACAAAGGG TAATATCCGG AAACCTCCTC
GGATTCCATT GCCCAGCTAT CTGTCACTTT
ATTGTGAAGA TAGTGGAAAA GGAAGGTGCG TCCTACAAAT
GCCATCATTG CGATAAAGGA AAGGCCATCG
TTGAAGATGC CTCTGCCGAC AGTGGTCCCA AAGATGGACC
CCCACCCCAAC GAGGAGCATC GTGGAAAAAG
AAGACGTTCG AACCACGTCT TCAAAGCAAG TGGATTGATG TGATA-3",
```

operably coupled directly to the −72 to +8 promoter fragment of the CaMV 35S gene, said −72 to +8 promoter fragment operably coupled to a structural gene.

*sequence corresponds to the complete domain B from −343 to −90 nucleotides of the 35S CaMV promoter.

**Claim 5**

A tissue-specific promoter fragment which functions in plants to cause tissue-specific expression in the leaves, stems, cotyledons and the vascular tissue of the hypocotyl and detachable levels of expression in root vascular tissue operably coupled directly to a DNA segment corresponding to the −72 to +8 promoter fragment of the Cauliflower Mosaic Virus 35S gene, said tissue-specific promoter fragment having the sequence from its 5′ to 3′ termini:

```
5′-TGAGACTTTT CAACAAAGGG TAATATCCGG AAACCTCCTC
GGATTCCATT GCCCAGCTAT CTGTCACTTT
ATTGTGAAGA TAGTGGAAAA GGAAGGTGCG TCCTACAAAT
GCCATCATTG CGATAAAGGA AAGGCCATCG
TTGAAGATGC CTCTGCCGAC AGTGGTCCCA AAGATGGACC
CCCACCCCAAC GAGGAGCATC GTGGAAAAAG
AAGACGTTCG AACCACGTCT TCAAAGCAAG TGGATTGATG TGATA-3"
```

*sequence corresponds to the complete domain B from −343 to −90 nucleotides of the 35S CaMV promoter.
nucleotides—of the 35S CaMV promoter.

Note: Patent information on this page was last updated on 15 March 2006.

Enforcement of rights

The 35S promoter is one of the most widely used promoters for driving the expression of genes in transgenic plants.

Entities outside the U.S. and Japan using the promoter even without a permit or license from the patent owners do not infringe the patents, because patents are jurisdiction-specific. However, laboratories in public and private institutes around the world using the promoter as claimed in these patents in their experiments and in products released into the market should bear in mind that unauthorized use or import into the U.S. or Japan could constitute infringement.

While the patent owners have not shown interest in prosecuting academic users of the promoter, they could choose to seek injunctions, damages and royalties for infringing uses, including use in any commercial product. For instance, Monsanto Company sued DNA Plant Technology Corporation in the U.S. for infringing the U.S. patent 5352605 by using the CaMV promoter in making and commercializing hybrid tomatoes with delayed ripening. Before going out of business, DNA Plant Technology Corporation had to acknowledge the infringement of this patent and also other Monsanto patents, and entered into a confidential legal settlement agreement that provided Monsanto with compensation.

Enhancer of CaMV 35S promoter

Scientific aspects

The subdomain B of the 35S promoter harbors an enhancer element that increases promoter activity. Initially, enhanced transcription in transgenic tobacco plants was obtained by duplicating the region from the –343 position to the –90 position, which is upstream of the TATA sequence. The sequences involved in the enhancement of transcription were localized to a 162 bp sequence fragment, from –208 to –46 bp. Like other enhancers, this fragment can function in an orientation-independent manner when located either upstream or downstream of a homologous or heterologous TATA box. The region contains binding sites for cellular trans-acting factors such as ASF 1, GATA 1 and CAF. An increase in transcription activity from three-fold up to six-fold is reported in monocot plants transformed with multiple copies of the enhancer region.

IP issues

The University of British Columbia was the initial applicant for several patents covering the use of a duplicated CaMV 35S enhancer sequence as part of a DNA construct for plant transformation. Monsanto Company is now the assignee of all the patents granted in the U.S. and in Canada. The assignee of the granted Brazilian patent is not identified, according to the information provided by the International Patent Documentation Center (INPADOC). If you are aware of updated licensing information, please provide it to other users via our comments interface.

How is enhancer defined?

The applicant defines an enhancer as a sequence which activates transcription and contains repetitive units of short sequences from about 4 to 16 bp. A repetitive unit includes a GTGG, where G may be replaced by A in some cases. A “natural enhancer” is described as comprising a DNA sequence that is upstream from and within about 600 bp of a promoter.

Nowadays, it is known that enhancers can be located thousands of base pairs upstream or downstream from the gene they control. They can even be located within the gene under control. In the specification, a promoter is described as the transcription initiation domain, that is, a sequence that contains the TATA box and the CAP site. So, a transcription initiation region is
formed by a transcription initiation domain or promoter and enhancer domain. In this way, the inventors divide a "full-length" or "complete" 35S promoter into two main areas, and the enhancer region becomes part of what is regarded in general as the promoter region.

Approximate scope of protection

In all the patents, the claims generally cover a fragment of the 35S upstream region, defined as AluI-EcoRV fragment. This is described as a truncated 35S enhancer, from –287 to –90 position. In the U.S., the claims are directed to:

- A DNA construct having:
  - a duplicated 35S enhancer, which corresponds to a duplication of the truncated 35S enhancer;
  - a promoter (with TATA box and CAP site);
  - a sequence of interest; and
  - a termination region.

- A plant cell having the mentioned construct.

- A chimeric transcriptional initiation region that, by having a tandemly duplicated 35S enhancer, "enhances" the amount of transcription compared to having one copy of the enhancer.

- A differentiated plant having the described chimeric transcriptional initiation region.

- A method for expressing a gene of interest by transforming a plant cell with the described chimeric transcriptional initiation region.

The Canadian patent claims the same type of DNA construct as the U.S. patents. Also claimed are:

- a chimeric transcriptional initiation region contains two to four copies of the truncated 35S enhancer;

- a method for producing a plant cell with enhanced expression of a gene of interest using the multicopy truncated 35S enhancer; and

- a plant cell having a DNA construct with two copies of the truncated 35S enhancer.

In addition, there are broader claims that encompass a transcription initiation region with a plant or viral enhancer domain having four units of enhancer sequences and a promoter. A DNA construct comprising such a transcription initiation region is also claimed. The patent numbers and a summary of the independent claims of each patent are presented in the following table.

<table>
<thead>
<tr>
<th>Patents on duplicated CaMV 35S enhancer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patent number</td>
</tr>
<tr>
<td>US 5164316</td>
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<tr>
<td>Patent</td>
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<tr>
<td>US 5196525</td>
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<tr>
<td>US 5322938</td>
</tr>
<tr>
<td>US 5359142</td>
</tr>
<tr>
<td>US 54234200</td>
</tr>
</tbody>
</table>
Claim 1
A method for expressing a DNA sequence of interest comprising transforming a plant cell with a DNA construct comprising (a) a tandemly duplicated CaMV 35S enhancer sequence comprising an AluI-EcoRV fragment of a CaMV 35S upstream region; (b) a promoter comprising an RNA polymerase binding site and an mRNA initiation site; (c) a nucleotide sequence of interest; and (d) a termination region; wherein said components are operably joined so that said sequence of interest is transcribed.

This patent is a divisional of **US 5359142**.
- A method for expressing a DNA sequence of interest by transforming a plant cell with a DNA construct as claimed in **US 53196525**.

Title – DNA construct for enhancing the efficiency of transcription

Claim 1
A transcriptional initiation region comprising:
(a) a plant or viral enhancer domain comprising a plurality of units each including a sequence of the group comprising: GTGG, ATGG, GTAG, GTGA, GTGGA(T)A(T)A(T), GTGTGGA(T)A(T)A(T)G, and complementary sequence thereof, said enhancer domain further comprising at least one additional unit including a sequence selected from the said group; and
(b) a transcription initiation domain comprising an RNA polymerase binding site and an mRNA initiation site, under the enhancing control of said enhancer domain.

Claim 6
A DNA construct comprising: a transcription initiation region comprising:
(a) an enhancer domain comprising a plurality of units each including a sequence selected from the group comprising: GTGG, ATGG, GTAG, GTGA, GTGGA(T)A(T)A(T), GTGTGGA(T)A(T)A(T)G, and complementary sequence thereof; and
(b) a transcription initiation domain comprising an RNA polymerase binding site and an mRNA initiation site under the enhancing control of said enhancer domain;
(c) a sequence of interest for transcription to mRNA; and
(d) a termination region.

Claim 22
A DNA sequence comprising a transcription initiation region capable of regulating transcription of a nucleotide sequence of interest in a plant cell and including, as operably joined components, (i) two to four copies in tandem of an enhancer sequence comprising an AluI–EcoRV fragment of a CaMV 35S upstream region; and (ii) a promoter comprising an RNA polymerase binding site and an mRNA initiation site.

Claim 29
A chimeric transcriptional initiation region comprising: as operably joined components, (i) two or more copies of a CaMV 35S enhancer sequence and (ii) a promoter comprising an RNA polymerase binding site and an mRNA initiation site, wherein when a nucleotide sequence of interest is transcribed under the regulatory control of said chimeric transcriptional initiation region, the amount of transcription product is
enhanced as compared to the amount of transcription obtained with a chimeric transcriptional initiation region comprising a single copy of said CaMV enhancer sequence and a promoter.

Claim 33

A DNA construct having as components, (a) a transcription initiation region including (i) a tandemly duplicated CaMV 35S enhancer sequence comprising an AluI–EcoRV fragment of a CaMV 35S upstream region; (ii) a promoter comprising an RNA polymerase binding site and an mRNA initiation site; (b) a nucleotide sequence of interest for transcription to mRNA; and (c) a termination region wherein said components are operably joined.

Claim 38

A method for producing a plant cell capable of enhanced expression of a nucleotide sequence of interest, said method comprising: operably linking to form an expression cassette said nucleotide sequence of interest and a transcription initiation region capable of regulating transcription of a DNA sequence of interest in a plant cell, wherein said transcription initiation region comprises (i) two to four copies in tandem of an enhancer sequence comprising an AluI–EcoRV fragment of a CaMV 35S upstream region; and (ii) a promoter comprising an RNA polymerase binding site and an mRNA initiation site; and introducing said expression cassette into said plant cell to form a plant cell capable of enhanced expression of a said nucleotide sequence of interest.

Claim 47

A plant cell comprising: a DNA construct having components, (a) a transcription initiation region including (i) a tandemly duplicated CaMV 35S enhancer sequence comprising an AluI–EcoRV fragment of a CaMV 35S upstream region; (ii) a promoter comprising an RNA polymerase binding site and an mRNA initiation site; (b) a nucleotide sequence of interest for transcription to mRNA; and (c) a termination region wherein said components are operably joined.

Claim elements:

- A DNA transcription region comprising 2 to 4 copies in tandem of enhancer 35S and a promoter region as generally claimed in US 5164316.
- A chimeric transcriptional initiation region comprising 2 or more copies of enhancer 35S and having the properties as generally claimed in US 5322938.
- A DNA construct as generally claimed in US 5196525.
- A method of producing a plant cell capable of enhanced expression of a gene of interest by having in an expression cassette with a gene of interest linked to a DNA transcription region as described in the first point.
- A plant cell comprising a DNA construct as generally claimed in US 5164316.

Remarks

A related patent has been granted in Brazil (BR 1101045).

Note: Patent information on this page was last updated on 6 March 2006.

Pending revocations of 35S patents
Challenges to patents on 35S promoter and duplicated CaMV 35S enhancer sequences

In September 2006, PUBPAT filed formal requests with the United States Patent and Trademark Office to revoke four patents owned by Monsanto Company. PUBPAT has issued a press release giving the opinion that these patents were a subject for action in the public interest because Monsanto is using these patents to intimidate and sue farmers.

The patents are U.S. Patents 5352605, 5164316, 5196525, and 5322938, all of which assert claims to the CaMV 35S promoter, double 35S enhancer, and/or constructs containing them. Like some other Monsanto technologies, the 35S promoter and double 35S enhancer have been used in a great deal of agricultural research worldwide, and as a result Monsanto's intellectual property does constrain the delivery of products.

In its filings, PUBPAT submitted putative prior art supporting an assertion that the patents should not have been granted, and therefore should be revoked. The USPTO will do a re-examination of the patents in view of the submission, and if it is found that the patents are invalid over the prior art, may require revision or revocation of specified claims. The independent claims are listed below for easy reference.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5352605</td>
<td><strong>Title</strong> - Chimeric genes for transforming plant cells using viral promoters</td>
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</tr>
<tr>
<td></td>
<td><strong>Claim 1</strong> A chimeric gene which is expressed in plant cells comprising a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV (35S) promoter isolated from CaMV protein–encoding DNA sequences and a CaMV (19S) promoter isolated from CaMV protein–encoding DNA sequences, and a structural sequence which is heterologous with respect to the promoter.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Claim 4</strong> A plant cell which comprises a chimeric gene that contains a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV (35S) promoter and a CaMV (19S) promoter, wherein said promoter is isolated from CaMV protein–encoding DNA sequences, and a structural sequence which is heterologous with respect to the promoter.</td>
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<tr>
<td></td>
<td><strong>Claim 7</strong> An intermediate plant transformation plasmid which comprises a region of homology to an Agrobacterium tumefaciens vector, a T–DNA border region from Agrobacterium tumefaciens and a chimeric gene, wherein the chimeric gene is located between the T–DNA border and the region of homology, said chimeric gene comprising a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and a structural sequence which is heterologous with respect to the promoter.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Claim 8</strong> A plant transformation vector which comprises a disarmed plant tumor inducing plasmid of Agrobacterium tumefaciens and a chimeric gene, wherein the chimeric gene contains a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and a structural sequence which is heterologous with respect to the promoter.</td>
<td>Monsanto</td>
</tr>
</tbody>
</table>
Claim 13
A DNA construct comprising: (A) a CaMV promoter selected from the group consisting of (1) a CaMV 35S promoter isolated from CaMV protein–encoding DNA sequences and (2) a CaMV 19S promoter isolated from CaMV protein–encoding DNA sequences, and (B) a DNA sequence of interest heterologous to (A), wherein (B) is under the regulatory control of (A) when said construct is transcribed in a plant cell.

Claim 14
A chimeric gene which is transcribed and translated in plant cells, said chimeric gene comprising a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of: a) a CaMV 35S promoter region free of CaMV protein–encoding DNA sequences and b) a CaMV 19S promoter region free of CaMV protein–encoding DNA sequences, and a DNA sequence which is heterologous with respect to the promoter.

Claim 15
A chimeric gene which is expressed in plant cells comprising a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter region free of CaMV protein–encoding DNA sequences and a CaMV(19S) promoter region free of CaMV protein–encoding DNA sequences, and a DNA sequence which is heterologous with respect to the promoter.

Claim 16
A chimeric gene which is transcribed in plant cells comprising a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter free of CaMV protein–encoding DNA sequences and a CaMV(19S) promoter free of CaMV protein–encoding DNA sequences, a DNA sequence which is heterologous with respect to the promoter and a 3' non–translated polyadenylation signal sequence.

Claim 17
A plant cell which comprises a chimeric gene where said chimeric gene comprises a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, wherein said promoter is free of CaMV protein–encoding DNA sequences, and a DNA sequence which is heterologous with respect to the promoter and a 3' non–translated polyadenylation signal sequence.

Claim elements:

- 35S and 19S promoters, as defined in the specification, are claimed as part of a chimeric construct, also containing a heterologous signal and a poly(A) signal, expressed in a plant
- There are also claims to intermediate plant transformation vectors and plant transformation vectors containing the chimeric gene for Agrobacterium–mediated plant transformation, and to plant cells having the chimeric gene.
1987
- Filed - 17 August 1989
- Granted - 17 November 1992
- Expected expiry - 17 November 2009

(a) a duplicated CaMV 35s enhancer sequence comprising
an AluI-EcoRV fragment of a CaMV 35S upstream region; and
(ii) a promoter comprising an RNA polymerase binding site and an
mRNA initiation site;
(b) a nucleotide sequence of interest for transcription to mRNA; and
(c) a termination region wherein said components are operably
joined.

**Title** – DNA construct for enhancing the efficiency of transcription

**Claim 1**

A DNA construct having as components,

(a) a transcription initiation region including

(i) a tandemly duplicated CaMV 35S enhancer sequence
comprising an AluI-EcoRV fragment of a CaMV 35S upstream region;

(ii) a promoter comprising an RNA polymerase binding site and an
mRNA initiation site;

(b) a nucleotide sequence of interest for transcription to mRNA; and
(c) a termination region wherein said components are operably
joined.

**Title** – DNA sequence for enhancing the efficiency of transcription

**Claim 1**

A chimeric transcriptional initiation region comprising:

as operably joined components

(i) a tandemly duplicated CaMV 35S enhancer sequence comprising an AluI-EcoRV fragment of a CaMV 35S upstream region; and

(ii) a promoter comprising an RNA polymerase binding site and an
mRNA initiation site, wherein when a nucleotide sequence of interest
is transcribed under the regulatory control of said chimeric
transcriptional initiation region, the amount of transcription product
is enhanced as compared to the amount of transcription obtained
with a chimeric transcriptional initiation region comprising a single
copy of said CaMV enhancer sequence and said promoter.

**Remarks**

Corresponding applications also be pending and there may be issued
claims in Brazil and Japan.

Note: Patent information on this page was last updated on 30 September 2006.

**CaMV 35S minimal promoter**

**Scientific aspects**

Part of the domain A of the CaMV 35S promoter, which contains the TATA box and extends from the −90
position to the transcription start site +1, is used by many as a "minimal promoter." Apart from the TATA
box, which is the binding site for RNA polymerase II, the region contains at least three CAAT-like boxes. These sequences potentiate the activity of upstream sequences and influence the efficiency of the promoter activity. These CAAT-like boxes alone, or attached to heterologous promoter regions, drive the expression of transgenes.

The so-called minimal promoter, which is often described as a sequence similar to that extending from nucleotides -60 or -46 to +1 of the 35S promoter, does not appear to drive the expression of a gene by itself. Additional sequences, such as an enhancer, are required. Thus, the 35S minimal promoter has often been used to find and define enhancer regions, and determine activity of heterologous promoters, and as part of trans-activating or inducible promoter systems.

IP issues

A careful reading of the patents on the CaMV 35S promoter, including the ones directed to the subdomains of the promoter and the enhancer region, suggests but does not confirm that the widely used "minimal promoter" may be unencumbered by patents. The most relevant claims to the 35S minimal promoter were granted to the Rockefeller University in the patent US 5097025. Several independent claims cover the minimal promoter of the CaMV 35S promoter, which the inventors define as sequence from -46 to +8, but the minimal promoter is not claimed by itself, rather as linked to one of the subdomains of domain B of the 35S promoter.

Because of the minimal amount of sequence actually derived from the 35S promoter, we suggest that scientists refer to it simply as a "minimal promoter."

Opine Promoters

Scientific Aspects

Under natural conditions, opine promoters drive the expression of opines (i.e. mannopine, octopine, nopaline), hormone–like compounds generated by the soil bacterium Agrobacterium through the use of the plant's expression machinery. Opines are utilized by the bacterium as a source of carbon, nitrogen and energy. Promoters from the nopaline synthase (nos), octopine synthase (ocs) and mannopine synthase (mas) genes have been isolated and inserted into transformation vectors upstream of foreign genes to control the expression of those genes.

Although these promoters are regarded as constitutive, their level of activity can be affected by hormones and wounding. Nevertheless, they are still frequently used, mainly for transformation of dicotyledonous (dicot) plants. Additionally, depending on the orientation, some functional parts of regulatory sequences associated with promoters, such as the mas promoters, have the ability to bind nuclear protein factors from different plants and can be used as enhancers or silencers.

IP issues

Granted patents related to transcriptional regulatory elements derived from opine synthase genes may be divided into three groups:

1. **Promoters and UAS from opine synthase genes**: Patents granted in the U.S. and Australia directed to promoters and upstream activating sequences (UAS) derived from opine synthase genes, especially from the ocs and mas genes of A. tumefaciens;

2. **35S-enhanced mas promoter**: Patents granted in the U.S. and Canada directed to a mas promoter enhanced by a UAS from CaMV 35S gene; and

3. **Enhancers from the ocs gene**: Patents granted in the U.S., Europe and Canada directed to enhancer sequences isolated from the ocs gene of particular use in the transformation of monocots.

### Promoters and UAS from opine synthase genes

The Biotechnology Research and Development Corporation and the Purdue Research Foundation have patents granted in the U.S., Australia and Russia. The United States and the Australian patents are directed to chimeric regulatory regions containing promoters and UAS derived from the opine genes mas and ocs of Agrobacterium tumefaciens. The UAS are essential for the transcription of the opine genes in natural conditions. The promoters of mas and ocs are themselves weak promoters, however linking UAS regions to
them increases the promoter activity and they behave in a more constitutive fashion.

**Definition of promoter and UAS**

A UAS is defined by the inventors in functional terms as a *cis*-acting element that in the native state is usually at least 100 bp upstream of the native transcriptional start site and can exert influence on gene expression.

A promoter is not precisely defined in the specification, but it is referred to as a DNA sequence required for the transcription of genes that contain a functional TATA box and directs the expression of a gene. Thus, the concept of promoter is both structural and functional.

**Approximate scope of protection**

The chimeric regulatory regions claimed in the U.S. patent combine promoters and UAS as follows:

- a promoter and an UAS from a mas gene and at least one UAS from an ocs gene;
- a promoter from a mas gene and at least 3 UAS from an ocs gene; and
- a promoter and an UAS from a mas gene and at least 3 UAS from an ocs gene.

**Examples of opine promoters - UAS constraints**

![Diagram of opine promoters and UAS constraints]

The Australian patent claims more combinations of promoters and UAS than the U.S. patent. In contrast with the U.S. patent, the promoters and UAS of some of the regulatory regions claimed in the Australian patent derive from *any opine synthase gene*. It also includes a claim to a regulatory region formed by a promoter and a UAS, both derived from a *mas* gene.

Cassettes and methods for the expression of a foreign gene in a plant using the described chimeric regulatory regions are also claimed in both the U.S. and the Australian patents. Some of the cassettes are used for *inducible* expression of a gene of interest in a plant. Transgenic plants containing some of the cassettes are also claimed.

Some bibliographic data and a summary of the independent claims of the granted patents are presented in the table on the following page.
**Title** – Chimeric regulatory regions and gene cassettes for expression of genes in plants

<table>
<thead>
<tr>
<th>Claim</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Claim 1</strong></td>
<td>A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising a promoter derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em>, an upstream activating sequence derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em>, and at least one upstream activating sequence derived from an octopine synthase gene of <em>Agrobacterium tumefaciens</em>.</td>
</tr>
<tr>
<td><strong>Claim 2</strong></td>
<td>A method for nematode inducible expression of a foreign gene in a plant, comprising: linking said foreign gene to a regulatory region comprising a promoter derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em> comprising 138 bases upstream of the transcription initiation site, and an upstream activating sequence derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em>; inserting said foreign gene and said regulatory region in said plant, wherein expression is induced by nematode attack on the plant.</td>
</tr>
<tr>
<td><strong>Claim 3</strong></td>
<td>A method for nematode inducible expression of a foreign gene in a plant, comprising: linking said foreign gene to a regulatory region comprising a promoter derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em>, an upstream activating sequence derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em>, and at least one upstream activating sequence derived from an octopine synthase gene of <em>Agrobacterium tumefaciens</em>; inserting said foreign gene and said regulatory region in said plant, wherein expression is induced by nematode attack on the plant.</td>
</tr>
<tr>
<td><strong>Claim 4</strong></td>
<td>A chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from an <em>Agrobacterium tumefaciens</em> octopine synthase gene operably linked to a promoter derived from an <em>Agrobacterium tumefaciens</em> mannopine synthase gene.</td>
</tr>
<tr>
<td><strong>Claim 5</strong></td>
<td>A cassette for expressing a foreign gene comprising the foreign gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from <em>Agrobacterium tumefaciens</em> octopine synthase genes operably linked to a promoter derived from an <em>Agrobacterium tumefaciens</em> mannopine synthase gene.</td>
</tr>
<tr>
<td><strong>Claim 6</strong></td>
<td>A plasmid comprising a cassette comprising a foreign gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from <em>Agrobacterium tumefaciens</em> octopine synthase genes operably linked to a promoter derived from an <em>Agrobacterium tumefaciens</em> mannopine synthase gene.</td>
</tr>
<tr>
<td><strong>Claim 7</strong></td>
<td>A method of expressing a foreign gene in a plant, comprising: linking said foreign gene to a chimeric regulatory region</td>
</tr>
</tbody>
</table>
comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene; and inserting said foreign gene and said chimeric regulatory region into a plant, wherein said plant expresses said foreign gene.

**Claim 8**

A transgenic plant comprising a cassette comprising a foreign gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* octopine synthase genes operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 9**

A chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to an upstream activating sequence derived from an *Agrobacterium tumefaciens* mannopine synthase gene that is operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 10**

A cassette for expressing a foreign gene comprising the foreign gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* octopine synthase genes operably linked to an upstream activating sequence derived from an *Agrobacterium tumefaciens* mannopine synthase gene that is operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 11**

A method of expressing a foreign gene in a plant, comprising: linking said foreign gene to a chimeric regulatory region comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* octopine synthase gene operably linked to an upstream activating sequence derived from an *Agrobacterium tumefaciens* mannopine synthase gene that is operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene; and inserting said foreign gene and said chimeric regulatory region into a plant, wherein said plant expresses said foreign gene.

**Claim 12**

A transgenic plant comprising a chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to an upstream activating sequence derived from an *Agrobacterium tumefaciens* mannopine synthase gene that is operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

- A cassette for inducible expression of a foreign gene operably linked to a regulatory region having
  - a promoter and an UAS from the *mas* gene and
  - at least one UAS from an *ocs* gene.
The term "operably linked" is not explicitly defined but in the context of the disclosure appears to refer to a gene sequence present on the same nucleic acid molecule as a promoter sequence and whose expression is under the control of that promoter sequence.

- A chimeric regulatory region for expressing genes in plants having at least 3 UAS derived from an ocs gene linked to a promoter derived from a mas gene. A cassette, a plasmid, a method of expressing a foreign gene in a plant using this regulatory region, methods for nematode inducible expression of a foreign gene, and a transgenic plant are also claimed.

- A chimeric regulatory region for expressing genes in plants having
  - 4 UAS,
  - at least 3 UAS from an ocs gene and one UAS from a mas gene linked to a mas gene promoter.
  - A cassette, a method of expressing a foreign gene in a plant using this regulatory region and a transgenic plant are also claimed.

The disclosure describes that a "foreign gene" includes "any DNA that is sought to be expressed in [a] transgenic plant. In this context, the gene, no matter the source, is inserted into the plant genome and is thus foreign to that plant in the location of insertion, even if the gene originated from the plant being transformed." So a foreign gene can be a plant gene and may even be from the plant being transformed if inserted into a different genomic location from where it is usually found.

A cassette, as described in the patent, does not necessarily include an origin of replication. As claimed, a cassette could encompass a plasmid or a portion of a plasmid (e.g., a linear fragment).

<table>
<thead>
<tr>
<th>Title</th>
<th>Chimeric regulatory regions and gene cassettes for expression of genes in plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claim 1</td>
<td>A chimeric regulatory region for expressing genes in plants comprising an upstream activating sequences derived from an Agrobacterium tumefaciens octopine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens mannopine synthase gene.</td>
</tr>
<tr>
<td>Claim 2</td>
<td>A cassette for expressing a gene comprising a gene operably linked to a chimeric regulatory region comprising an upstream activating sequences derived from an Agrobacterium tumefaciens octopine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens mannopine synthase gene.</td>
</tr>
<tr>
<td>Claim 4</td>
<td>A chimeric regulatory region for expressing genes in plants comprising at least two upstream activating sequences derived from an Agrobacterium tumefaciens opine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.</td>
</tr>
</tbody>
</table>
Claim 8
A cassette for expressing a gene comprising a gene operably linked to a chimeric regulatory region comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.

Claim 13
A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising:

a) a promoter derived from a mannopine synthase gene of *Agrobacterium tumefaciens* by deletion to nucleotide position -138, and

b) an upstream activating sequence derived from a mannopine synthase gene of *Agrobacterium tumefaciens*.

Claim 15
A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising:

a) a promoter derived from a mannopine synthase gene of *Agrobacterium tumefaciens*,

b) an upstream activating sequence derived from a mannopine synthase gene of *Agrobacterium tumefaciens*, and

c) an upstream activating sequence derived from an octopine synthase gene of *Agrobacterium tumefaciens*.

Claim 16
A method for expressing a gene in a plant, comprising the steps of:

a) linking said gene to a chimeric regulatory region comprising an upstream activating sequence derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene;

b) inserting said gene and said chimeric regulatory region into a plant; and

c) allowing said plant to express said gene.

Claim 17
A method for expressing a gene in a plant, comprising the steps of:

a) linking said gene to a chimeric regulatory region comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter;

b) inserting said gene and said chimeric regulatory region into a plant; and

c) allowing said plant to express said gene.

Claim 21
A method of inducible expression of a foreign gene in a plant, comprising:

a) linking said foreign gene to a regulatory region comprising:
i. a promoter derived from a mannopine synthase gene of *Agrobacterium tumefaciens* by deletion to nucleotide position -138, and

ii. an upstream activating sequence derived from a mannopine synthase gene of *Agrobacterium tumefaciens*;

b) inserting said foreign gene and said regulatory region in said plant; and
c) inducing expression of said foreign gene.

**Claim 23**
A method for inducible expression of a foreign gene in a plant, comprising:

a) linking said foreign gene to a regulatory region comprising:

   i. a promoter derived from a mannopine synthase gene of *Agrobacterium tumefaciens*,

   ii. an upstream activating sequence derived from a mannopine synthase gene of *Agrobacterium tumefaciens*; and

   iii. an upstream activating sequence derived from an octopine synthase gene of *Agrobacterium tumefaciens*;

b) inserting said foreign gene and said regulatory region in said plant; and
c) inducing expression of said foreign gene.

**Claim 32**
A chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 33**
A cassette for expressing a gene comprising a gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* octopine synthase genes operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 34**
A method of expressing a gene in a plant, comprising the steps of:

a) linking said gene to a chimeric regulatory region comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase;
b) inserting said gene and said chimeric regulatory region into a plant; and
c) allowing said plant to express said gene.

**Claim 37**
A chimeric regulatory region for expressing a gene in a plant comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream
activating elements are derived from a different opine synthase gene than said promoter.

Claim 39

A cassette for expressing a gene in a plant comprising a gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* opine synthase genes operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.

Claim 40

A chimeric regulatory region for expressing a gene in a plant comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene.

Claim 41

A chimeric regulatory region for expressing a gene in a plant comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene.

- A chimeric regulatory region for expressing genes in plants comprising a promoter derived from a *mas* gene and an UAS derived from an *ocs* gene.
- A chimeric regulatory region for expressing genes in plants having at least 2 UAS elements and a promoter from an opine synthase gene. One of the UAS elements derives from a different opine gene than the promoter.
- A cassette for inducible expression of a foreign gene linked to a regulatory region having a promoter and a UAS from a *mas* gene.
- A cassette for inducible expression of a foreign gene linked to a regulatory region having a promoter and a UAS from a *mas* gene and a UAS from an *ocs* gene.
- A chimeric regulatory region for expressing genes in plants having at least 3 UAS elements from an *ocs* gene linked to a promoter from a *mas* gene.
- A chimeric regulatory region for expressing genes in plants having at least 3 UAS elements and a promoter from an opine synthase gene. One of the UAS is from a different opine gene than the promoter.
- Chimeric regulatory regions comprising at least 2 and at least 3 UAS elements from an opine synthase gene and a promoter also from an opine synthase gene.
- Cassettes containing the mentioned chimeric regulatory regions and methods for expressing a gene in a plant are also claimed.

EP 729514 B1

- Earliest

Title – Chimeric regulatory regions and gene cassettes for expression of genes in plants
The number of claims in this granted European patent was reduced to nine with only one independent claim as follows:

**Claim 1**
A chimeric regulatory region for expressing genes in plants comprising an upstream activating sequence of an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from a second *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter of an *Agrobacterium tumefaciens* mannopine synthase.

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**Title** – Chimeric regulatory regions and gene cassettes for expression of genes in plants

**Claim 1**
A chimeric regulatory region for expressing genes in plants comprising an upstream activating sequence derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 2**
A chimeric regulatory region for expressing genes in plants comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.

**Claim 3**
A chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 7**
A cassette for expressing a gene in plants, said cassette comprising a gene operably linked to chimeric regulatory region comprising an upstream activating sequence derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 8**
A cassette for expressing a gene in plants, said cassette comprising a gene operably linked to chimeric regulatory region comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.
<table>
<thead>
<tr>
<th>Claim 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A cassette for inducible expression of a foreign gene in plants, said cassette comprising said foreign gene operably linked to a regulatory region comprising a promoter derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em> by deletion to base pair position −138 and an upstream activating sequence derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em>.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>A cassette for inducible expression of a foreign gene in plants, said cassette comprising said foreign gene operably linked to a regulatory region comprising a promoter derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em>, an upstream activating sequence derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em>, and an upstream activating sequence derived from an octopine synthase gene of <em>Agrobacterium tumefaciens</em>.</td>
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</table>

<table>
<thead>
<tr>
<th>Claim 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>A cassette for expressing a gene in plants, said cassette comprising a gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from <em>Agrobacterium tumefaciens</em> octopine synthase genes operably linked to a promoter derived from an <em>Agrobacterium tumefaciens</em> mannopine synthase gene.</td>
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</tbody>
</table>

<table>
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<tr>
<th>Claim 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>A method for expressing a gene in a plant, comprising the steps of: linking said gene to a chimeric regulatory region comprising an upstream activating sequence derived from an <em>Agrobacterium tumefaciens</em> octopine synthase gene operably linked to a promoter derived from an <em>Agrobacterium tumefaciens</em> mannopine synthase gene; inserting said gene and said chimeric regulatory region into a plant; and allowing said plant to express said gene.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>A method for expressing a gene in a plant, comprising the steps of: linking said gene to a chimeric regulatory region comprising at least two upstream activating sequences derived from an <em>Agrobacterium tumefaciens</em> opine synthase gene operably linked to a promoter derived from an <em>Agrobacterium tumefaciens</em> opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter; inserting said gene and said chimeric regulatory region into a plant; and allowing said plant to express said gene.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>A method for inducible expression of a foreign gene in a plant, comprising: linking said foreign gene to a regulatory region comprising a promoter derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em> by deletion to base pair position −138 and an upstream activating sequence derived from a mannopine synthase gene of <em>Agrobacterium tumefaciens</em>.</td>
</tr>
</tbody>
</table>
synthase gene of Agrobacterium tumefaciens;
inserting said foreign gene and said regulatory region in said plant;
and
inducing expression of said foreign gene.

**Claim 22**

A method for inducible expression of a foreign gene in a plant,
comprising:
linking said foreign gene to a regulatory region comprising a
promoter derived from a mannopine synthase gene of
Agrobacterium tumefaciens, an upstream activating sequence
derived from a mannopine synthase gene of Agrobacterium
tumefaciens, and an upstream activating sequence derived from an
eptopine synthase gene of Agrobacterium tumefaciens;
inserting said foreign gene and said regulatory region in said plant;
and
inducing expression of said foreign gene.

**Claim 24**

A method of expressing a gene in a plant, comprising the steps of:
linking said gene to a chimeric regulatory region comprising at
least three upstream activating sequences derived from an
Agrobacterium tumefaciens octopine synthase gene operably linked
to a promoter derived from an Agrobacterium tumefaciens
mannopine synthase;
inserting said gene and said chimeric regulatory region into a plant;
and
allowing said plant to express said gene.

- The Canadian patent claims an additional method of
expressing a gene in a plant using a chimeric regulatory region
with at least 3 UAS from ocs gene and a promoter from a mas
gene.

**Remarks**

Related patents were also granted in Russia (RU 2142998 C1), in
China (CN 1061376) and in Japan (JP 9505205). An application was
filed in Brazil but was abandoned on March 6, 2001.

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**35S-enhanced mas promoter**

Calgene has two granted patents, one in the U.S. and one in Canada on a promoter region comprising a UAS of the CaMV 35S gene linked to a transcription initiation region. The patent defines a transcription/translation region of mas as including the sequences responsible for initiating transcription and effecting translation of the mas gene derived from the octopine Ti plasmid of A. tumefaciens. A mas promoter is included within the transcription/translation region. The region comprises the TATA box, an upstream activating region (UAS) and a transcription start site. As described in the patents, the DNA sequences of the region can correspond to:

- from –625 bp upstream the transcriptional start site to +60 bp downstream of the start site;
- from –300 bp upstream the transcriptional start site to +60 bp downstream of the start site; or
- from –300 bp to the start site.

The UAS or upstream activating region (UAR) of the CaMV 35S–enhanced mas promoter can correspond to a DNA sequence:

- from the –25 position of the CaMV 35S promoter to the –360 position;
• from the −45 position to −90 position; or
• from the −25 position or −45 position to the −168 position.

The \textit{mas} promoter alone is a weak promoter that is active in all tissues but which drives gene expression at different levels, being highly expressed in apical shoots and roots. The promoter of the invention, which combines the \textit{mas} promoter with the UAR of the CaMV 35S increases levels of expression to about 5 to 10–fold higher than the strong double CaMV 35S promoter, according to the patent.

Some bibliographic data and a summary of the independent claims of the granted patents are presented in the following table.

### 35S–enhanced \textit{mas} promoter – Specific Patent Information*

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5106739</td>
<td>Title – CaMV 35S–enhanced mannopine synthase promoter and method for using same</td>
<td>Originally assigned to Calgene (now owned by Monsanto)</td>
</tr>
<tr>
<td></td>
<td>• A DNA sequence comprising from 5' to 3' direction: UAR of CaMV 35S and a mas transcription initiation region.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• A chimeric CaMV 35S–enhanced mas promoter regulating the expression of a gene of interest in a plant. The expression level is at least 5–fold higher than the expression level achieved under the control of a CaMV 35S-enhanced 35S promoter. [Should describe how the patent defines a 35S–enhanced promoter]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• A method to increase the expression of a gene of interest by using the described chimeric promoter.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A DNA sequence comprising, in the 5' to 3' direction, a first element linked to a second element, said first element comprising an upstream activating region of CaMV 35S and said second element comprising a mannopine synthase transcription initiation region.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A chimeric promoter comprising a CaMV 35S enhanced mannopine synthase promoter, wherein upon expression of a DNA sequence of interest in a plant cell under the regulatory control of said promoter, said DNA sequence of interest is expressible at a level of at least 5–fold higher than expression of said gene of interest in a plant cell under the regulatory control of a CaMV 35S enhanced CaMV 35S promoter.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A method to increase the expression of an expressible gene of interest under the regulatory control of a mannopine synthase promoter comprising the steps of: providing a CaMV 35S upstream activating region to the 5' end of a DNA sequence comprising the mannopine synthase promoter; and allowing said gene to be expressed.</td>
<td></td>
</tr>
</tbody>
</table>

| CA 1334175    | Title – Plant promoter and method for using same |          |
|               | The independent claims are the same as the U.S. patent. |          |
Enhancers from the *ocs* gene

Most of the patents in this group were filed and initially granted to Agrigenetics and the Commonwealth Scientific and Industrial Research Organization (CSIRO) in U.S., Europe and Canada. The patents have been re-assigned to other institutes (see table next page).

Approximate scope of protection

This group of patents encompasses two enhancer elements. One of them is a consensus DNA sequence from the non–transcribed region of the promoter region of *seven opine synthase genes* and the *plant viral promoters* from cauliflower mosaic virus (CaMV), figwort mosaic virus (FMV) and carnation etched ring virus (CERV). The 20 bp consensus sequence is: 5’–TGACGTAAGCGATGACGTAA–3’.

The other enhancer element is a 16 bp palindromic sequence isolated from the 5’-untranscribed region of the *ocs* gene. The nucleotide sequence is 5’–ACGTAAGCGCTTACGT–3’.

The enhancer’s elements are linked to any *plant–expressible promoter* and any *structural gene of interest* to form recombinant DNA molecules. The elements are useful for enhancing the expression of a nearby plant–expressible gene. By "enhancing the expression" the inventors mean that the gene is activated only when the enhancer is present or gene expression is increased by the enhancer.

The claims of the European and the Canadian patents cover sequences that are at least 50% homologous to the 16 bp OCS enhancer element. Also, the target plants for transformation with the recombinant molecules are limited to *monocots*. Some bibliographic data and a summary of the independent claims of the granted patents are presented in the table on the following page.

### Enhancers from the *ocs* gene – Specific Patent Information

<table>
<thead>
<tr>
<th>Patent Number</th>
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<th>Assignee</th>
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</thead>
<tbody>
<tr>
<td>US 5573932</td>
<td><strong>OCS element</strong></td>
<td>Mycogen Plant Sciences, (now ow by Dov AgroScien)</td>
</tr>
<tr>
<td></td>
<td><strong>Claim 1</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A recombinant DNA molecule <strong>comprising</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a DNA fragment which fragment is a plant enhancer element capable of being bound by an OCS transcription factor and displaying the upper band binding pattern characteristic of the wild–type ocs enhancer in gel retardation assays, said fragment consisting essentially of a consensus sequence selected from the group consisting of</td>
<td></td>
</tr>
</tbody>
</table>
A recombinant DNA molecule comprising

a DNA fragment which fragment is a plant enhancer element capable of being bound by an OCS transcription factor and displaying the upper band binding pattern characteristic of the wild-type ocs enhancer in gel retardation assays, said fragment comprising a consensus sequence selected from the group consisting of

5′-ACGTAAGCGCTTACGT-3′ and its reverse sequence, in combination with

(a) a plant-expressible promoter heterologous to said plant enhancer element wherein said promoter is placed 3′ to said enhancer element, and

(b) a plant-expressible structural gene wherein said gene is placed 3′ to said promoter and under the regulatory control of said enhancer element and said plant-expressible promoter.

The claims are generally drawn to:

- A recombinant DNA molecule comprising in 5′–3′ direction an OCS enhancer with opine synthase enhancer consensus sequence, a plant-expressible promoter and a plant-expressible structural gene. The structural gene is under the control of the enhancer and the promoter.

- A recombinant DNA molecule having the same elements as mentioned above, but the enhancer element has the sequence

5′-ACGTAAGCGCTTACGT-3′.
(b) a plant-expressible structural gene wherein said gene is placed 3' to said promoter and under the regulatory control of said enhancer element and said plant-expressible promoter.

**Claim 18**

A recombinant DNA molecule comprising

a DNA fragment which fragment is a plant enhancer element capable of being bound by an OCS transcription factor and displaying the upper band binding pattern characteristic of the wild-type ocs enhancer in gel retardation assays, said fragment comprising at least two consensus sequences selected from the group consisting of

5'-A-C-G-T-A (A)-G-C-G-A (G)-T (A)-G-C (T)-A-C (G)-T (C)-3'

and its reverse sequence, in combination with

(a) a plant-expressible promoter placed 3' to said enhancer element, and

(b) a plant-expressible structural gene wherein said gene is placed 3' to said promoter and under the regulatory control of said enhancer element and said plant-expressible promoter.

**Claim 33**

A recombinant DNA molecule comprising

a DNA fragment which fragment is a plant enhancer element capable of being bound by an OCS transcription factor and displaying the upper band binding pattern characteristic of the wild-type enhancer in gel retardation assays, wherein said fragment comprises the sequence

5'--ACGTAAGCGCTACGT--3'

and its reverse sequence, in combination with

(a) a plant-expressible promoter heterologous to said plant enhancer element wherein said promoter is placed 3' to said enhancer element, and

(b) a plant-expressible structural gene wherein said gene is placed 3' to said promoter and under the regulatory control of said enhancer element and said plant-expressible promoter.

This patent is a Continuation of **US 5573932**.

The claims are generally directed to three different recombinant DNA molecules having the same elements as mentioned in **US 5573932**. The difference is in the nucleotide sequence of the enhancer element.

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**US 5837849**

- Earliest priority – 6 February 1987
- Filed – 2 June 1995
- Granted – 17 November 1998
- Expected expiry – 17 November 2015

**Title** – OCS element

**Claim 1**

An isolated DNA fragment which is a plant enhancer capable of activating or enhancing the transcription level of a plant-expressible gene, said enhancer element comprising a consensus sequence selected from the group consisting of

5'--T--G--A--C--G--T(C)--A--A--G--C(G)--G(A)--A(C)--T--G(T)--A--C--G--T(C)--A(C)--A(C)--3'

and its reverse sequence.

This patent is a Division of **US 5573932**.

The claim is drawn to an isolated plant enhancer element capable of enhancing the expression of a gene of interest in a plant having the consensus sequence

5'-- TGACGTAAGCGATGACGTAA--3'
Title – OCS enhancer

Claim 1
A monocotyledonous plant transformed with a recombinant DNA molecule comprising a plant transcription activating element capable of activating or enhancing the transcription level of a gene comprising a sequence having 50% to 100% homology with an identifying sequence selected from 5'-ACGTAAGCGCTTACGT-3' and its reverse sequence.

Claim 15
A method for enhancing the expression of a plant-expressible gene in plant tissue comprising the steps of
(a) inserting a transcription activating element comprising a sequence having 50% to 100% homology to an identifying sequence selected from 5'-ACGTAAGCGCTTACGT-3' and its reverse sequence, in such a way that said transcription activating element modulates the expression of said gene, and
(b) introducing said recombinant DNA molecule into plant tissue.

The claims are directed to:

- A monocot transformed with a recombinant DNA molecule having an enhancer element with a sequence that is 50 to 100% homologous to the sequence 5'-ACGTAAGCGCTTACGT-3'.
- A method for enhancing the expression of a gene in a monocot by introducing a recombinant molecule with an enhancer element as described above.

Remarks
Patents have lapsed in Austria, Greece, Belgium, France, Germany, Netherlands, Spain, Sweden and UK.

Applications were also filed in Japan (JP 63276492 A2) and South Africa (ZA 8800319 A).

The claims are the same as the claims of the European patent EP 278659 B1 except that transformation of a plant by the recombinant molecule is not limited to monocots.
Plant Ubiquitin promoter (Ubi)

Scientific aspects

Ubiquitin is a protein found in eukaryotic cells and its sequence is highly conserved among organisms as diverse as humans and the fruit fly. The protein is implicated in processes such as protein turnover, chromatin structure, cell cycle control, DNA repair, and response to heat shock and other stresses.

In 1992, Christensen et al. identified two out of the 8 to 10 loci encoding ubiquitin in maize. Both characterized genes, *Ubi-1* and *Ubi-2*, contain an open reading frame of 1599 bp arranged as seven tandem, head-to-tail repeats of 228 bp.

The regulatory region which controls the expression of the *Ubi-1* gene of maize extends from -899 bp 5' of the transcription start site (+1) to about 1093 bp 3' of the transcription start site.

This sequence of approximately 2 kb comprises:

- a TATA box sequence located at -30,
- two overlapping sequences that are similar to the consensus heat shock element found in heat-inducible genes, located at the -214 and -204 position from the transcription start site,
- an 83 bp untranslated exon sequence 3' of and adjacent to the transcription start site and
- an intron of around 1 kb, which extends from 84 to 1093 position.

The heat shock elements of the regulatory region enhance the expression of the ubiquitin protein in response to temperature stress.

![Plant Ubiquitin Regulatory Region Diagram](diagram.png)

IP issues

**Mycogen Plant Science** (now owned by Dow Agro Sciences) and **Monsanto PLC** are entities that have patents and patent applications on the plant ubiquitin regulatory system. The **Mycogen** patents evaluated in this report were granted in:

- the United States (4 patents)
- Europe (1 patent), and
- Canada (1 patent)

**Monsanto** has granted patents in the United States and Australia. Related patent applications were also filed in Europe and Canada.

**Prodigene** owns a United States patent and also filed a patent application in Australia.

How is promoter defined?

The description of a eukaryotic promoter in both **Mycogen** patents and **Monsanto** patent applications includes functional and structural components. That is, a promoter is defined as the region
upstream of a gene containing the binding site for RNA polymerase II that initiates transcription of the DNA. It contains a TATA box, a CAAT box or an AGGA box, and the CAP site. The inventors also acknowledge that there are ancillary regulatory sequences or regions that are part of a regulatory system in general. Those regions include enhancers and upstream activating sequences.

One of the regulatory elements set out by the inventors of the Mycogen patents is the heat shock element, which transiently enhances the level of downstream gene expression in response to sudden temperature elevation.

Thus, the inventions described in the patents do not refer to a "simple" ubiquitin promoter, but to an ubiquitin regulatory system that includes the promoter as described above and the additional elements that participate in the modulating gene expression.

In the Monsanto patents, the ubiquitin regulatory system is modified by excising the heat shock elements. While in the Prodigene patent, the ubiquitin regulatory system is modified by making the two overlapping heat shock elements adjacent.

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Summary of Claims and Independent Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5510474</td>
<td><strong>Title – Plant ubiquitin promoter system</strong></td>
<td>Mycogen Plant Sciences</td>
</tr>
</tbody>
</table>
|               | **Claim 1**
|               | An isolated DNA fragment, useful in effecting expression in both monocots and dicots of coding sequences placed 3' to said fragment, wherein said DNA is approximately 2 kb in length, and said DNA fragment further comprises, in the following order beginning with the 5' most element and proceeding toward the 3' terminus of said DNA fragment: |
|               | (a) two heat shock elements, which overlap; |
|               | (b) a promoter comprising a transcription start site; |
|               | (c) an intron of about 1 kb in length; and |
|               | (d) a translation start site; wherein said DNA fragment comprising said elements (a)-(d) regulates gene expression in both dicots and monocots, and wherein said DNA fragment comprises the nucleotide sequence shown from position –899 to 1092 of the maize ubiquitin sequence listed in FIG. 2. |
|               | Independent claim 1 is drawn to: |
|               | • An isolated DNA fragment from –899 to +1092 of the 5' flanking region of a maize ubiquitin sequence comprising from 5' to 3': two overlapping heat shock elements, a promoter with a transcription start site, an intron of about 1 kb, and a translation start site. |
|               | • The DNA fragment has to be capable of regulating gene expression (of any gene) in both dicots and monocots; and |
|               | • must include a portion of the sequence shown in Figure 2 of the patent (i.e., the sequence from position –899 to 1092). |
| US 5614399    | **Title – Plant ubiquitin promoter system**    | Mycogen Plant Sciences |
|               | **Claim 1**
|               | A method for selective heat shock induced enhancement of the constitutive expression of a structural gene in a plant cell comprising the steps of: |
|               | (a) transforming said plant cell with a DNA construct comprising an approximately 2 kb plant ubiquitin regulatory region operably joined to a
DNA sequence of interest, wherein said plant ubiquitin regulatory region is from a plant ubiquitin gene and comprises at least one heat shock element, a promoter, a transcription start site, and an intron; and

(b) selectively applying stress conditions of high temperature to said transformed plant cell thereby inducing enhancement in expression of said DNA sequence of interest.

This patent is a Division of US 5510474. The claims are drawn to:

A method for rendering a constitutively expressed structural gene of a plant cell heat shock inducible by transforming the plant cell with a DNA construct which comprises a plant ubiquitin regulatory region and selectively applying high temperature to the transformed cell.

The plant ubiquitin regulatory region comprises:

• at least one heat shock element
• a promoter
• a transcription start site
• and an intron

The plant can be any type but the gene must be a structural gene (defined in the patent as one which encodes a protein, polypeptide or portion thereof) and so the claim does not cover constructs for expressing siRNAs.

The term "high temperature" is not defined but the patent describes that heat shock can occur at 40°C.

The patent discloses a single type of ubiquitin regulatory system – the sequence from a single maize ubiquitin gene – though the claims encompass ubiquitin regulatory systems from any kind of plant.

Claim 1

A DNA construct comprising:

(a) a DNA sequence no larger than 2 kb, said DNA sequence comprising a plant ubiquitin regulatory system, wherein said regulatory system contains a heat shock element and an intron, said intron being located at 3’ to said heat shock element, and

(b) a plant-expressible structural gene wherein said structural gene is placed under the regulatory control of said plant ubiquitin regulatory system.

This patent is a Division of US 5614399. The claims generally relate to:

• A DNA construct having a plant ubiquitin regulatory sequence no larger than 2 kb that contains an intron located 3’ of a heat shock element, and a plant-expressible structural gene under the control of the ubiquitin regulatory sequence.

Although the claim recites that the construct must contain a plant expressible gene this does not necessarily mean that the gene is from a plant.

However, the claim does require that the gene is a structural gene (e.g., must encode a protein, polypeptide or portion thereof).

The patent describes that placing a structural gene under the regulatory control of a promoter or a regulatory element "means positioning the
structural gene such that the expression of the gene is controlled by these sequences. In general, promoters are found positioned 5' (upstream) to the genes that they control."

**Title** – Plant ubiquitin promoter system

**Claim 1**

A DNA fragment approximately 2 kb in length, said DNA fragment comprising a plant ubiquitin regulatory system, wherein said regulatory system contains a promoter comprising

- a transcription start site,
- one or more heat shock elements positioned 5' to said transcription start site, and
- an intron positioned 3' to said transcription start site, wherein said regulatory system is capable of regulating constitutive and inducible gene expression in both dicots and monocots such that the level of said constitutive gene expression in monocots is about one-third that obtained in said inducible gene expression in monocots.

**Claim 9**

A recombinant DNA construct comprising:

a. A DNA fragment approximately 2 kb in length, said DNA fragment comprising a plant ubiquitin regulatory system, wherein said plant ubiquitin regulatory system contains a promoter comprising

- a transcription start site,
- one more heat shock elements positioned 5' to said transcription start site,
- a translational start site, and
- an intron positioned 3' to said transcription start site and 5' to said translational start site, wherein said plant ubiquitin regulatory system is capable of regulating constitutive and inducible gene expression in both dicots and monocots such that said constitutive gene expression in monocots is at a level about one-third that obtained in said inducible gene expression in monocots, and

b. a plant-expressible heterologous structural gene positioned 3' to said plant ubiquitin regulatory system and a polyadenylation signal positioned 3' to said structural gene, wherein said heterologous gene is placed under the regulatory control of said plant ubiquitin regulatory system.

**Claim 18**

A DNA fragment, useful in effecting expression in both monocots and dicots of coding sequences placed 3' to said fragment, wherein said DNA is isolated or incorporated into a larger piece of DNA but in a position other than in the 5' sequence of a plant ubiquitin gene, is approximately 2 kb in length, and said DNA fragment further comprises, in the following order beginning with the 5' most element and proceeding toward the 3' terminus of said DNA fragment:

- (a) one or more heat shock elements, which elements may or may not be overlapping;
- (b) a promoter comprising a transcription start site; and
- (c) an intron of about 1 kb in length; and wherein said DNA fragment comprising said elements (a)–(c) is capable of regulating gene expression in both dicots and monocots.

This patent is a Division of **US 6020190**. The claims generally relate to:

- A DNA fragment of approximately 2 kb comprising a plant ubiquitin.
A regulatory system comprising: one or more heat shock elements 5' to a promoter with a transcription start site and an intron 3' to the transcription start site. The system can be used to regulate constitutive and inducible expression for both dicots and monocots; however, constitutive expression must be 1/3 the level of heat-shock inducible expression in monocots.

- A recombinant DNA construct having a plant ubiquitin regulatory system as described and a plant expressible heterologous structural gene under control of the regulatory region which also includes a polyadenylation sequence 3' of the structural gene.

- A DNA fragment of approximately 2kb as described above which can be part of a larger sequence so long as it is not 5' of a plant ubiquitin gene. The level of heat-shock inducibility in monocots is not an element of this claim.

A particular level of heat shock induction in dicots is not an element of any of the claims.

The ubiquitin regulatory system can be from any plant although the patent does not describe any sequences other than those from maize.
The claims are generally drawn to:

- A plant ubiquitin regulatory system of around 2 kb capable of constitutive and inducible regulation of gene expression in both monocots and dicots. The system contains an overlapping heat shock elements and intron.

- A recombinant DNA construct having a plant ubiquitin regulatory system as described and a plant expressible structural gene under control of the regulatory region.

- A method for both constitutive and stress–inducible enhanced expression of a structural gene in a plant cell. The transformed cell with the plant ubiquitin system is subjected to high temperature to induce enhancement in gene expression.

The construct used in method claim 23 does not require overlapping heat shock elements but encompasses constructs with a single heat shock element.

The claims don't require a particular level of induction after heat shock.

As with the above patents, the ubiquitin regulatory system could be from any plant so long as that system comprises a heat shock element.

**EP 342926 B1**

<table>
<thead>
<tr>
<th>Title</th>
<th>Plant ubiquitin promoter system</th>
</tr>
</thead>
</table>
| **Claim 1** | A DNA sequence no larger than 2 kb, said DNA sequence comprising a plant ubiquitin regulatory system, wherein said regulatory system contains:  
  a) a heat shock element and  
  b) an intron. |
| **Claim 10** | A DNA construct comprising:  
  a) a DNA sequence no larger than 2 kb, said DNA sequence comprising a plant ubiquitin regulatory system, wherein said regulatory system contains:  
    1. a heat shock element, and  
    2. an intron; and  
  b) a plant–expressible structural gene wherein said structural gene is placed under the regulatory control of said plant ubiquitin regulatory system. |
| **Claim 12** | A method for the constitutive expression of a structural gene and the selected stress–induced enhancement in expression of said structural gene in a plant cell comprising the steps of:  
  a) transforming said plant cell with a DNA construct comprising:  
    i. a plant ubiquitin regulatory system, wherein is found a heat shock element and an intron, and  
    ii. a plant–expressible structural gene that is under the regulatory control of said plant ubiquitin regulatory system, and  
  b) selectively applying stress conditions to said transformed plant cell thereby inducing enhancement in expression of said structural gene. |
### Patents and applications relating to the ubiquitin regulatory region owned by Monsanto

<table>
<thead>
<tr>
<th>Patent/application number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US 6878818</strong></td>
<td><strong>Title</strong> - Modified ubiquitin regulatory system</td>
<td>Monsanto UK Ltd</td>
</tr>
<tr>
<td></td>
<td><strong>Claim 1</strong></td>
<td></td>
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<tr>
<td></td>
<td>An isolated DNA sequence <strong>comprising</strong> a ubiquitin regulatory system lacking heat shock elements wherein the ubiquitin regulatory system <strong>comprises</strong> the nucleotide sequence according to <strong>SEQ.ID.NO. 8</strong>.</td>
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</tr>
<tr>
<td></td>
<td><strong>The claims are to:</strong></td>
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<tr>
<td></td>
<td>• DNA sequences which comprise a specific nucleotide sequence (<strong>SEQ.ID.NO. 8</strong>), a 2033 bp sequence which includes the promoter, the 83 bp untranslated first exon and the intron of the maize Ubi-1 gene.</td>
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<tr>
<td></td>
<td>• Dependent claims are drawn to host cells, transgenic plants (of any kind), and seeds comprising the sequences, as well as constructs comprising the sequence operably linked to a plant-expressible structural coding sequence and vectors comprising the constructs.</td>
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<tr>
<td></td>
<td>Claim 1 requires that the DNA sequence has at least the 2033 bp sequence but allows for the inclusion of additional sequences – such as the remainder of the coding region of the Ubi-1 gene or heterologous sequences not usually found in association with the Ubi-1 promoter and intron. However, the heat shock elements cannot be included.</td>
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<tr>
<td></td>
<td>Because the claims do require <strong>SEQ.ID.NO. 8</strong> as an element of the sequence, a regulatory system which included portions of the promoter and intron sequence required for expression, but which deleted other parts of the sequence would not literally be covered by these claims.</td>
<td></td>
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<tr>
<td><strong>AU 769567 B2</strong></td>
<td><strong>Title</strong> - Modified ubiquitin regulatory system</td>
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<tr>
<td></td>
<td><strong>Claim 1</strong></td>
<td></td>
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<tr>
<td></td>
<td>A DNA sequence <strong>comprising</strong> a ubiquitin regulatory system lacking heat shock elements wherein the ubiquitin regulatory system <strong>comprises</strong> the nucleotide sequence according to <strong>SEQ.ID.NO. 8</strong>.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 1 of the Australian patent is identical to that of the US patent.</td>
<td></td>
</tr>
<tr>
<td><strong>EP 1210446 A1</strong></td>
<td><strong>Title</strong> - Modified ubiquitin regulatory system</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Claim 1</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Patent owned by Prodigene

<table>
<thead>
<tr>
<th>Patent/application number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US 6977325</strong></td>
<td><strong>Title – Plant promoter sequences and methods of use for same</strong></td>
<td>Prodigene Inc.</td>
</tr>
</tbody>
</table>
| Earliest priority – 9 Jun 2000 | **Claim 1**  
An engineered ubiquitin promoter sequence capable of directing expression of a nucleotide sequence in a plant cell, said engineered ubiquitin promoter sequence comprising: a heat shock region, wherein said heat shock region has the sequence as set forth in **SEQ ID NO: 4.** | |
| Filed – 28 Feb 2002        | **Claim 2**  
A method for causing expression of a heterologous structural gene or open reading frame in a plant cell, said method comprising: introducing to a plant cell an expression construct comprising an engineered ubiquitin promoter sequence operably linked to said heterologous structural gene or open reading frame, wherein said engineered ubiquitin promoter sequence comprises a heat shock region, wherein said heat shock region has the sequence as set forth in **SEQ ID NO: 4.** | |
| Granted – 20 Dec 2005      | **Remarks**  
The patent application filed in Australia (AU 200175433) lapsed. The related PCT application is WO 2001/094394. | |
| Expected expiry – 16 Jun 2020 (adjusted) | | |
Ubiquitin promoters from rice and sugarcane

Apart from patents on the ubiquitin regulatory system from maize, there are also patents claiming specifically for ubiquitin promoters from rice and sugarcane.

The Board of Supervisors of Louisiana State University and Agricultural and Mechanical College holds a United States patent (US 6528701) on rice ubiquitin promoters. This patent claims for a rice ubiquitin promoter and a method for using it in controlling gene expression in plants. It shows in the description that the rice ubiquitin promoter is stronger than the maize ubiquitin promoter in driving gene expression in rice.

United States of America as represented by the Secretory of Agriculture & University of Hawaii has three United States patents (US 6706948, US 6686513 and US 6638766) on sugarcane ubiquitine promoters (UBI4 and UBI9).

### rice ubiquitin promoters

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title – Rice ubiquitin–derived promoters</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6528701</td>
<td></td>
<td>Board of Supervisors of Louisiana State University and Agricultural and Mechanical College</td>
</tr>
</tbody>
</table>

**Claim 1**

An isolated nucleotide sequence comprising a rice ubiquitin promoter capable of controlling constitutive expression of a nucleic acid encoding a polypeptide, wherein said nucleotide sequence comprises at least a portion of SEQ ID NO:3 which is upstream of position 2785 and wherein said portion retains promoter activity.

The claims of this patent are generally drawn to:

- An isolated nucleotide sequence comprising a rice ubiquitin promoter (upstream of position 2785 of SEQ ID NO:3), and
- A method of expressing a heterologous nucleic acid sequence encoding a polypeptide in a plant or in its progeny under the control of the rice ubiquitin promoter (claim 6).

### Patents and applications on sugarcane ubiquitine promoters

<table>
<thead>
<tr>
<th>Patent/application number</th>
<th>Title – Sugarcane UBI9 gene promoter and methods of use thereof</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6706948</td>
<td></td>
<td>United States of America as represented by the Secretory of Agriculture &amp; University of Hawaii</td>
</tr>
</tbody>
</table>

**Claim 1**

A substantially purified nucleic acid sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:7, the complement of SEQ ID NO:7, SEQ ID NO:10, and the complement of SEQ ID NO:10.
<table>
<thead>
<tr>
<th>Claim 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A substantially purified nucleic acid sequence comprising the HindIII/XbaI fragment isolated from plasmid pubi9–GUS contained in Escherichia coli cells deposited as NRRLB-30116.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A transgenic plant cell comprising a nucleic acid sequence comprising a double-stranded nucleotide sequence listed as <a href="http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggregator/insight/claim_search.html?beg_pos=189&amp;end_pos=203&amp;claim=5">SEQ ID NO:10</a>, wherein said nucleotide sequence is operably linked to a nucleic acid sequence of interest.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A method for expressing a nucleic acid sequence of interest in a plant cell, comprising:</td>
</tr>
<tr>
<td>a) providing:</td>
</tr>
<tr>
<td>i) a plant cell;</td>
</tr>
<tr>
<td>ii) a nucleic acid sequence of interest; and</td>
</tr>
<tr>
<td>iii) a nucleotide sequence selected from the group consisting of <a href="http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggregator/insight/claim_search.html?beg_pos=409&amp;end_pos=423&amp;claim=6">SEQ ID NO:10</a> and the complement of <a href="http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggregator/insight/claim_search.html?beg_pos=409&amp;end_pos=423&amp;claim=6">SEQ ID NO:10</a>;</td>
</tr>
<tr>
<td>b) operably linking said nucleic acid sequence of interest to said nucleotide sequence to produce a transgene; and</td>
</tr>
<tr>
<td>c) introducing said transgene into said plant cell to produce a transgenic plant cell under conditions such that said nucleic acid sequence of interest is expressed in said transgenic plant cell.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>A method for expressing a nucleic acid sequence of interest in a plant cell, comprising:</td>
</tr>
<tr>
<td>a) providing:</td>
</tr>
<tr>
<td>i) a plant cell;</td>
</tr>
<tr>
<td>ii) a nucleic acid sequence of interest; and</td>
</tr>
<tr>
<td>iii) a promoter comprising <a href="http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggregator/insight/claim_search.html?beg_pos=598&amp;end_pos=612&amp;claim=7">SEQ ID NO:10</a>;</td>
</tr>
<tr>
<td>b) operably linking said nucleic acid sequence of interest to said promoter to produce a transgene;</td>
</tr>
<tr>
<td>c) introducing said transgene into said plant cell to produce a transgenic plant cell under conditions such that said nucleic acid sequence of interest is expressed in said transgenic plant cell, and</td>
</tr>
<tr>
<td>d) identifying said transgenic plant cell.</td>
</tr>
</tbody>
</table>

The claims of this patent are directed to:
Title – Sugarcane ubi9 gene promoter sequence and methods of use thereof

Claim 1
A transgenic plant cell comprising a promoter comprising SEQ ID NO:10, wherein said promoter is operably linked to a nucleic acid sequence of interest, and said plant cell is selected from sugar cane, tobacco, sorghum, pineapple, rice, maize, tomato, soybean, banana, and garlic.

Claim 6
A method for expressing a nucleic acid sequence of interest in a plant cell selected from sugar cane, tobacco, sorghum, pineapple, rice, maize, tomato, soybean, banana, and garlic, comprising:

a) providing:
   i) said plant cell;
   ii) a nucleic acid sequence of interest; and
   iii) a promoter comprising SEQ ID NO: 10;

b) operably linking said nucleic acid sequence of interest to said promoter to produce a transgene; and

c) introducing said transgene into said plant cell to produce a transgenic plant cell under conditions such that said nucleic acid sequence of interest is expressed in said transgenic plant cell.

This patent is a Continuation in part of US 6706948.

Title – Promoter of the sugarcane UBI4 gene

Claim 1
A substantially purified nucleic acid sequence comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:7 and the complement thereof.

Claim 4
A transgenic plant cell comprising a nucleic acid sequence comprising the double-stranded nucleotide sequence listed as SEQ ID NO:7, wherein said nucleotide sequence is operably linked to a nucleic acid sequence of interest.

This patent is a Continuation of US 6706948.

Remarks
Related patent applications were also filed in Australia (AU 3192799 A1), Europe (EP 1063880 A1), Canada (CA 2324520) and Israel (IL 138580). However, the applications in Australia, Europe and Canada all lapsed.

Plant Actin promoters

Scientific aspects

In the quest to improve the expression of foreign genes in transformed cereals, several promoter regions originating from monocot plants have been tested. In 1990, McElroy and colleagues from Cornell University reported that the 5' region of the rice actin 1 (Act-1) gene successfully directed the expression of a reporter gene in transformed rice protoplasts. Since then, the promoter of the rice Act-1 gene has been used as a strong constitutive promoter driving the expression of genes of interest in monocots.

Actin is a fundamental component of the cell cytoskeleton present in all tissues. Cell shape determination, cell division, organelle movement and extension growth are all believed to involve actin proteins.

The Act-1 gene from rice has a short 5' non-coding exon, separated by a 447 bp intron (intron 1) from the first coding exon. The presence of the first intron of the gene has proved to be fundamental for the efficient gene expression from the Act-1 promoter. The region is active in almost all plant tissues except sporophytic and gametophytic pollen tissues.

All the necessary cis-acting regulatory elements for the activity of the Act-1 promoter are in the region 834 bp upstream of the transcription start site. Apart from the TATA box, there are two regions that play a role in the regulation of the constitutive expression of the promoter. A poly (dA-dT) element of 38 bp between -245 and -152 position is the binding sequence for a trans-acting protein that works as a positive regulator of Act-1 promoter activity. A region between -300 and -260 positions contains CCCAA pentamer repeats that appear to be involved in negative regulation of promoter activity. Its action seems to be tissue-specific, particularly in roots.

The portion of the rice Act-1 gene used in vectors for monocot transformation normally contains:

- approximately 1 kb of regulatory sequences located 5' of the transcribed region,
- the 5' non-coding exon 1,
- the intron 1, and
- the coding exon 2 of the Act-1 gene.

The regulatory region of rice Act-1 gene has been successfully used for expressing diverse genes of interest after transformation of cereals, i.e. maize, rice, barley, wheat and rice.

IP issues

1. Promoter region derived from the rice Act-1 gene

The rice Act-1 promoter region is protected under a U.S. patent granted to the Cornell Research Foundation, Inc. In the description of the invention, the definition of a promoter regulatory region includes more sequence than the minimal elements of a promoter region: TATA box, AGGA box and transcription start site. The claimed promoter region encompasses sequences located 5' to the translational start site that are essential for conferring a high level of expression of the gene under its control.

Approximate scope of protection

The claimed isolated nucleotide sequence of the Act-1 promoter construct extends 2.1 kb in the 5' direction from the translation initiation codon. The construct contains:
1.3 kb of 5' untranscribed sequence
- the 5' transcribed but untranslated exon
- the 5' intron, and
- a part of the first coding exon of the gene.

From the description of the invention it can be concluded that the presence of the 5' intron is vital for the efficient performance of the promoter construct.

### Specific patent information

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5641876</td>
<td>Title – Rice actin gene and promoter</td>
<td>Cornell Research Foundation, Inc.</td>
</tr>
<tr>
<td></td>
<td><strong>Claim 1</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>An isolated nucleic acid molecule encoding a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>promoter region from rice actin 1 gene.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Claim 2</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>An isolated nucleic acid molecule encoding a</td>
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</tr>
<tr>
<td></td>
<td>promoter region from rice actin 1 gene wherein</td>
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<tr>
<td></td>
<td>said nucleic acid molecule has a nucleotide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sequence as shown in nucleotides 1-2180 of</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SEQ ID NO:5.</td>
<td></td>
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</tbody>
</table>

The claims are directed to:

- An isolated DNA sequence encoding a promoter from rice *Act–1* gene. The nucleotide sequence of 2.18 kb is provided.

**Remarks**

The PCT application WO 91/09948 was not converted into national applications.
The patent application in Australia (AU 71827/91) lapsed.


### 2. Elements of the 5' region of the rice Act–1 gene.

As mentioned before, apart from the promoter region itself (5' upstream of the transcription start site) of the rice *Act–1* gene, there are elements of the 5' region of the gene that play an important regulatory role in driving expression of a downstream linked gene.

The regulatory elements are used in a modular way, in conjunction with other promoters or regulatory transcriptional regions. Patents and patent applications directed to the use of rice *Act–1* derived elements have claims that include:

- the intron 1 of the rice *Act–1* gene, and
- the exon 1 of the same gene.

#### A. Intron 1

- as part of a wound-inducible promoter construct for monocots. A U.S. patent granted to Cornell Research Foundation protects:
  - the use of a 5' intron sequence of the rice Act–1 gene linked to a wound-inducible potato proteinase inhibitor II gene promoter to form a promoter region and
  - a construct, a monocot plant and a rice plant containing a foreign gene of interest under the control of the mentioned promoter region.
as part of a chimeric promoter for driving the expression of a gene of interest in transformed monocots. There is a European patent application and an Australian patent application filed by Rhone Poulenc disclosing a chimeric regulatory region comprising the first intron of the rice Act–1 gene and a promoter derived from the maize histone H3C4 gene. In some of the filed independent claims, the gene of interest encodes an optimized transit peptide–calcium–dependent protein kinase (OTP/CP4).

B. Exon 1

Granted Australian, European and U.S. patents assigned to the Max–Planck Institute (Gottingen, DE) are directed to a modular promoter construct for plant transformation having a DNA sequence from exon 1 of the rice Act–1 gene regulatory region and a promoter. The promoter of the modular construct is any but the rice Act–1 gene promoter. Derivatives of the modular promoter construct are also covered.

The U.S. patent also claims a vector for plant cell transformation containing the modular promoter construct, a transformed plant and its descendants, and a method for preparing plants with an elevated gene expression level by transforming the plant with a vector comprising the claimed modular promoter construct.

Specific patent information

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5684239</td>
<td>Title – Monocot having dicot wound–inducible promoter</td>
<td>Cornell Research Foundation, Inc.</td>
</tr>
<tr>
<td></td>
<td>Claim 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A wound inducible promoter construct for use in monocotyledonous plants consisting essentially of, in 5' to 3' order, a potato proteinase inhibitor II gene promoter and a 5' intron of rice actin 1 gene promoter.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A nucleic acid construct comprising: a wound inducible promoter construct consisting essentially of, in 5' to 3' order, a potato proteinase inhibitor II gene promoter and a 5' intron of rice actin 1 gene promoter; and a foreign gene of interest under regulatory control of said wound inducible promoter construct.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A monocotyledonous plant comprising: a wound inducible promoter construct consisting essentially of, in 5' to 3' order, a potato proteinase inhibitor II gene promoter and a 5' intron of rice actin 1 gene promoter; and a foreign gene of interest under regulatory control of said wound inducible promoter construct.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A rice plant comprising: a wound inducible promoter construct consisting essentially of, in 5' to 3' order, a potato proteinase inhibitor II gene promoter and a 5' intron of rice actin 1 gene promoter; and a foreign gene of interest under regulatory control of said wound inducible promoter construct.</td>
<td></td>
</tr>
</tbody>
</table>

Remarks

The related European application EP 666921 was withdrawn. Application also filed in Japan (JP 7503126 T2).

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6750378</td>
<td>Title – Maize H3C4 promoter combined with the first intron of rice actin, chimeric gene comprising it and transformed plant</td>
<td>Rhone–Poulenc Agrochimie (then named Aventis CropScience SA and now Bayer)</td>
</tr>
<tr>
<td></td>
<td>Claim 1</td>
<td></td>
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<td>An isolated DNA sequence comprising, in the direction of</td>
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</tbody>
</table>
transcription, a fragment of the sequence of the maize H3C4 promoter wherein said fragment of the sequence of the maize H3C4 promoter has the sequence shown in **SEQ ID NO: 1** and a fragment of the sequence of the first intron of rice actin wherein said fragment of the sequence of the first intron of rice actin has the sequence shown in **SEQ ID NO: 2**.

**Claim 2**

An isolated DNA sequence comprising the DNA sequence shown in **SEQ ID NO: 3**.

**Claim 1**

DNA sequence, a 5' regulatory element allowing the expression of a heterologous gene in a plant cell from a monocotyledonous plant, characterized in that it comprises, in the direction of transcription, a first DNA sequence, which is a functional fragment of the sequence of the maize H3C4 promoter, and a second DNA sequence, which is a functional fragment of the sequence of the first intron of rice actin.

**Claim 8**

DNA sequence, a 5' regulatory element allowing the expression of a heterologous gene in a plant cell from a monocotyledonous plant, characterized in that it **comprises** the DNA sequence represented by the sequence identifier No. 3 (**SEQ ID NO: 3**) or a sequence homologous to the said sequence.

**Claim 21**

DNA sequence encoding a fusion protein OTP/CP4.

**Claim 22**

Fusion protein OPT/CP4.

**Claim 23**

Chimeric gene characterized in that it **comprises**, in the direction of transcription, an appropriate 5' regulatory sequence for ensuring the expression of a coding sequence in plants, functionally linked to a sequence encoding a fusion protein OTP/CP4, optionally linked to a 3' regulatory sequence.

**Title** – Maize H3C4 promoter combined with the first intron of rice actin, chimeric gene comprising it and transformed plant

This patent application is a Continuation of **US 6750378**.

**Claim 2**

Fusion protein OTP/CP4.

**Claim 4**

An isolated DNA sequence **comprising**, in the direction of transcription, a functional fragment of the sequence of the maize H3C4 promoter and a functional fragment of the first intron of rice actin.
### AU 759003 B2

**Title** – Maize H3C4 promoter associated with first rice actin intron, chimeric gene containing it and transformed plant

<table>
<thead>
<tr>
<th>Claim 1</th>
</tr>
</thead>
</table>
| A DNA sequence which is a 5' regulatory element allowing the expression of a heterologous gene in a plant cell from a monocotyledonous plant, characterized in that it comprises, in the direction of transcription,  
  - a first DNA sequence, which is a functional fragment of the sequence of the maize H3C4 promoter, and  
  - a second DNA sequence, which is a functional fragment of the sequence of the first intron of rice actin. |

<table>
<thead>
<tr>
<th>Claim 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>A DNA sequence which is a 5' regulatory element allowing the expression of a heterologous gene in a plant cell from a monocotyledonous plant, characterized in that it comprises the DNA sequence represented by the sequence identifier No.3 (SEQ ID NO: 3) or a sequence homologous to the said sequence.</td>
</tr>
</tbody>
</table>

**Remarks**

Related patents were granted in Europe ([EP 1042491 B1](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggregator?docid=EP1042491B1)) and China ([CN 98813783](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggregator?docid=CN98813783)). A patent application in Canada ([CA 2315677](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggregator?docid=CA2315677)) is still pending. Both the European patent and the Canadian patent application are in French with an abstract in English.

### US 5859331

**Title** – Modular promoter construct

<table>
<thead>
<tr>
<th>Claim 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A modular promoter construct, comprising a promoter which is active in plant cells and a DNA sequence of at least 30 bases from exon 1 of the rice actin 1 gene, or derivatives of this modular promoter construct which have promoter activity, wherein said promoter is not a rice actin 1 gene promoter.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A vector comprising a promoter construct which comprises a promoter which is active in plant cells and a DNA sequence of at least 30 bases from exon 1 of the rice actin 1 gene, or derivatives of this modular promoter construct which have promoter activity, wherein said promoter construct is coupled to a gene which is expressed in a plant cell, and wherein said promoter is not a rice actin 1 gene promoter.</td>
</tr>
</tbody>
</table>
Claim 6

A plant cell which is transformed with a vector comprising a promoter construct which comprises a promoter which is active in plant cells and a DNA sequence of at least 30 bases from exon 1 of the rice actin 1 gene, or derivatives of this modular promoter construct which have promoter activity, wherein said promoter construct is coupled to a gene which is to be expressed in a plant cell, and wherein said promoter is not a rice actin 1 gene promoter.

Claim 7

A plant or its descendants, regenerated from a plant cell comprising a promoter construct which comprises a promoter which is active in plant cells and a DNA sequence of at least 30 bases from exon 1 of the rice actin 1 gene, or derivatives of this modular promoter construct which have promoter activity, wherein said promoter construct is coupled to a gene which is to be expressed in a plant cell, and wherein said promoter is not a rice actin 1 gene promoter.

Claim 8

A method for preparing plants having elevated gene expression, said method comprising transforming a plant cell with a vector comprising a promoter construct which comprises a promoter which is active in plant cells and a DNA sequence of at least 30 bases from exon 1 of the rice actin 1 gene, or derivatives of this modular promoter construct which have promoter activity, wherein said promoter construct is coupled to a gene which is to be expressed in a plant cell, and wherein said promoter is not a rice actin 1 gene promoter.

Title – Modular promoter construct

Claim 1

Modular promoter construct, which has a promoter which is active in plant cells and a DNA sequence from exon 1 of the rice actin 1 gene, and the alleles and gene expression-stimulating derivatives of this modular promoter construct, with the proviso that the promoter is not a promoter of the rice actin 1 gene.

Notes:

- The European patent was converted in Austria (AT), France (FR), Germany (DE), Italy (IT), Spain (ES), and the Netherlands (NL). The European patent has abstract and claims in English and the description of the invention is in German. The granted Australian patent (AU 687004 B2) and the Canadian patent (CA 2139846) were both lapsed. Application also filed in Japan (JP 7508653 T2).


3. Promoter region derived from the rice Act–2 gene

Cornell Research Foundation and Dekalb Genetics have jointly filed patent applications on the regulatory elements from the rice Act–2 gene. The promoter region is referred to as the region that directs the
transcriptional activity of the coding region. As in the rice Act-1 patent, the intron is deemed essential for promoter efficacy and, in combination with the promoter, is required for the efficient expression of a gene.

A PCT application (WO 2000/070067) was filed containing 120 claims, 15 of them independent. The application discloses an isolated sequence of the 5' region of the rice Act-2 gene. The region comprises:

- the promoter sequence of about 743 bp,
- exon 1 of the gene,
- intron 1,
- exon 2, and
- the translation initiation codon.

The claims of the PCT application recite:

- isolated rice Act-2 promoter and intron from the 5' region described above,
- an expression vector comprising the rice Act-2 promoter,
- an expression vector comprising the rice Act-2 intron,
- a fertile transgenic plant stably transformed with the rice Act-2 promoter,
- methods to prepare crossed fertile transgenic plants comprising selected DNA comprising Act-2 promoter and the intron, and
- methods for expressing an exogenous gene in a plant by transforming the plant with a construct having the gene of interest linked to a rice Act-2 promoter and a rice Act-2 intron.

Note that claims of PCT applications do not provide enforceable rights and the claim scope may vary in countries that ultimately grant a patent.

The granted United States patent have similar claim elements but methods for preparing crossed fertile transgenic plants were excluded.

Specific patent information

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US 6429357</strong></td>
<td>Title – Rice actin 2 promoter and intron and methods for use thereof</td>
<td>Dekalb Genetics Corp. and Cornell Research Foundation Inc.</td>
</tr>
<tr>
<td></td>
<td>Claim 1: An isolated nucleic acid comprising from 40 to about 743 contiguous nucleotides of the nucleic acid sequence of <strong>SEQ ID NO:2</strong>.</td>
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</tr>
<tr>
<td></td>
<td>Claim 8: An expression vector comprising an isolated rice actin promoter comprising the nucleic acid sequence of <strong>SEQ ID NO:1</strong> or <strong>SEQ ID NO:2</strong> or a fragment thereof having promoter activity.</td>
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</tr>
<tr>
<td></td>
<td>Claim 15: A fertile transgenic plant stably transformed with a selected nucleic acid comprising a rice actin promoter, wherein said rice actin promoter comprises the nucleic acid sequence of <strong>SEQ ID NO:1</strong> or <strong>SEQ ID NO:2</strong> or a fragment thereof having promoter activity.</td>
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<td></td>
<td>Claim 26: A method of expressing an exogenous nucleic acid in a plant comprising the steps of: (i) preparing a construct comprising said exogenous nucleic acid operably linked to a rice actin promoter, wherein said rice actin promoter comprises the nucleic acid sequence of <strong>SEQ ID NO:1</strong> or <strong>SEQ ID NO:2</strong> or a fragment of <strong>SEQ ID</strong></td>
<td></td>
</tr>
</tbody>
</table>
Title – Rice actin 2 promoter and intron and methods for use thereof

The claims are from the corresponding PCT application WO 2000/070067.

Claim 1
An isolated rice actin 2 promoter isolatable from the nucleic acid sequence of SEQ ID NO:1.

Claim 2
An isolated rice actin 2 promoter isolatable from the nucleic acid sequence of SEQ ID NO:2.

Claim 3
An isolated nucleic acid comprising from 40 to about 743 contiguous nucleotides of the nucleic acid sequence of SEQ ID NO:2.

Claim 10
An isolated rice actin 2 intron isolatable from the nucleic acid sequence of SEQ ID NO:1.

Claim 11
An isolated rice actin 2 intron isolatable from the nucleic acid sequence of SEQ ID NO:3.

Claim 12
An isolated nucleic acid comprising from 40 to about 1763 contiguous nucleotides of the nucleic acid sequence of SEQ ID NO:3.

Claim 19
An expression vector comprising an isolated rice actin 2 promoter.

Claim 32
An expression vector comprising an isolated rice actin 2 intron.

Claim 41
A fertile transgenic plant stably transformed with a selected DNA comprising an actin 2 promoter.

Claim 58
A crossed fertile transgenic plant prepared according to the method comprising the steps of:
(i) obtaining a fertile transgenic plant comprising a selected DNA comprising an actin 2 promoter;
(ii) crossing said fertile transgenic plant with itself or with a second plant lacking said selected DNA to prepare the seed of a crossed fertile transgenic plant comprising said selected DNA; and
(iii) planting said seed to obtain a crossed fertile transgenic plant.

Claim 74
A crossed fertile transgenic plant prepared according to the method comprising:
(i) obtaining a fertile transgenic plant comprising a selected DNA comprising an actin 2 intron;
(ii) crossing said fertile transgenic plant with itself or with a second plant lacking said selected DNA to prepare seed of a crossed fertile transgenic plant comprising said selected DNA; and (iii) planting said seed to obtain a crossed fertile transgenic plant comprising said selected DNA.

Claim 91

A method of expressing an exogenous gene in a plant comprising the steps of:
(i) preparing a construct comprising said exogenous gene operably linked to an actin 2 promoter;
(ii) transforming a recipient plant cell with said construct; and
(iii) regenerating a transgenic plant expressing said exogenous gene from said recipient cell.

Claim 98

A method of expressing an exogenous gene in a plant comprising the steps of:
(i) preparing a construct comprising an actin 2 intron and an exogenous gene;
(ii) transforming a recipient plant cell with said construct; and
(iii) regenerating a transgenic plant expressing said exogenous gene from said recipient cell.

Claim 107

A method of plant breeding comprising the steps of:
(i) obtaining a transgenic plant comprising a selected DNA comprising an actin 2 promoter; and
(ii) crossing said transgenic plant with itself or a second plant.

Claim 114

A method of plant breeding comprising the steps of
(i) obtaining a transgenic plant comprising a selected DNA comprising an actin 2 intron; and
(ii) crossing said transgenic plant with itself or a second plant.

CA 2372859

Title – Rice actin 2 promoter and intron and methods for use thereof

The claims are the same as EP 1179081.

Remarks

The patent application in Australia (AU 57231/00) lapsed.

Note: Patent information was last updated on 23 May 2006. Search terms: "Rice actin" in abstract. Patent database: PatentLens and esp@cenet in combination with INPADOC.

Other plant actin promoters

Rice actin promoters have been shown to be strong in directing gene expression in graminaceous
monocotyledonous plants. However, their function in certain non-graminaceous monocots are variable suggesting that the activity of actin promoters may be limited to closely related species. Therefore, Researches were also carried out to identify actin promoters from other plant species. So far, patents have been issued and patent applications have been filed on actin promoters from the plant species including banana, melon, maize  and Arabidopsis.

Queensland University of Technology filed patent applications in the United States and Australia on an actin promoter from banana and its use.

Exelixis Plant Sciences, Inc. obtained patents in United States and Australia on a melon actin promoter.

Monsanto owns two United States patents and one Australian patent claiming Arabidopsis actin promoters and their use as part of the invention. In addition, Monsanto also filed patent applications on actin regulatory elements from rice and maize in the United States and Europe.

**Exelixis Plant Sciences, Inc. patent family**

<table>
<thead>
<tr>
<th>Patent/Application No</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US 6642438</strong></td>
<td><strong>Title – Melon promoters for expression of transgenes in plants</strong></td>
<td>Exelixis Plant Sciences, Inc.</td>
</tr>
<tr>
<td></td>
<td><strong>Claim 1</strong> An isolated nucleic acid molecule comprising a promoter, wherein the promoter comprises the nucleic acid sequence presented as SEQ ID NO:4.</td>
<td></td>
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<tr>
<td></td>
<td><strong>Claim 2</strong> An isolated nucleic acid molecule comprising a promoter, wherein the promoter consists of a portion of the nucleic acid sequence presented as SEQ ID NO:4 that, when operably linked to a protein-encoding polynucleotide sequence, directs expression of the protein in a plant cell.</td>
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<tr>
<td></td>
<td>The claims are directed to a promoter isolated from melon (Cucumis melo) and its use including:</td>
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<tr>
<td></td>
<td>• An isolated nucleic acid molecule comprising a promoter</td>
<td></td>
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<tr>
<td></td>
<td>• A method for producing a transgenic plant with constitutive expression of protein encoding polynucleotide sequence under the control of the promoter (Claim 7).</td>
<td></td>
</tr>
<tr>
<td><strong>AU 782602</strong></td>
<td><strong>Title – Banana and melon promoters for expression of transgenes in plants</strong></td>
<td></td>
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<tr>
<td></td>
<td>The independent claims of this Australian patent are the same as that in <strong>US 6642438</strong>.</td>
<td></td>
</tr>
<tr>
<td><strong>Remarks</strong></td>
<td>Patent applications were also filed in Europe (EP 1165755 A1), Canada (CA 2365259) and Japan (JP 2002539779 T2).</td>
<td></td>
</tr>
</tbody>
</table>


**Monsanto patent family on Arabidopsis actin promoters**
### Title - Plant expression constructs

**Claim 1**

A DNA construct comprising: a first expression cassette, a second expression cassette, and a third expression cassette, wherein said first expression cassette in operable linkage comprises

(i) a Figwort mosaic virus 35S promoter;
(ii) a heterologous 5' untranslated leader;
(iii) a chloroplast transit peptide DNA sequence;
(iv) a DNA sequence encoding a glyphosate tolerant EPSPS; and
(v) a transcriptional terminator; and

said second expression cassette comprising in operable linkage

(a) an Arabidopsis Elongation factor 1α promoter with homologous EF1α intron;
(b) a chloroplast transit peptide DNA sequence;
(d) a DNA sequence encoding a glyphosate tolerant EPSPS; and
(e) a transcriptional terminator; and

said third expression cassette comprising in operable linkage

(I) an Arabidopsis Act2 promoter with homologous Act2 intron;
(II) a chloroplast transit peptide DNA sequence;
(III) a DNA sequence encoding a glyphosate tolerant EPSPS; and
(IV) a transcriptional terminator.

---

### Title - Chimeric cauliflower mosaic virus 35S-arabidopsis actin 8 promoters and methods of using them

**Claim 1**

A DNA construct comprising a chimeric promoter DNA sequence comprising **SEQ ID NO:29**; a structural DNA sequence; and a 3' non-translated region that functions in plants to cause the addition of polyadenylated nucleotides to the 3' end of the RNA sequence; wherein the structural DNA sequence is operably linked to the chimeric promoter and the 3' non-translated region, and the chimeric promoter DNA sequence is heterologous with respect to the structural DNA sequence.

**Claim 7**

A DNA construct comprising a chimeric promoter DNA sequence comprising **SEQ ID NO:29**; an aroA:CP4 structural DNA sequence; and a 3' non-translated region that functions in plants to cause the addition of polyadenylated nucleotides to the 3' end of the RNA sequence; wherein the structural DNA sequence is operably linked to the chimeric promoter and the 3’ non-translated region, and the chimeric promoter DNA sequence...
Monstanto patent family on actin regulatory elements from rice and maize

<table>
<thead>
<tr>
<th>Patent/Application No</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 2006/162010 A1</td>
<td>Title - Actin regulatory elements for use in plants</td>
<td>MONSANTO TECHNOLOGY LLC</td>
</tr>
<tr>
<td></td>
<td>Claim 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>An isolated polynucleotide molecule having gene regulatory activity and comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1−7.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A DNA construct comprising an isolated polynucleotide molecule having gene regulatory activity and comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1−7, wherein said isolated polynucleotide molecule is operably linked to a transcribable polynucleotide molecule.</td>
<td></td>
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<td></td>
<td>Claim 15</td>
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<td>A method of inhibiting weed growth in a field of transgenic glyphosate tolerant crop plants comprising: i) planting the transgenic plants transformed with an expression cassette comprising (a) an isolated polynucleotide molecule having gene regulatory activity and comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1−7 and operably linked to a DNA molecule encoding a glyphosate tolerance gene and ii) applying glyphosate to the field at an application rate that inhibits the growth of weeds, wherein the growth and yield of the transgenic crop plant is not substantially affected by the glyphosate application.</td>
<td></td>
</tr>
</tbody>
</table>

Remarks: Related European patent application (EP 1664311 A2) was also filed. Its PCT application is WO 2005030968.

Maize alcohol dehydrogenase promoter (Adh)

Scientific aspects

Some plants, e.g. rice, have the ability to synthesize proteins that allow them to thrive under transient anaerobic conditions. One of those proteins is alcohol dehydrogenase, which is involved in ethanolic fermentation.

In maize, there are two proteins identified: alcohol dehydrogenase I (ADH–I) and II (ADH–II), of which ADH–I is the most important. In the 5’ untranslated region of the maize ADH genes anaerobic regulatory elements and an intron are important for driving gene expression in monocots. The regulatory elements responsible for the anaerobic response of the genes are within a 247 bp segment immediately upstream of the CAP site, more specifically between positions −140 and −99 of the maize Adh–I promoter. Within this 40 bp segment, there are two essential regions, each of around 15 bp, required for expression under low oxygen conditions.

The whole Adh–I promoter has been used in cereals such as rice, oat and barley and in dicots such as tobacco, obtaining very low levels of expression in the dicot crop. The Adh–I promoter in conjunction with the intron has proved to be much superior for transformation of some cereals such as rice.
Basically only two regions of the regulatory transcription region of maize ADH genes are currently used to drive gene expression in transformed plants, mainly monocots:

- the **anaerobic regulatory elements (ARE)** of the Adh-1 promoter, and
- the **first intron** of the maize Adh-1 gene.

The two elements are the basis of a recombinant promoter called **Emu**. **pEmu** is formed in the 5' to 3' direction by:

- a truncated Adh-1 promoter having **multiple** copies of ARE,
- **enhancer elements** from the octopine synthase (OCS) gene of A. tumefaciens,
- a TATA box from a promoter expressed in plants, and
- the maize Adh-1 **intron 1**.

The promoter has been tested in transient assays in various monocot crops obtaining a very high rate of activity compared to the 35S CaMV promoter. It has also been used for the development of transgenic rice and sugarcane. However, in some stable transformations the response has been poor and the recovery of the transgenic plants difficult. Problems have been attributed to the presence of duplicated OCS enhancer elements.

**IP issues**

The **Commonwealth Scientific and Industrial Research Organization (CSIRO)** of Australia and the companies **Lubrizol Enterprises** and **Mycogen Plant Science** (U.S.) have jointly filed and been granted patents related to the Adh promoter and "regulatory elements". Actual assignment of the patent rights varies among jurisdictions according to Inpadoc. The table shows the entity identified by Inpadoc as owning the patents granted in Australia, Canada, Europe and the United States. These may now be controlled by Dow.

**Approximate scope of protection**

The patents **do not** refer to the maize **Adh-1 promoter as such**. They cover **some elements** derived from the **promoter regions** of maize aldolase gene, maize Adh-1 and Adh-2 genes. The patents are in general directed to two different aspects:

- the ARE regions of the promoters of maize ADH genes and maize aldolase gene, and
- the chimeric pEmu having ARE regions and an intron

1. **Patents directed to the ARE enhancer regions**

- Patents directed to these regions were granted in the U.S., Canada, and Europe. The enhancer regions are part of a recombinant promoter molecule that has, from 5' to 3' direction, at least:
  - the ARE regions
  - a promoter expressible in plants, which is basically reduced to a TATA box, and
  - a structural gene under the control of the above mentioned elements

- The claims of the European patent encompass the DNA sequences of the ARE regions of:
  - maize aldolase gene,
  - maize adh-1 and adh-2 genes, and
  - sequences that are at least 66% homologous to the mentioned genes

- The Canadian patent also claims a method for the expression of a structural gene in a plant under conditions of low oxygen.

2. **Patents directed to a chimeric promoter known as pEmu**

- The patents on a recombinant promoter for enhancing-expression of structural genes in **monocot** plant cells have been granted in the U.S., Europe and Australia. The minimum elements of the promoter in direction 5' to
3', claimed in all three jurisdictions, are:

- several ARE enhancer elements
- a TATA box
- a transcription start site
- an intron and
- a structural gene;

- In addition, the independent claims of the U.S. and the Australian patents comprise enhancer elements derived from the ocs promoter region.

- Although the examples provided in the specification refer to the ARE regions and intron 1 derived from the maize aldolase and maize adh genes, the ARE regions and the intron of the recombinant promoter claimed in the patents in all three jurisdictions are not limited to the elements of the maize adh genes. Thus, the use of other ARE enhancer elements and introns might be protected by the claims of these patents. The prosecution histories of the patents might shed light on this aspect.

The patent information, a summary of the independent claims and the actual independent claims of each patent are presented in the following tables.

### Patents on the ARE of maize ADH and maize aldolase promoters

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Independent Claims and Summary of Claims</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US 5001060</strong></td>
<td>Title – Plant Anaerobic regulatory element</td>
</tr>
<tr>
<td><strong>Claim 1</strong></td>
<td>A recombinant DNA molecule comprising:</td>
</tr>
<tr>
<td></td>
<td>(a) an anaerobic regulatory element;</td>
</tr>
<tr>
<td></td>
<td>(b) a plant–expressible promoter located 3' to said anaerobic regulatory element, and</td>
</tr>
<tr>
<td></td>
<td>(c) a plant–expressible structural gene located 3' to said plant–expressible promoter such placed under the regulatory control of said promoter and said anaerobic regulatory element not in nature under the regulatory control of said anaerobic regulatory element.</td>
</tr>
<tr>
<td></td>
<td>• A recombinant DNA molecule comprising from 5' to 3' direction an ARE, a plant– express plant–expressible structural gene.</td>
</tr>
</tbody>
</table>

| **CA 1338858** | Title – Plant Anaerobic regulatory element |
| **Claim 1** | A recombinant DNA molecule comprising: |
| | (a) an anaerobic regulatory element; (b) a plant–expressible promoter located 3' to said anaerobic regulatory element, and (c) a plant–expressible structural gene located 3' to said plant–expressible promoter such that said structural gene is placed under the regulatory control of said promoter and said anaerobic regulatory element not in nature under the regulatory control of said anaerobic regulatory element. |
| **Claim 25** | A method for selective expression of a plant–expressible structural gene under anaerobic conditions comprises the steps of: |
| | (i) constructing a recombinant DNA molecule which comprises (a) an anaerobic regulatory element, (b) a plant–expressible promoter located 3' to said anaerobic regulatory element, and (c) a plant–expressible structural gene such that said structural gene is placed under the regulatory control of said promoter and said anaerobic regulatory element not in nature under the regulatory control of said anaerobic regulatory element. |
plant–expressible promoter such that said structural gene is placed under the regulatory control of an anaerobic regulatory element.

(ii) transforming said plant tissue with said recombinant DNA molecule, and

(iii) placing said transformed plant cell under anaerobic conditions so that said plant–expressed.

- A recombinant DNA molecule the same as described in the US patent US 5001060.

- A method for the anaerobic expression of a plant–expressible structural gene. The met plant tissue with a recombinant DNA molecule as described and placing the tissue under anaerobic expression.

A recombinant DNA molecule the same as described in the US patent

Claim 1
A method for selective expression of a plant–expressible structural gene under anaerobic conditions, the method comprises using as an anaerobic regulatory element a recombinant DNA molecule comprising:

1) 5' -GCTGGTTTCT-3'
2) 5' -CGTGGTTTGCTTGCC-3',
or a sequence having about 66% or greater homology thereto
3) 5' -CGAGCCTTTCTTCCC-3'
4) 5' -CTGCCTCCCTGGTTTCT-3', and
5) 5' -CTGCAGCCCCGGTTTCG-3',
or a sequence having about 66% or greater homology thereto,

a plant–expressible promoter being located 3' to said anaerobic regulatory element, and a promoter being located 3' to said plant–expressible promoter such that said structural gene is placed under regulatory control of said recombinant promoter molecule.

A method for the anaerobic expression of a plant–expressible structural gene using a recombinant DNA molecule described in the Canadian patent. The ARE sequences are selected from maize aldolase of maize adh–1 and adh–2. Also, sequences with at least 66% of homology to the mentioned sequences are included.

Remarks
The granted Japanese patent JP 8826414 B2 assigned to Lubrizol Enterprises & CSIRO was not extended in Belgium (BE), France (FR), Germany (DE), Netherlands (NL), Spain (ES), Sweden (SE). An application is pending in South Africa (ZA 8800320 A).

Patents on the recombinant promoter pEMU

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Summary of Claims and Independent Claims</th>
<th>Assignee</th>
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<tbody>
<tr>
<td><strong>EP 459643 B1</strong></td>
<td><strong>Title – A recombinant promoter for gene expression in monocotyledonous plants</strong></td>
<td><strong>Mycogen Plant Science Inc. &amp; CSIRO</strong></td>
</tr>
<tr>
<td><strong>Claim 1</strong></td>
<td>A recombinant promoter molecule for enhancing expression of a plant–expressible structural gene in a monocot plant cell comprising: (a) a plurality of ARE enhancer elements (b) a truncated, plant expressible promoter providing a TATA box region necessary to initiate transcription positioned 3' to said plurality of enhancer elements; and (c) a nucleotide sequence naturally found as an intron positioned between the transcription start site and the translation start site in a plant–expressible gene; whereby a plant–expressible structural gene placed 3' to said recombinant promoter molecule is expressed in said monocot plant cell under regulatory control of said recombinant promoter molecule.</td>
<td><strong>Mycogen Plant Science Inc. &amp; CSIRO</strong></td>
</tr>
<tr>
<td><strong>Expected expiry – 8 May 2011</strong></td>
<td></td>
<td><strong>Mycogen Plant Science Inc. &amp; CSIRO</strong></td>
</tr>
</tbody>
</table>
intron. A plant-expressible gene is located 3' to and under the control of the mentioned recombinant molecule. The gene is expressed in a monocot plant cell.

**US 5290924**

**Title** – Recombinant promoter for gene expression in monocotyledonous plants

**Claim 1**

A recombinant promoter molecule, useful for enhancing expression of a plant-expressible structural gene in a monocot plant cell, said promoter molecule comprising: (a) a plurality of enhancer elements selected from the group consisting of only ARE elements, only OCS elements, and combinations of ARE and OCS elements; (b) a truncated, plant expressible promoter, providing a TATA box region and a transcription start site, said promoter selected from the group consisting of ∆35S and ∆ADH positioned 3' to said plurality of enhancer elements wherein said truncated promoter excludes the presence of enhancer sequences and wherein said truncated promoter is recombined with said plurality of enhancer elements positioned 5' to said truncated promoter; and (c) a maize Adh1 intron positioned 3' to said transcription start site whereby a plant-expressible structural gene, placed 3' to said recombinant promoter molecule, is expressed in said monocot plant cell under regulatory control of said recombinant promoter molecule.

- A recombinant promoter molecule for expression in monocots similar to the one claimed in the European patent. The enhancer elements are selected from ARE, OCS, and a combination of both. There is also a plant-expressible termination signal 3' to the gene of interest.

**CA 2042831**

**Title** – Recombinant promoter for gene expression in monocotyledonous plants

**Claim 1**

A recombinant promoter molecule for enhancing expression of a plant-expressible structural gene in a monocot plant cell comprising: (a) a plurality of enhancer elements selected from the group consisting of ARE and OCS elements; (b) a truncated, plant expressible promoter providing a TATA box region necessary to initiate transcription positioned 3' to said plurality of enhancer elements; and (c) a nucleotide sequence naturally found as an intron positioned between the transcription start site and the translation start site in a plant-expressible gene; whereby a plant-expressible structural gene placed 3' to said recombinant promoter molecule is expressed in said monocot plant cell under regulatory control of said recombinant promoter molecule.

- A recombinant promoter molecule for enhancing the expression of a gene of interest in monocots comprising several ARE and OCS elements, a promoter having a TATA box, an intron. A structural gene is located 3' to the recombinant promoter.

**Remarks**

The granted Australian patent (AU 643521 B2) has lapsed. The European patent registered in Denmark (DK), France (FR), Germany (DE), Italy (IT), and Spain (ES). A patent was also granted in Japan (JP 3325589 B2) and an application in China (CN 1063506) was withdrawn.

Note: Patent information on this page was last updated on 13 March 2006.
Synthetic promoters

Scientific aspects

As mentioned in the introductory information about promoters, a set of minimum elements are required for an active eukaryotic promoter. Among those elements are the TATA box, the transcription start site or CAP site and the CCAAT consensus sequence, which is required for accurate transcription. From the sequences of these elements in diverse organisms, it is possible to synthesize consensus sequences that may work across different organisms and are not necessarily derived from a particular organism. The group of patents under this section present promoters whose parts are synthesized as consensus sequences of the promoter elements found in nature.

IP issues

Two families of patents directed to synthetic promoters and promoter elements have been filed by Pioneer Hi-Bred international Inc. The first patent family includes two granted patents in the U.S. and Australia and a couple of European applications and one Canadian application. The second patent family consists of patent applications filed in Australia, United States and a PCT application. The two patent families are unrelated.

First patent family

Definition of promoter and some of its elements

Promoters are defined by the inventors in functional and structural terms. They are the transcription control units that contain the signals for RNA polymerase to begin transcription so that protein synthesis can go ahead. Promoters are located in the 5' flanking or upstream region of the transcribed gene. The most common motifs present in promoters are:

- The TATA element, which is the site where the TATA–binding protein (TBP) binds. This protein is part of a complex of polypeptides that recruit the RNA polymerase II to begin transcription;
- the transcription start site; and
- the CCAAT consensus sequence.

A core promoter or minimal promoter contains the TATA box and the transcription start site. This core promoter may or may not have detectable activity in the absence of sequence that enhance the activity or confer tissue specific activity.

Other elements of promoters include:

- INR, sequences near the transcription start site of some genes that provide an alternate site for binding factors to activate transcription;
- enhancers; and
- upstream elements.

The enhancers are not classified as upstream elements by the inventors. The upstream elements are position and orientation dependent, interact with specific binding factors and are less common. The upstream elements also may be exchanged with other elements while maintaining their characteristic control over gene expression. In contrast, the enhancers can increase the efficiency of transcription regardless of their distance and orientation to the transcription start site.

Approximate scope of protection

The synthetic promoters that are the subject of the Pioneer Hi–Bred's inventions contain:

- a TATA motif;
- a GC–rich region (at least 64% GC); and
- a transcription start site.
According to the inventors, the GC-rich region located between the TATA motif and the transcription start site in plant promoters acts as a very strong inducer of constitutive expression. It increases transcriptional activation efficiency. Plant-expressible promoters contain a region of about 40% GC, while a 64% or greater GC content is characteristic of animal promoters. The maize ubiquitin 1 gene (Ubi-1) core promoter, which produces high levels of activity in monocots, has a GC content more similar to animal promoters (64%). The GC content of promoters covered by the claims is at least 64%.

Both the U.S. and the Australian patents claim a synthetic promoter as described. Expression cassettes containing a structural gene linked to the promoter and a poly(A) signal are also part of the claimed inventions.

In addition, both patents claim:

- A synthetic upstream element comprising at least 3 octopine synthase (OCS) binding motifs (TGACG) with an intervening sequence. This motif has been identified from several opine synthase genes, i.e., octopine, nopaline, mannopine, and from other genes such as histone genes.
- Expression cassettes containing the synthetic upstream element linked to the synthetic promoter.
- Expression cassettes where the synthetic upstream element is linked to a promoter which does not necessarily have the structural elements described above.

The U.S. patent also claims a promoter construct having a core promoter and the upstream activating region (UAR) of the Ubi-1 gene. An expression cassette containing these elements, a structural gene linked to the promoter and a poly(A) signal is also claimed.

A summary of the independent claims of the granted patents and some bibliographic data are shown in the following table.
Claim 1
A synthetic DNA promoter sequence functional in a plant cell, said promoter sequence comprising:

- a TATA motif,
- a transcription start site, and
- a region between said TATA motif and said start site that is at least 64% GC-rich;

wherein said region is not a region between a TATA motif and a transcription start site of native maize ubiquitin promoter, and wherein said promoter sequence is set forth in SEQ ID NO:10.

Claim 2
A synthetic DNA promoter sequence functional in a plant cell, said promoter sequence comprising:

- a TATA motif,
- a transcription start site, and
- a region between said TATA motif and said start site that is at least 64% GC-rich;

wherein said region is not a region between a TATA motif and a transcription start site of native maize ubiquitin promoter, and wherein said promoter sequence is set forth in SEQ ID NO:1.

Claim 3
An expression cassette comprising:

- a synthetic promoter comprising:
  - a TATA motif,
  - a transcription start site and
  - a "region" between said TATA motif and said start site that is at least 64% GC rich,

wherein said "region" is not a region between a TATA motif and a transcription start site of native maize ubiquitin promoter, and wherein sequence of said promoter is set forth in SEQ ID NO:1.

Claim 4
An expression cassette comprising:

- a synthetic promoter comprising:
  - a TATA motif,
  - a transcription start site and
  - a region between said TATA motif and said start site that is at least 64% GC rich,

wherein said region is not a region between a TATA motif and a transcription start site of native maize ubiquitin promoter, and wherein sequence of said promoter is set forth in SEQ ID NO:10.

Claim 5
An expression cassette comprising:

- a synthetic promoter comprising:
Second Patent Family

This patent family has patent applications filed in the United States, Europe, Australia and Canada. However, the applications in the US, Europe and Canada have lapsed. For the purpose of providing the disclosed art as prior art, only brief information about this patent family is provided here, but it should be noted that the application may still be pending in Australia, and continuations may still be pending elsewhere.

Some bibliographic data is outlined in the following table:

<table>
<thead>
<tr>
<th>Applicant</th>
<th>Title</th>
<th>Publication No.</th>
<th>Publication Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WO 01/53476 A2</td>
<td>26 July 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1252304</td>
<td>30 October 2002</td>
</tr>
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<td></td>
<td></td>
<td>AU 34507/01</td>
<td>31 July 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2390753</td>
<td>26 July 2001</td>
</tr>
</tbody>
</table>

What's disclosed in this patent family

The patent applications are directed to nucleotide sequences of plant promoters comprising synthetic multimeric promoter elements regions (SMPERs). The different sequences of the SMPERs contain the following promoter elements:

- the dehydration or drought responsive element (DRE) of Arabidopsis, which is part of a stress–responsive gene promoter;
- the abscisic acid responsive element (ABRE) of maize, which acts in embryo development;
- the activating sequence (AS–1) of the Cauliflower mosaic virus (CaMV), which mediates both salicylic acid– and auxin–inducible transcriptional activation;
- the DNA–binding domain of the rice transcription factor GT–2; and
- the proliferating cell nuclear antigen (PCNA) IIA gene promoter element of rice, which confers meristematic tissue–specific expression.

The SMPERs can be linked to any other promoters, native or synthetic. The above–mentioned promoter elements function as transcription factor binding sites. Plants or parts of plants containing one SMPER linked to an encoding sequence and enhancing the expression of the coding sequence are claimed. Methods for selecting promoter elements and for creating SMPERs active in a tissue of interest are also claimed.

Note: Patent information on this page was last updated on 29 March 2006.

Chapter 3

Inducible promoters

Summary

As their name says, the activity of these promoters is induced by the presence or absence of biotic or abiotic factors. Inducible promoters are a very powerful tool in genetic engineering because the expression of genes operably linked to them can be turned on or off at certain stages of development of an organism or in a particular tissue.

This section presents a general view of patents directed to promoters whose activity is triggered by either chemical or physical factors. The patents discussed in this section are about general promoter systems and their uses and do not encompass particular promoters. There are virtually hundreds of inducible promoters that vary according to the organism source and cells or tissues where they regulate gene transcription. Thus, this section only discusses patents drawn to inducible promoter systems that are regulated by particular chemical or physical factors.
The analyzed patents on inducible promoters are grouped as:

- **Chemically-regulated promoters**, including promoters whose transcriptional activity is regulated by the presence or absence of alcohol, tetracycline, steroids, metal and other compounds.

- **Physically-regulated promoters**, including promoters whose transcriptional activity is regulated by the presence or absence of light and low or high temperatures.

### Chemically-regulated promoters

**Summary**

The transcription activity of this class of promoters is modulated by chemical compounds that either turn off or turn on gene transcription. As prerequisites, the chemicals influencing promoter activity typically

- should not be naturally present in the organism where expression of the transgene is sought;
- should not be toxic;
- should affect only the expression of the gene of interest;
- should be of easy application or removal; and
- should induce a clear expression pattern of either high or very low gene expression.

Preferably, chemically-regulated promoters should be derived from organisms distant in evolution to the organisms where its action is required. Thus, promoters to be used in plants are mostly derived from organisms such as yeast, E. coli, Drosophila or mammalian cells.

This section presents an analysis of patents disclosing some of the main chemically-inducible promoters actually used to modulate expression in plants and animals. The promoter types analyzed are grouped as follows:

- **Alcohol-regulated** Syngenta has several patents and patent applications in Europe and Australia directed to the transcriptional system containing the alcohol dehydrogenase I (alcA) gene promoter and the transactivator protein AlcR. Different agricultural alcohol-based formulations are used to control the expression of a gene of interest linked to the alcA promoter.

- **Tetracycline-regulated** Yale University and BASF AG have several patents and patent applications filed in the United States, Europe, Australia and Canada covering aspects of tetracycline-responsive promoter systems, which can function either as an activating or repressing gene expression system in the presence of tetracycline. Some of the elements of the systems are a tetracycline repressor protein (TetR), a tetracycline operator sequence (tetO) a tetracycline transactivator fusion protein (tTA), which is the fusion of TetR and a herpes simplex virus protein 16 (VP16) activation sequence. Eukaryotic cells transformed with the transactivation systems including animal cells are part of the protected inventions.

- **Steroid-regulated** Numerous patent and patent applications are directed to steroid-responsive promoters for the modulation of gene expression in plant and animal cells. Analysis on patents on this type of promoters include:
  - McGill University patents on promoters based on the rat glucocorticoid receptor (GR);
  - Rockefeller University patents on promoters based on the human estrogen receptor (ER);
  - Syngenta and Pioneer Hi-Bred patents directed to promoters based on ecdysone receptors derived from different moth species; and
  - a group of patents filed by different entities covering promoters from the steroid/retinoid/thyroid receptor superfamily.

- **Metal-regulated** Promoters derived from metallothionein (proteins that bind and sequester metal ionic) genes from yeast, mouse and human are the subject matter of several United States patents granted to Genentech, University Patents Inc. and University of California (Berkeley). DNA constructs having metal-regulated promoters and eukaryotic cells transformed with them are part of the protected inventions.
Pathogenesis-related (PR) Pathogen-related (PR) proteins are induced in plants by the presence of exogenous chemicals besides pathogen infection. Salicylic acid, ethylene and benzothiadiazole (BTH) are some of the inducers of PR proteins. Promoters derived from Arabidopsis and maize PR genes are the subject matter of patents granted to Novartis and Pioneer Hi-Bred in the United States, Australia and Europe.

Alcohol-regulated promoters

Scientific aspects

The promoter system in its natural environment

In a bacterial positive-mode operon the genes are off by default and are turned on by activators. The interaction between the regulatory protein and the regulatory region on the DNA turns transcription on.

The ethanol utilization pathway of the filamentous fungus Aspergillus nidulans is a well-characterized positive operon system. It controls the cellular response to ethanol and other related chemicals. The first enzyme in the path of ethanol utilization is alcohol dehydrogenase I (Adh–I) encoded by the alcA gene. The transcriptional activator protein AlcR binds target sequences within the alcA gene promoter in the presence of ethanol, ethyl methyl ketone or other alcohols/ketones. These compounds act as inducers of the gene expression.

The promoter system transferred into plants

The system transferred into plants uses the alcR gene, which encodes the transcriptional activator protein AlcR, and the alcA promoter. The alcR gene is under the control of a strong constitutive promoter such as CaMV 35S. In the absence of the inducer, the transactivator protein AlcR cannot bind the specific sequences of the modified alcA promoter, which is linked to a gene of interest. The modified or chimeric target promoter comprises the regulatory sequences of the alcA promoter and a core promoter region (a TATA box and a transcription start site) of a plant–expressible gene promoter.

Among the advantages of the system are:

- the lack of an equivalent alcR gene in plants, which rules out interference with plant endogenous Adh activity,
- the chemical inducers are relatively simple molecules with low toxicity, and
- under normal growth conditions of a plant, the levels of natural inducers, (e.g. ethanol) that are produced when plants are waterlogged or under artificial anoxia are very low and do not induce alcA expression.

The alcohol-inducible system can be used for:

- production of high level recombinant proteins, especially those that interfere with growth or biomass production,
- inducible male sterility system, and
- inducible expression of resistance genes.

Nevertheless there are still some difficulties in using the system for agricultural purposes. Specifically, the means to introduce the inducer, e.g. by spraying or drenching and its volatility impact the effectiveness of the system for field work.

IP issues

Syngenta has filed patent applications related to alcohol-inducible expression systems for plants. The applications have been filed in Europe, Australia, Great Britain and with the World Intellectual Property Organization (WIPO). There are no granted patents in the U.S.

Approximate scope of protection

The only patent grant is in Europe. The patent claims:
a chemically-inducible plant gene expression cassette that contains:
  - a promoter linked to the AlcR encoding gene, and
  - an inducible promoter linked to a gene of interest.

- a method for controlling plant gene expression by using the mentioned expression cassette, and
- a chimeric chemically-inducible promoter containing:
  - regulatory sequences of the alcA gene promoter, and
  - a transcription initiation sequence from a plant-expressible gene promoter.

Note that the inducible promoter of the expression cassette is not restricted to any particular promoter (claim 1). The promoter is conditioned to activation by the regulatory protein (AlcR protein) and the presence of an effective inducer. The inducible promoter may be derived from aldehyde dehydrogenase gene or other alcohol dehydrogenase genes that are involved in the ethanol utilization pathway.

The transcription initiation sequence of the chimeric chemically-inducible promoter refers to the CAAT box, TATA box and surrounding sequences that define the transcription start point for the structural gene.

### Alcohol-inducible expression cassettes

<table>
<thead>
<tr>
<th>pACN1</th>
<th>alcR binding site</th>
<th>min 35S</th>
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Specific Patent Information

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<th>Patent Number</th>
<th>Title, Summary of Claims and Independent Claims</th>
<th>Assignee</th>
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<tbody>
<tr>
<td>EP 637339 B1</td>
<td>Title – DNA constructs and plants incorporating them</td>
<td>Syngenta Ltd.</td>
</tr>
</tbody>
</table>

**Claim 1**

A chemically-inducible plant gene expression cassette comprising
  - a first promoter operatively linked to the alcR regulator sequence obtainable from *Aspergillus nidulans* which encodes the AlcR regulator protein, and
  - an inducible promoter operatively linked to a target gene, the inducible promoter being activated by the regulator protein in the presence of an effective exogenous inducer whereby application of the inducer causes expression of the target gene.
Claim 11
A method for controlling plant gene expression comprising
transforming a plant cell with a chemically-inducible plant gene expression cassette which has
- a first promoter operatively linked to the alcR regulator sequence obtainable from Aspergillus nidulans which encodes the AlcR regulator protein, and
- an inducible promoter operatively linked to a target gene, the inducible promoter being activated by the regulator protein in the presence of an effective exogenous inducer whereby application of the inducer causes expression of the target gene.

Claim 12
A chimeric promoter comprising
- an upstream region containing a promoter regulatory sequence obtainable from the alcA gene promoter of Aspergillus nidulans and
- a downstream region containing a transcription initiation sequence, characterised in that said upstream and downstream regions are heterologous, the promoter is chemically inducible and the transcription initiation sequence is obtainable from the core promoter region of a promoter which is active in plant cells.

The claims are generally to:
- A chemically-inducible expression cassette comprising a first promoter linked to the alcR sequence and an inducible promoter linked to a target gene. The AlcR protein activates the inducible promoter in the presence of an inducer and causes the expression of the gene.
- A chimeric promoter comprising an upstream region with a chemically-inducible alcA gene promoter and a downstream region with a transcription initiation sequence from a core plant-expressible promoter region, in which the two regions are heterologous.
- A method for controlling plant gene expression by transforming a plant with an expression cassette as described.
an inducible promoter from an ALCR-activatable gene, which gene is the alcA gene from *Aspergillus nidulans*, operatively linked to a target gene, said inducible promoter being activated by the ALCR regulator protein in the presence of an alcohol and/or ketone inducer, so that application of a sufficient amount of a suitable inducer causes expression of the target gene.

**Claim 8**

A plant cell containing a chimeric promoter operatively linked to a target gene, said chimeric promoter comprising an upstream region containing a promoter from an ALCR-activatable alcA gene from *Aspergillus nidulans* and a downstream region containing a transcription initiation sequence, wherein the upstream region and the downstream region are heterologous and the chimeric promoter is inducible by an alcohol and/or ketone, so that application of a sufficient amount of a suitable inducer, in the presence of the ALCR regulator protein encoded by the alcR gene from *Aspergillus nidulans*, causes expression of the target gene.

The claims of this US patent is generally directed to:

- Plant cells containing a chemically-inducible plant gene expression cassette or chimeric promoter operatively linked to a target gene as described in **EP 637339 B1**.

- A method for controlling plant gene expression by transforming a plant cell with the chemically-inducible gene expression cassette as described so that gene expression is controlled by an alcohol and/or ketone inducer.

**Remarks**
The application in Australia (AU 39019/93 A1) has lapsed.


### Tetracycline-regulated promoters

#### Scientific aspects

Transposons are mobile genetic elements which can insert at random into plasmids or the bacterial chromosome independently of the host cell recombination system. Transposons carry genes that confer new phenotypes on the host cell such as antibiotic resistance.

**Tetracycline resistance operon** is carried by the *Escherichia coli* transposon (Tn) 10. This operon has a **negative mode** of operation, where the interaction between a *repressor protein*, the Tet repressor (TetR) and the tet operator (tetO), a DNA sequence next to the promoter or juxtaposed, prevents transcription of the genes. **Tetracycline** acts as an *inducer* by switching on the transcription system. It binds to the TetR, changing its conformation, so it can no longer remain attached to the operator.

In plants, the system has been adapted in two ways

- as a promoter repressing system, basically using the system as described, and
- as a promoter activating system, where the TetR is modified to activate gene transcription, instead of inhibiting transcription.
For this system, a CaMV 35S promoter is modified by introducing a tet operator sequence upstream and downstream of the TATA box. In the absence of tetracycline, over expressed TetR binds to the tet operators and prevents gene expression. In tobacco, the expression of the tetracycline-inducible promoter could be modulated up to 500-fold. The inducible promoter has also worked in tomato and potato.

Unfortunately, the system presents some problems. For tetracycline to work as an inducer, it must be supplied continuously to the medium due in part to the short-half life of the antibiotic. In addition, TetR must be in high concentration to be effective as a repressor as it has to compete with at least forty proteins that assemble around the TATA box. For some plants, such as Arabidopsis, high concentrations of the repressor are toxic and alter the photosynthetic physiology of the plant.

Due to the drawbacks of the promoter repressing system, the TetR has been converted to an activator of gene expression. In one version of this system, the TetR is fused to the acidic activation sequence of the herpes simplex virus protein 16 (VP16), forming a tetracycline transactivator (tTA) fusion protein. In the absence of tetracycline, the tTA binds to the tet operator sequences, which are upstream of a TATA box in a target promoter. The added tetracycline forms a complex with the tTA and releases the operator, thus, turning off gene transcription.

In contrast to the wild type TetR, tTA does not need to compete with endogenous transcription factors for binding sites. The system has worked in tobacco and in Arabidopsis.

Despite the advantages of the Tet activating system, the plants must be in the presence of tetracycline to turn transcription off. That implies a continuous supply of the antibiotic. Also the original promoter that contains the tet operators is prone to silencing over time. Work has been done to improve the efficacy of the promoter.
IP issues

Yale University and BASF AG have several patents and patent applications related to tetracycline-regulated promoter systems. The inventions claimed by these institutes include the use of a wild-type TetR and fusion proteins formed by a wild type TetR or a mutated TetR linked to either an activator or an inhibitor of transcription.

1. Yale University patents

Yale has a United States granted patent and a European patent application directed to an auto-regulated tetracycline transactivator tTA placed under the control of a minimal promoter having at least one tetO sequence.

The inventions

In United States patent US 5851796 and European application EP 832254 A1, the production of tTA is regulated by the presence of tetracycline: in its presence, tTA is not produced. In the absence of tetracycline, tTA binds the tetO sequence promoting the expression of the gene(s) under the control of an inducible promoter.

Claims are drawn to:

A polynucleotide:

- encoding a tetracycline transactivator fusion protein and
- operably linked to an inducible minimal promoter with at least one tetO sequence

The patent describes that a gene that's operably linked to a promoter sequence is placed under the control of that sequence.

The encoded protein contains:

- a prokaryotic tet repressor (the claim is not limited to only the DNA binding portion) and a
- eukaryotic transcriptional activator protein (the activator is not limited to the VP16 activator).

Although patent describes that different activator sequences can be used (listing acidic rich domains such as in VP16, proline rich domains of CTF/NF-1, serine/threonine rich domains of Oct-2, or glutamine rich domains, such as found in Sp1), the only construct tested is one in which the activator is VP16.

The specification describes that a "eukaryotic transcriptional activator" is capable of activating transcription in eukaryotes.

Methods for decreasing or enhancing the expression of a heterologous protein in a eukaryotic cell are also claimed. The methods generally include inserting two DNA molecules into the cell: One of them is the polynucleotide described above.; the other encodes a heterologous protein to be regulated by the system linked to a minimal promoter, which also has at least a tetO sequence. When the transformed eukaryotic cell is cultivated in the presence of tetracycline or a tetracycline analogue, the expression of the heterologous protein is inhibited because the activator tTA does not remain bound to the minimal promoter having the tetO sequence that drives the expression of the heterologous gene. Conversely, in the absence of tetracycline, the heterologous protein is expressed in the eukaryotic cell because the tTA activator remains bound

The specification defines "heterologous" as is a protein that does not naturally occur in the specific host organism in which it is present.

Kits containing the two types of molecules used in the method are also claimed. However, the kits have to have:

- at least two container means each containing a different one of the two molecules;
• the means have to be in close confinement in the kit.

An additional kit claim requires that:

• the mentioned polynucleotide encoding the transactivator fusion protein is within a eukaryotic cell.

The patent doesn't describe what a "container means" is (is it anything different from a container?) or how close "close confinement" has to be.

The specific patent information of US 5851796 and EP 832254 A1 is presented in the following table.

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Summary of Claims and Independent Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5851796</td>
<td>Title – Autoregulatory tetracycline–regulated system for inducible gene expression in eucaryotes</td>
<td>Yale University</td>
</tr>
<tr>
<td></td>
<td>Claim 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A polynucleotide comprising a nucleotide sequence encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A method to inhibit expression of a heterologous protein in a eucaryotic cell comprising (a) obtaining a eucaryotic cell comprising (i) a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; (ii) a second polynucleotide molecule encoding the heterologous protein, said second polynucleotide molecule being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and (b) cultivating the eucaryotic cell in a medium comprising tetracycline or a tetracycline analogue such that expression of the heterologous protein is inhibited.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A method to enhance the expression of a heterologous protein in a eucaryotic cell comprising (a) obtaining a eucaryotic cell comprising (i) a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; (ii) a second polynucleotide molecule encoding the heterologous protein, said second polynucleotide molecule being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and (b) cultivating the eucaryotic cell in a medium lacking tetracycline or a tetracycline analogue such that expression of the heterologous protein is enhanced.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A method to activate the expression of a heterologous protein in a eucaryotic cell comprising (a) obtaining a eucaryotic cell comprising (i) a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; (ii) a second</td>
<td></td>
</tr>
</tbody>
</table>
polynucleotide molecule encoding the heterologous protein, said second polynucleotide molecule being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and (b) cultivating the eucaryotic cell in a medium lacking tetracycline or a tetracycline analogue such that expression of the heterologous protein is activated.

**Claim 27**

A kit comprising a carrier means having in close confinement therein at least two container means, wherein a first container means contains a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a procaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; and a second container means contains a second polynucleotide molecule encoding said inducible minimal promoter, which promoter contains at least one tet operator sequence, which tet operator sequence is strategically positioned for being operably linked to a heterologous polynucleotide sequence encoding a polypeptide.

**Claim 28**

A kit comprising a carrier means having in close confinement therein at least two container means, wherein a first container means contains a eucaryotic cell transfected with a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a procaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; and a second container means contains a second polynucleotide molecule comprising an inducible minimal promoter, which promoter contains at least one tet operator sequence, which tet operator sequence is strategically positioned for being operably linked to a heterologous polynucleotide sequence encoding a heterologous polypeptide.

### EP 832254 A1

- **Title** – Autoregulatory tetracycline-regulated system for inducible gene expression in eucaryotes

The independent claims are from the PCT application (WO 96/40946):

**Claim 1**

A composition of matter comprising a polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a procaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence.

**Claim 11**

A method to decrease or shut off expression of a heterologous protein comprising

- (a) transforming a eucaryotic cell with (i) a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a procaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; (ii) a second polynucleotide molecule encoding the heterologous protein, said protein being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and
- (b) cultivating the eucaryotic cell in a medium comprising tetracycline or a...
tetracycline analogue.

**Claim 13**
A method to activate or enhance the expression of a heterologous protein comprising
(a) transforming a eucaryotic cell with (i) a first polynucleotide molecule encoding tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible promoter, which promoter contains at least one tet operator sequence; (ii) a second polynucleotide molecule encoding the heterologous protein, said protein being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and
(b) cultivating the eucaryotic cell in a medium lacking tetracycline or a tetracycline analogue.

**Claim 17**
A composition of matter consisting essentially of the plasmid pTet-Splice.

**Claim 18**
A composition of matter consisting essentially of the plasmid pTet-tTAK.

**Claim 19**
A kit comprising a carrier means having in close confinement therein at least two container means, wherein a first container means contains a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; and a second container means contains a second polynucleotide molecule encoding said inducible minimal promoter, which promoter contains at least one tet operator sequence, which tet operator sequence is strategically positioned for being operably linked to a heterologous polynucleotide sequence encoding a polypeptide.

**Claim 20**
A kit comprising a carrier means having in close confinement therein at least two container means, wherein a first container means contains a eucaryotic cell transfected with a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; and a second container means contains a second polynucleotide molecule comprising an inducible minimal promoter, which promoter contains at least one tet operator sequence, which tet operator sequence is strategically positioned for being operably linked to a heterologous polynucleotide sequence encoding a heterologous polypeptide.

**Remarks**
The application filed in Australia (AU 62745/96) has lapsed. Related application also filed in Japan (JP 11507539 T2).

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2. BASF AG patents

This company has a large portfolio of granted patents and patent applications related to tetracycline-regulated promoters. Patents have been granted in Australia (1), Canada (1) and in the United States (16). Additional applications have been filed in Australia, Canada, China and Europe. The inventions cover different versions of Tet-activating systems, transgenic organisms transformed with tetracycline-regulated promoters including eukaryotes in general, and plants and animals in particular.

**Granted patents**

**Bibliographic data**
The protected inventions

The group of patents entitled "Tight control of gene expression in eucaryotic cells by tetracycline–responsive promoters" are all directed to sequence encoding a tTA fusion protein comprising a prokaryotic TetR and a transcriptional activator domain. The claimed tTA binds to tetO sequences in the absence of tetracycline activating the transcription of a gene linked to a tTA responsive promoter.

The US patent 5 464 758 claims, in addition to the tTA–encoding sequence:

- a eukaryotic cell transfected with a tTA–encoding sequence and a sequence containing a minimal promoter with tetO sequences linked to a gene of interest; and
- kits containing
  - the tTA–encoding sequence and a second polynucleotide sequence linked to a minimal promoter having at least a tetO sequence; and
a eukaryotic cell transfected with the polynucleotide coding for the tTA fusion protein.

The definition of eukaryotic cells provided by the inventors includes yeast, plant cells, insects, mammalian and human cells.

The claims don't limit the types of regulatory sequences provided with the tTA-encoding sequence.

The patent specification only describes constructs that are constitutively expressed and so although at least some of the claims of this patent dominate those of the Yale patent (US 5 851 796) discussed above, this patent does not defeat the novelty of the claims of the Yale patent.

The kit claims notably have the same peculiarities of those of the Yale patent, e.g., requiring:

- at least two container means each containing the a different one of the two molecules (and in one of the kit claims, the tTA-encoding molecule is already in a cell);
- the means have to be in close confinement in the kit.

This claim format may have been copied in the Yale patent from the BASF patent which it referenced by Application No. in the specification. It's of note that the same law firm listed on the front page of the BASF patent is also listed on the front page of the Yale patent.

BASF's United States patent 5 650 298 claims in addition:

- an isolated DNA sequence coding for a tTA that is recombined with a target DNA molecule. The tTA-coding gene is flanked at each end by DNA sequences that allow homologous recombination with the target sequence.
- In additional claims, these sequences are:
  - a 5′ flanking regulatory region of a gene of interest, and
  - a portion of a gene of interest linked to a tTA-responsive promoter.

In this system, the expression of the tTA is controlled by the promoter of the gene of interest and the target gene is controlled by the tTA-responsive promoter.

Host cells (including mouse stem cells, and human cells of unrestricted type) containing the mentioned sequences, methods for producing the host cells and methods for producing gene products using such sequences are also claimed.

The Canadian patent CA 2165162 claims in addition to the polynucleotide molecules of the United States patent 5 650 298:

- the use of tetracycline or a tetracycline analogue for the inhibition of the transcription of a gene of interest linked to a tTA-responsive promoter in a transgenic animal; and
- a method
  for producing a transgenic animal (non-human) by introducing a tTA coding gene into a fertilized oocyte and implanting the oocyte in a foster mother allowing the oocyte to develop into a transgenic animal.

The Australian patent AU 684 524 B2 claims the same type of tTA fusion protein that recombines in a particular location in the host cell as the Canadian patent and the United States patent 5 650 298. In addition, the Australian patent claims a transgenic animal (non-human) having the tTA fusion protein integrated at a predetermined location in a chromosome of the animal cells.

The United States patent US 5 589 362 is directed to a sequence comprising a mutated version of a TetR protein having at least one amino acid mutation, which has the ability to bind a certain class (B) of tetO sequences having a nucleotide substitution at position +4 or +6. The mutated TetR is linked to a polypeptide that regulates transcription in eukaryotic cells. A method for regulating transcription of a class B tetO–linked gene in a cell by introducing into the cell the fusion protein described above.

View Independent Claims
The fusion proteins, having a first and second polypeptide, claimed in the United States patents 5 654 168 and 5 789 156 are the opposite from each other. In the first patent, the fusion protein works as a transcriptional activator, whereas in the second, the fusion protein works as a transcriptional inhibitor. The differences between the fusion proteins are as follows:

<table>
<thead>
<tr>
<th>Patent No.</th>
<th>First polypeptide</th>
<th>Second polypeptide</th>
<th>Tetracycline</th>
<th>Transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5 654 168</td>
<td>mutated TetR</td>
<td>Activator</td>
<td>presence</td>
<td>activated</td>
</tr>
<tr>
<td>US 5 789 156</td>
<td>TetR</td>
<td>inhibitor or silencer</td>
<td>presence</td>
<td>inhibited</td>
</tr>
</tbody>
</table>

The United States patent 5 654 168 also claims a kit carrying in separate containers:

- a tTA-encoding gene as described above; and
- a nucleotide sequence with a cloning site linked to a tetO sequence of first class type.

The United States patent 5 789 156 also claims a kit carrying in separate containers:

- a fusion protein as described above or a eukaryotic cell line into which the fusion protein has been introduced; and
- a nucleotide sequence with a cloning site linked to at least a tetO sequence.

View Independent Claims

The independent claims of the patents entitled "Methods for regulating gene expression" are methods for regulating the expression of a tetO-linked gene in a cell. The expression is regulated by the concentration of tetracycline administered to the cell. The differences between the independent claims of the three United States patents are:

- **US 5 814 618**
  - in one method the fusion protein encoded by the gene introduced in the cell contains a TetR and a second polypeptide that inhibits transcription in the cell; and
  - in a second method two different nucleotide molecules are introduced in a cell:
    - a fusion protein–encoding gene as mentioned above and
    - a nucleotide sequence linked to at least a tetO sequence.

- **US 5 888 981**
  the fusion protein used in the methods claimed is a tetracycline-controllable transactivator (tTA), which activates the transcription in eukaryotic cells.

- **US 6 004 941**
  the TetR of the fusion protein binds to the tetO sequences in the presence of tetracycline, activating transcription of the gene linked to the tetO sequences.

In addition, there are two product claims directed to recombinant vectors containing:

- in one case, two different nucleotide sequences comprising each a cloning site linked to the same tetO sequence for bidirectional regulation of the transcription; and
- in another case, each of the two cloning sites is linked to different class of tetO sequence for independent regulation of the transcription.

View Independent Claims

The United States patents US 5 859 310, US 5 866 755, US 5 912 411 and US 5 922 927 are all directed to transgenic mice containing a tetracycline-responsive transcriptional regulator. The transgenic mice of the inventions have integrated in their genome:

- a transgene encoding a fusion protein, and
- a gene of interest linked to a tetO sequence.

The transcription of the gene of interest is either activated or inhibited by the binding of the fusion protein to the tetO sequences. The fusion protein formed by two different polynucleotides differs between the patents.
as follows:

<table>
<thead>
<tr>
<th>First polypeptide</th>
<th>Second polypeptide</th>
<th>Tetracycline</th>
<th>Transcription</th>
<th>Special feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tet repressor (TetR)</td>
<td>activator</td>
<td>absence</td>
<td>activated</td>
<td>Specific homologous recombination of tTA (fusion protein)–encoding gene and gene of interest</td>
</tr>
<tr>
<td>Tet R</td>
<td>inhibitor</td>
<td>absence</td>
<td>inhibited</td>
<td>–</td>
</tr>
<tr>
<td>mutated TetR</td>
<td>inhibitor</td>
<td>presence</td>
<td>inhibited</td>
<td>–</td>
</tr>
<tr>
<td>mutated TetR</td>
<td>activator</td>
<td>presence</td>
<td>activated</td>
<td>–</td>
</tr>
<tr>
<td>prokaryotic TetR</td>
<td>activator</td>
<td>absence</td>
<td>activated</td>
<td>Specific homologous recombination of tTA (fusion protein)–encoding gene and gene of interest</td>
</tr>
</tbody>
</table>

The United States patent US 5 922 927 claims methods for producing a transgenic mouse with a transgene coding for a fusion protein as described in the table. Some of the methods involve the introduction of a tTA–encoding sequence in a fertilized oocyte and its implantation in a foster mother, allowing the development of a transgenic mouse. In other methods, the tTA–encoding gene is introduced in embryonic stem cells of a mouse. For homologous recombination between the tTA–encoding transgene and the gene of interest in the cells of a mouse, the DNA molecule carrying the transgene comprises:

- a 5' regulatory region of the gene of interest linked to,
- a tTA–encoding sequence, and
- a tTA–responsive promoter linked to
- at least a portion of the gene of interest of sufficient length to mediate homologous recombination.

View Independent Claims

The fusion protein of the United States patent 6 136 954 activates transcription in the presence of tetracycline. A portion of the fusion protein binds to the tetO. It’s not limited to the tetR and the patent describes that mutated tetR proteins are part of the invention. The second polypeptide of the fusion protein is a transcriptional activator. In the broadest claims of the patent, the activator is also not limited as to structure or amino acid sequence so long as it functions as an activator (though the level of activation required is not defined).

Conversely, the second polypeptide of the fusion protein claimed in the United States patent 6 271 348 is a transcriptional inhibitor and therefore, the protein as a whole inhibits transcription in eukaryotic cells. The inhibitor portion also has no structural limitations in the broadest claims. The term "inhibition" is defined in the patent as "a diminution in the level or amount of transcription of a target gene compared to the level or amount of transcription prior to regulation by the transcriptional inhibitor protein." The patent describes that transcriptional inhibition may be partial or complete.

View Independent Claims

The United States patents entitled "Transgenic organisms having tet racycline–regulated transcriptional regulatory systems" are directed to transgenic plants containing:

- a tetO–linked gene of interest and
- a transgene comprising a fusion protein having a first and a second polypeptide that either activates or inhibits the transcription of the gene linked to the tetO sequence as follows:
Applications

<table>
<thead>
<tr>
<th>Title</th>
<th>Publication No.</th>
<th>Filing date</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline–regulated transcriptional modulators</td>
<td>CA 2193122 A</td>
<td>June 29, 1995</td>
<td>January 18, 1996</td>
</tr>
<tr>
<td></td>
<td>EP 1 092 771 A2</td>
<td>June 29, 1995</td>
<td>April 18, 2001</td>
</tr>
</tbody>
</table>

Remarks
- Applications also filed in Australia (AU 30923/95 & AU 44566/99), China (CN 1167504 A), Finland (FI 9605287 A), Japan (JP 9500526 T2 & JP 11506901 T2) and Norway (NO 9605623 A).
- The European applications EP 705 334 A1 and EP 804 565 A1 were withdrawn on July 23, 2001 and February 21, 2002, respectively.

The claims as filed in the Canadian application CA 2193122 recite:

- An isolated DNA sequence coding for a fusion protein comprising:
  - a first polypeptide that binds to tetO in the presence of tetracycline, and
  - a transcriptional activator for eukaryotic cells.
- An isolated sequence coding for a fusion protein where the second polypeptide inactivates transcription.
- A host cell and an organism (except humans) comprising both types of fusion proteins (activating and inhibiting transcription proteins) and a sequence of interest to be transcribed linked to a tetO sequence.
- A recombinant vector for bidirectional transcription of genes of interest. There are cloning sites at each end of a tetO sequence for the introduction of the sequences to be transcribed.
- A recombinant vector for independent regulation of transcription of two genes of interest where the tetO sequences linked to the genes are of a different class.
- A kit comprising in separate containers
  - a fusion protein that activates transcription in the presence of tetracycline and
  - a DNA sequence comprising a cloning site for inserting the gene of interest linked to a tetO sequence.

The first polypeptide of the fusion proteins is not specified in the independent claims as filed.

The only independent claim as filed of the EP application 1 092 771 recites a DNA sequence encoding a fusion protein that inhibits transcription in eukaryotic cells. The first polypeptide of the fusion protein is defined as a TetR.

View Independent Claims

The protected inventions

The group of patents entitled "Tight control of gene expression in eucaryotic cells by tetracycline-responsive promoters" are all directed to sequence encoding a tTA fusion protein comprising a prokaryotic TetR and a transcriptional activator domain. The claimed tTA binds to tetO sequences in the absence of tetracycline activating the transcription of a gene linked to a tTA responsive promoter.

The US patent 5464758 claims, in addition to the tTA-encoding sequence:

- a eukaryotic cell transfected with a tTA-encoding sequence and a sequence containing a minimal
promoter with tetO sequences linked to a gene of interest; and

- kits containing
  - the tTA-encoding sequence and a second polynucleotide sequence linked to a minimal promoter having at least a tetO sequence; and
  - a eukaryotic cell transfected with the polynucleotide coding for the tTA fusion protein.

The definition of eukaryotic cells provided by the inventors includes yeast, plant cells, insects, mammalian and human cells.

The claims don't limit the types of regulatory sequences provided with the tTA-encoding sequence.

The patent specification only describes constructs that are constitutively expressed and so although at least some of the claims of this patent dominate those of the Yale patent (US 5851796) discussed above, this patent does not defeat the novelty of the claims of the Yale patent.

The kit claims notably have the same peculiarities of those of the Yale patent, e.g., requiring:

- at least two container means each containing the a different one of the two molecules (and in one of the kit claims, the tTA-encoding molecule is already in a cell);
- the means have to be in close confinement in the kit.

This claim format may have been copied in the Yale patent from the BASF patent which it referenced by Application No. in the specification. It's of note that the same law firm listed on the front page of the BASF patent is also listed on the front page of the Yale patent.

BASF's United States patent 5650298 claims in addition:

- an isolated DNA sequence coding for a tTA that is recombinated with a target DNA molecule. The tTA-coding gene is flanked at each end by DNA sequences that allow homologous recombination with the target sequence.
- In additional claims, these sequences are:
  - a 5' flanking regulatory region of a gene of interest, and
  - a portion of a gene of interest linked to a tTA-responsive promoter.

In this system, the expression of the tTA is controlled by the promoter of the gene of interest and the target gene is controlled by the tTA-responsive promoter.

Host cells (including mouse stem cells, and human cells of unrestricted type) containing the mentioned sequences, methods for producing the host cells and methods for producing gene products using such sequences are also claimed.

The Canadian patent CA 2165162 claims in addition to the polynucleotide molecules of the United States patent 5650298:

- the use of tetracycline or a tetracycline analogue for the inhibition of the transcription of a gene of interest linked to a tTA-responsive promoter in a transgenic animal; and
- a method for producing a transgenic animal (non-human) by introducing a tTA coding gene into a fertilized oocyte and implanting the oocyte in a foster mother allowing the oocyte to develop into a transgenic animal.

The Australian patent AU 684524 B2 claims the same type of tTA fusion protein that recombines in a particular location in the host cell as the Canadian patent and the United States patent 5650298. In addition, the Australian patent claims a transgenic animal (non-human) having the tTA fusion protein integrated at a predetermined location in a chromosome of the animal cells.

View Independent Claims

The United States patent US 5589362 is directed to a sequence comprising a mutated version of a TetR protein having at least one amino acid mutation, which has the ability to bind a certain class (B) of tetO

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sequences

having a nucleotide substitution at position +4 or +6. The mutated TetR is linked to a polypeptide that regulates transcription in eukaryotic cells. A method for regulating transcription of a class B tetO–linked gene in a cell by introducing into the cell the fusion protein described above.

**View Independent Claims**

The fusion proteins, having a first and second polypeptide, claimed in the United States patents [5654168](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggr...) and [5789156](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggr...) are the opposite from each other. In the first patent, the fusion protein works as a transcriptional activator, whereas in the second, the fusion protein works as a transcriptional inhibitor. The differences between the fusion proteins are as follows:

<table>
<thead>
<tr>
<th>Patent No.</th>
<th>First polypeptide</th>
<th>Second polypeptide</th>
<th>Tetracycline</th>
<th>Transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5654168</td>
<td>mutated TetR</td>
<td>Activator</td>
<td>presence</td>
<td>activated</td>
</tr>
<tr>
<td>US 5789156</td>
<td>TetR</td>
<td>inhibitor or silencer</td>
<td>presence</td>
<td>inhibited</td>
</tr>
</tbody>
</table>

The United States patent [5654168](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggr...) also claims a kit carrying in separate containers:

- a tTA–encoding gene as described above; and
- a nucleotide sequence with a cloning site linked to a tetO sequence of first class type.

The United States patent [5789156](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggr...) also claims a kit carrying in separate containers:

- a fusion protein as described above or a eukaryotic cell line into which the fusion protein has been introduced; and
- a nucleotide sequence with a cloning site linked to at least a tetO sequence.

**View Independent Claims**

The independent claims of the patents entitled "Methods for regulating gene expression" are methods for regulating the expression of a tetO–linked gene in a cell. The expression is regulated by the concentration of tetracycline administered to the cell. The differences between the independent claims of the three United States patents are:

- **US 5814618**
  - in one method the fusion protein encoded by the gene introduced in the cell contains a TetR and a second polypeptide that inhibits transcription in the cell; and
  - in a second method two different nucleotide molecules are introduced in a cell:
    - a fusion protein–encoding gene as mentioned above and
    - a nucleotide sequence linked to at least a tetO sequence.

- **US 5888981**
  - the fusion protein used in the methods claimed is a tetracycline–controllable transactivator (tTA), which activates the transcription in eukaryotic cells.

- **US 6004941**
  - the TetR of the fusion protein binds to the tetO sequences in the presence of tetracycline, activating transcription of the gene linked to the tetO sequences.

  In addition, there are two product claims directed to recombinant vectors containing:
  - in one case, two different nucleotide sequences comprising each a cloning site linked to the same tetO sequence for bidirectional regulation of the transcription; and
  - in another case, each of the two cloning sites is linked to different class of tetO sequence for independent regulation of the transcription.

**View Independent Claims**

The United States patents [5859310](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggr...), [5866755](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggr...), [5912411](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggr...) and [5922927](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggr...) are all directed to transgenic mice containing a tetracycline–responsive transcriptional regulator. The transgenic mice of the inventions have integrated in their genome:
• a transgene encoding a fusion protein, and
• a gene of interest linked to a tetO sequence.

The transcription of the gene of interest is either activated or inhibited by the binding of the fusion protein to the tetO sequences. The fusion protein formed by two different polynucleotides differs between the patents as follows:

<table>
<thead>
<tr>
<th>First polypeptide</th>
<th>Second polypeptide</th>
<th>Tetracycline</th>
<th>Transcription</th>
<th>Special feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tet repressor (TetR)</td>
<td>activator</td>
<td>absence</td>
<td>activated</td>
<td>Specific homologous recombination of tTA (fusion protein)-encoding gene and gene of interest</td>
</tr>
<tr>
<td>Tet R</td>
<td>inhibitor</td>
<td>absence</td>
<td>inhibited</td>
<td>–</td>
</tr>
<tr>
<td>mutated TetR</td>
<td>inhibitor</td>
<td>presence</td>
<td>inhibited</td>
<td>–</td>
</tr>
<tr>
<td>mutated TetR</td>
<td>activator</td>
<td>presence</td>
<td>activated</td>
<td>–</td>
</tr>
<tr>
<td>prokaryotic TetR</td>
<td>activator</td>
<td>absence</td>
<td>activated</td>
<td>Specific homologous recombination of tTA (fusion protein)-encoding gene and gene of interest</td>
</tr>
</tbody>
</table>

The United States patent US 5922927 claims methods for producing a transgenic mouse with a transgene coding for a fusion protein as described in the table. Some of the methods involve the introduction of a tTA-encoding sequence in a fertilized oocyte and its implantation in a foster mother, allowing the development of a transgenic mouse. In other methods, the tTA-encoding gene is introduced in embryonic stem cells of a mouse. For homologous recombination between the tTA-encoding transgene and the gene of interest in the cells of a mouse, the DNA molecule carrying the transgene comprises:

• a 5' regulatory region of the gene of interest linked to,
• a tTA-encoding sequence, and
• a tTA-responsive promoter linked to
• at least a portion of the gene of interest of sufficient length to mediate homologous recombination.

The United States patents entitled "Transgenic organisms having tetracycline-regulated transcriptional regulatory systems" are directed to transgenic plants containing:

• a tetO-linked gene of interest and
a transgene comprising a fusion protein having a first and a second polypeptide that either activates or inhibits the transcription of the gene linked to the tetO sequence as follows:

<table>
<thead>
<tr>
<th>First polypeptide</th>
<th>Second polypeptide</th>
<th>Tetracycline</th>
<th>Transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>mutated TetR</td>
<td>activator</td>
<td>present</td>
<td>activated</td>
</tr>
<tr>
<td>mutated TetR</td>
<td>inhibitor</td>
<td>present</td>
<td>inhibited</td>
</tr>
<tr>
<td>TetR</td>
<td>activator</td>
<td>absence</td>
<td>activated</td>
</tr>
</tbody>
</table>

**View Independent Claims**

**Applications**

<table>
<thead>
<tr>
<th>Title</th>
<th>Publication No.</th>
<th>Filing date</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline-regulated transcriptional modulators</td>
<td>CA 2193122 A</td>
<td>June 29, 1995</td>
<td>January 18, 1996</td>
</tr>
<tr>
<td></td>
<td>EP 1092771 A2</td>
<td>June 29, 1995</td>
<td>April 18, 2001</td>
</tr>
</tbody>
</table>

**Remarks**

Applications also filed in Australia (AU 30923/95 & AU 44566/99), China (CN 1167504 A), Finland (FI 9605287 A), Japan (JP 9500526 T2 & JP 11506901 T2) and Norway (NO 9605623 A).

The European applications EP 705 334 A1 and EP 804 565 A1 were withdrawn on July 23, 2001 and February 21, 2002, respectively.

The claims as filed in the Canadian application CA 2193122 recite:

- An isolated DNA sequence coding for a fusion protein comprising:
  - a first polypeptide that binds to tetO in the presence of tetracycline, and
  - a transcriptional activator for eukaryotic cells.

- An isolated sequence coding for a fusion protein where the second polypeptide inactivates transcription.

- A host cell and an organism (except humans) comprising both types of fusion proteins (activating and inhibiting transcription proteins) and a sequence of interest to be transcribed linked to a tetO sequence.

- A recombinant vector for bidirectional transcription of genes of interest. There are cloning sites at each end of a tetO sequence for the introduction of the sequences to be transcribed.

- A recombinant vector for independent regulation of transcription of two genes of interest where the tetO sequences linked to the genes are of a different class.

- A kit comprising in separate containers a fusion protein that activates transcription in the presence of tetracycline and a DNA sequence comprising a cloning site for inserting the gene of interest linked to a tetO sequence.

The first polypeptide of the fusion proteins is not specified in the independent claims as filed.

The only independent claim as filed of the EP application 1092771 recites a DNA sequence encoding a fusion protein that inhibits transcription in eukaryotic cells. The first polypeptide of the fusion protein is defined as a TetR.

**View Independent Claims**

**Steroid–regulated promoters**

**Scientific aspects**

Among the chemically–inducible gene expression systems for plants and animals are the ones based on...
steroid hormone receptors. The mammalian glucocorticoid receptor (GR) is a member of the family of animal steroid hormone receptors. GR also acts as a transcription factor by activating gene transcription from promoters containing glucocorticoid response elements (GRE). In the cellular environment, GR exists in a complex in the cytoplasm with the 90-kilodaltons (kDa) heat shock protein (HSP90), which dissociates once GR binds its ligand (hormone).

A system comprised of the GR and GREs resulted in transient expression in tobacco cells in the presence of dexamethasone, a strong synthetic glucocorticoid. In stably transformed Arabidopsis plants, however, the system did not induce expression.

The hormone binding domain (HBD) of GR and other steroid receptors can also be used to regulate heterologous proteins in cis, that is, operatively linked to protein-encoding sequences upon which it acts. Thus, the HBD of GR, estrogen receptor (ER) and an insect ecdysone receptor have shown relatively tight control and high inducibility.

The HBD of the rat GR has been linked to the DNA–binding domain of the yeast GAL4 transcription factor and to the acidic transactivating domain of the herpes viral protein VP16. This chimeric transcriptional factor, named GVG, has driven the expression of a number of genes in several plants in the presence of the ligand dexamethasone. In some cases however, it appears to be toxic, which is caused by a high concentration of and extended exposure to dexamethasone and induction of untargeted genes (e.g. defense–related genes).

An ER–based inducible system for use in transgenic plants includes a chimeric transactivator called XVE, assembled by fusion of the regulatory region of the human ER, the DNA–binding domain of the bacterial repressor LexA and the acidic transactivating domain of VP16. In transgenic tobacco and Arabidopsis, XVE has induced the expression of genes in the presence of estradiol to levels 8–fold higher than observed when expression is driven by a constitutive promoter, such as 35S CaMV. The XVE system does not exhibit the toxic effects found with the GVG system, but in some legumes (e.g., soybean) it appears to be deregulated, presumably due to the presence of phyto–estrogens.

Since none of the systems described are induced by compounds suitable for agricultural use, a transcription system inducible by non–steroidal agrochemicals was developed based on the insect ecdysone receptor. Ecdysones are insect steroidal hormones that trigger the expression of critical genes during larval development and have been proposed as safer alternatives to pesticides. An advantage of the ecdysone receptor is that can also bind non–steroidal ecdysone agonists (agonists are molecules that improve the activity of a different molecule). One hybrid transactivator system contains the DNA–binding domain and the receptor activation of GR and the hormone–regulatory domain of the Heliothis virescens ER. In stable transgenic tobacco plants, the system induced the expression of a reporter gene over 400–fold greater than the activity of the 35S CaMV promoter. The system is highly responsive to RH5992 (tebufenozide), a non–steroidal ecdysone agonist that lacks phytoxicity and is used as a lepidopteran control agent on vine and horticulture plants. A drawback of the system is a relatively high background expression.

Promoters based on glucocorticoid receptor (GR)

One of the best characterized steroid–responsive promoter systems is the GR. It has been used widely in transgenic animal cells, in particular mammalian cells. Granted patents claiming the basic principle of the glucocorticoid–responsive transcriptional factors were not found. Neither were patents or patent applications claiming a GR–based system for plants.

Specific Patent Information
<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5 512 483</td>
<td>Title – Expression responsive to steroid hormones</td>
<td>McGill University</td>
</tr>
<tr>
<td></td>
<td>The system for expression in animal cells is part of an invention disclosed in a U.S. patent granted to McGill University.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The invention comprises an animal expression vector containing a synthetic promoter having at least glucocorticoid response elements (GREs) placed upstream of a minimal promoter TATA region. The vector also contains a transcriptional initiator site and a restriction endonuclease site for the insertion of a gene to be expressed from the promoter.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The promoter itself, which is also claimed, is responsive to several steroid receptors including glucocorticoid, progesterone, androgen and mineralocorticoid receptor. There is no limit on the types of cells into which the promoter is transfected.</td>
<td></td>
</tr>
<tr>
<td>Remarks</td>
<td>The related PCT application WO 94/28150 and the Australian application AU 67918/94 were withdrawn in 1996.</td>
<td></td>
</tr>
</tbody>
</table>

Promoters based on estrogen receptor (ER)

The Rockefeller University has filed two European patent applications directed to methods for transforming plant cells with a vector comprising a regulatory region from an ER.

Specific Patent Information

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP 1 232 273 A2</td>
<td>Title – Chemical inducible promoters used to obtain transgenic plants with a silent marker</td>
<td>The Rockefeller University</td>
</tr>
<tr>
<td></td>
<td>The claims of the European application EP 1 232 273 A2 recite methods for selecting transgenic plants by inserting a vector having a gene of interest under the control of an ER. Upon the application of an inducer compound, the transformed plants express the gene of interest. The genes under the control of the inducible promoter are:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• genes promoting shoot formation;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• genes promoting somatic embryogenesis;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• an antibiotic resistance gene; and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• a herbicide resistance gene.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The compounds 17–beta–estradiol and 4–hydroxy tamoxifen are cited as inducers of the transcription system.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Although the title of the application reads chimeric promoters, these are not subject matter of the independent claims as filed. Some dependent claims mention the use however of the DNA–binding domain of the bacterial repressor LexA assembled with the regulatory domain of the ER.</td>
<td></td>
</tr>
<tr>
<td>EP 1 242 604 A2</td>
<td>Title – Chemical inducible promoters used to obtain transgenic plants with a silent marker and organisms and cells and methods of using same for screening for mutations</td>
<td>The Rockefeller University</td>
</tr>
<tr>
<td></td>
<td>The claims range from organisms or cells comprising a transgenic inducible promoter to a specific chimeric inducible promoter corresponding to the XVE promoter mentioned above in scientific aspects and to lettuce plants transformed with an inducible promoter driving particular genes promoting shoot development and antibiotic resistance.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The broadest claims recite:</td>
<td></td>
</tr>
</tbody>
</table>
an organism or cell comprising a transgenic inducible promoter, any, which controls a gene whose natural promoter is inoperative or lacking; and
a method for screening mutations in an organism or cell gene by putting that gene under the control of a transgenic inducible promoter.

a nucleic acid containing a chemically-inducible promoter having an ER, and a vector and a transgenic plant or plant cell containing such chemically-inducible promoter are part of the filed claims. In other claims, the chimeric inducible promoter comprises:

- a constitutive promoter;
- a DNA-binding domain of bacterial repressor LexA;
- a transactivating domain of VP16;
- an estrogen receptor; and
- at least one LexA binding sites.

In addition, the claims recite either a transgenic plant cell or specifically a transgenic lettuce plant comprising an inducible promoter, which in some cases explicitly comprises an ER. The same genes disclosed in EP 1 282 273 A2 under the control of the inducible promoter are in the claims as filed, with the exception of genes promoting somatic embryogenesis.

Remarks
Although these applications have a similar scope and were filed by the same institute, they are not related.
Application also filed in Australia (AU 14851/01) and (AU 14856/01).

Chimeric transcriptional factor GVG

The United States patent US 6 063 985 does not belong to the patent family of EP 1 242 604 A2, but it is also directed to transformation of a plant with genes promoting shoot formation under the control of an inducible promoter. As this patent does not claim as part of independent claims steroid-responsive promoters in particular, it is not included in the analysis.

Promoters based on ecdysone receptor

Zeneca (now Syngenta) and Pioneer Hi-Bred have patents and patent applications directed to:

- isolated DNA sequences of ecdysone receptors derived from diverse moth species. Methods for inducing the expression of proteins in plants by using an ecdysone receptor, and
- the use of other inducible receptor such as the retinoid X receptor and the ultraspiracle receptor.

The patents granted to Syngenta protect the isolated DNA and amino acid sequences of the ecdysone receptor from the moth species *Heliothis virescens.*
<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6 379 945</td>
<td>Title – Gene switch  &lt;br&gt;The United States patent claims an isolated or synthetic DNA sequence encoding the mentioned ecdysone receptor and any of the following domains of the ecdysone receptor:  &lt;br&gt;• the transactivation domain;  &lt;br&gt;• the DNA binding domain;  &lt;br&gt;• the ligand binding domain;  &lt;br&gt;• the hinge binding domain; and  &lt;br&gt;• the carboxy terminus of the receptor.</td>
<td>Syngenta</td>
</tr>
</tbody>
</table>

| AU 711 391 B2 | Title – A gene switch comprising an ecdysone receptor  <br>The Australian patent claims both the DNA and the amino acid sequence of Heliothis ecdysone receptor and the comprising domains of the receptor. In this patent the species of Heliothis is not limited to H. virescens. In addition, DNA sequences that hybridize to the claimed sequences and any allelic variant or derivatives of the polypeptide sequence of the complete receptor and its domains are part of the protected invention. |  |

| EP 828 829 A1 | Title – A gene switch comprising an ecdysone receptor  <br>The claims as filed are quite similar to the claims of the Australian granted patent. Several DNA and amino acid sequences of the Heliothis ecdysone receptor and its domains are part of the invention. Like the Australian patent, sequences that show homology as low as 65% hybridize to the sequences shown and allelic variants and derivatives of the protein sequences are part of the filed claims.  <br>The claims of the European patent application, in addition, recite DNA sequences encoding a Spodoptera ecdysone receptor from the moth genus Spodoptera. | Syngenta |

| CA 2219121 | Title – A gene switch comprising an ecdysone receptor  <br>The claims as filed are quite similar to the claims of the Australian granted patent. Several DNA and amino acid sequences of the Heliothis ecdysone receptor and its domains are part of the invention. Like the Australian patent, sequences that show homology as low as 65% hybridize to the sequences shown and allelic variants and derivatives of the protein sequences are part of the filed claims. |  |

| Remarks | Related applications also filed in Bulgaria (BG 102124 A), Brazil (BR 9608897 A), China (CN 1191568 AA), Czech Republic (CZ9703722 A3), Hungary (HU 9802225 AB), Japan (JP 11506319 T2), Norway (NO 975419 A), New Zealand (NZ 308162 A), Poland (PL 323587 A1) and Turkey (TR 9701436 T1). |  |

| EP 1 112 360 A1 | Title – Ecdysone receptors and methods for their use  <br>The claims of the European patent application recite the isolated DNA sequences and the polypeptide sequences of an ecdysone receptor and an ultraspiracle derived from insects of the family Pyralidae, which is a moth family. Ecdysone response elements found in the promoters of the target genes are activated by heterodimers formed by an ultraspiracle and the ecdysone receptor.  <br>The claims as filed also recite methods of inducing gene expression in a plant where the gene of interest is linked to a transcriptional regulatory region that responds to the ecdysone receptor–ligand complex and is activated by the complex. Expression vectors comprising a promoter | Pioneer Hi-Bred |
Other patents related to steroid-responsive promoters

There is an increasing number of patent documents related to the steroid/retinoid/thyroid receptor superfamily and their multiple applications, which are beyond the scope of this paper. The disclosure of the European application EP 1 112 360 A1 discusses several patent applications and patents related to this topic. As such, it is a good reference to those who would like to assess the patent landscape in this field in a much broader context.

The following list of patent documents refers to chimeric inducible receptors that combine domains from steroid-responsive promoters such as glucocorticoid, mineralocorticoid, and estrogen, among others. Some of them also combine receptors that can respond to both steroid and metal compounds making it a doubly inducible promoter system. Finally, ecdysone receptors isolated from different insects and new receptors based on the retinoic acid receptor are also included. In no way do these documents represent the total of patents directed to this extensive topic, but they give an idea of the diverse applications in this field of technology.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title</th>
<th>Topic</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>WO 01/62780</td>
<td>Gene expression system based on chimeric receptors</td>
<td>System to modulate the expression of a target gene by the interaction of two chimeric proteins each having a dimerization domain member of the steroid/thyroid hormone nuclear receptor. The dimerization domain is required for functional transactivation. The chimeric proteins also contain a DNA-binding domain and a transcription-modulating domain.</td>
<td>The Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>AU-B-734 051</td>
<td>Hormone-mediated methods for modulating expression of exogenous genes in mammalian systems, and products related thereto</td>
<td>Methods for modulating the expression of an exogenous gene in a mammalian cell by using modified ecdysone receptors that is paired with a silent partner of the steroid/thyroid superfamily of receptors.</td>
<td></td>
</tr>
<tr>
<td>US 5 599 904</td>
<td>Chimeric steroid hormone superfamily receptor proteins</td>
<td>Chimeric receptor having component domains derived from at least two different members of the steroid hormone superfamily of receptors, with one of the domains derived from a human retinoic acid receptor alpha.</td>
<td>Daiichi Seiyaku Co. Ltd</td>
</tr>
<tr>
<td>US 4 981 784</td>
<td>Retinoic acid receptor composition and method for identifying ligands</td>
<td>Methods for identifying ligands for receptor proteins by replacing the DNA-binding domain of a putative novel receptor with the DNA-binding domain of a known receptor. The expression of the hybrid receptor depends on the presence of the new ligand. The European patent also claims isolated DNA sequences of a ligand–binding and a transcription–activating domain with the properties of retinoic acid receptor protein.</td>
<td></td>
</tr>
<tr>
<td>US 5 646 013</td>
<td>Method of producing foreign products</td>
<td>Inducible promoter system for mammalian cells comprising a first plasmid having a mouse mammary tumor virus (MMTV) long terminal repeat (LTR) linked to a glucocorticoid receptor and a second plasmid with a MMTV–LTR linked to a gene of interest.</td>
<td></td>
</tr>
<tr>
<td>AU-B-734 051</td>
<td>And EP 910 652 A1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patent Number</td>
<td>Date Filed</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td></td>
<td>The plasmids interact for the expression of the gene of interest in the presence of a glucocorticoid. The claimed invention of the European patent is similar to the United States patent but includes the mentioned plasmids, methods to produce the plasmids and animal cells co-transfected with the plasmids.</td>
<td></td>
</tr>
<tr>
<td>US 5 877 018</td>
<td>2 March 1999</td>
<td>Synthetic inducible promoters containing a metal-responsive element and a glucocorticoid responsive element for driving the expression of a gene in a eukaryotic system.</td>
<td></td>
</tr>
<tr>
<td>US 5 559 027</td>
<td>24 September 1996</td>
<td>Synthetic eukaryotic promoters containing two inducible elements. Inducible eukaryotic promoters derived from a human metallothionen gene and mouse mammary tumor virus. The first one contains metal-responsive elements and the second one glucocorticoid-responsive elements. The synthetic inducible promoter of the European patent contains inducible elements responsive to hormones, metals, heat-shock and interferon.</td>
<td></td>
</tr>
<tr>
<td>EP 633 941 B1</td>
<td>15 May 2002</td>
<td>Polynucleotides encoding insect steroid hormone receptor polypeptides and cells transformed with same. Polynucleotide encoding insect ecdysone receptor. Isolated polynucleotide encoding a Drosophila ecdysone receptor and an insect, bacterial or mammalian host cell transformed with the receptor. Recombinant insect ecdysone receptors where at least one of the domains (e.g. DNA-binding or hormone-binding) is from a Drosophila ecdysone receptor.</td>
<td></td>
</tr>
<tr>
<td>US 5 514 578</td>
<td>7 May 1996</td>
<td>Gene expression modulation systems with two expression cassettes, one having a DNA-binding and ligand-binding domain and in the other one a transactivation domain and a ligand-binding domain. The ligand-binding domains are derived from a retinoid X receptor, an ultraspiracle and an ecdysone receptor. The DNA-binding domain is derived from GAL4 and LexA. Mutations to the ecdysone or retinoid receptor enhance the ligand-binding activity and non-steroid binding activity.</td>
<td></td>
</tr>
<tr>
<td>WO 01/70816 A2</td>
<td></td>
<td>Metal–regulated promoters</td>
<td></td>
</tr>
<tr>
<td>WO 01/70816 A3</td>
<td></td>
<td>Scientific aspects</td>
<td></td>
</tr>
</tbody>
</table>

Metallothioneins are proteins that bind and sequester ionic forms of certain metals in fungi (yeast), plants and animals. Such metals include copper, zinc, cadmium, mercury, gold, silver, cobalt, nickel and bismuth. The specific metals sequestered by metallothioneins vary in different organisms. Typically, these proteins are cysteine (cys)–rich and lack aromatic amino acids. The presence of cys motifs is related to the capability of binding metal ions. For instance, Cys–Xaa–Cys motifs have been implicated in the binding and sequestration of copper. The location of these residues within...
the protein is highly conserved between different species showing a tight evolutionary relationship. In plants, metallothioneins have roles in metal accumulation, metal detoxification and embryogenesis.

In the early 1990's a gene expression system for plants was devised based on the copper-dependent yeast metallothionein gene, named ACE1 (activating copper-metallothionein expression). When a tfactor-binding site for ACE1 gene was linked to the 35S CaMV promoter, regulates the expression of beta-glucuronidase (GUS) reporter gene was regulated in transformed tobacco plants. Moreover, the GUS expression depended on the presence of copper ions. The yeast copper-inducible system has been also introduced in Arabidopsis thaliana driving the expression of green fluorescent protein (GFP).

Several metallothionein genes have been isolated from plants such as pea, maize, barley, soybean and Arabidopsis and different plant tissues such as maize root. Although the metal-responsive system is useful for regulation of gene expression in a particular tissue and development stage, effects that the metal could have in other tissues in the transformed organisms should be assessed.

IP issues

A eukaryotic metal-responsive promoter in general was claimed in the European patent EP 94428, granted to University Patents Inc. (now Competitive Technologies Inc.) on February 12, 1992. The invention consisted of processes for securing the transcription of a selected gene sequence in a host cell under the regulation of a eukaryotic metal-responsive promoter. This European patent expired in all European states where the patent was converted. The disclosed invention is now in the public domain.

Other patents relevant to metal-responsive promoters are directed to metallothionein gene promoters isolated from different sources such as yeast, mouse, human and maize. In general the patents claim:

- isolated sequences of the metallothionein promoters;
- vectors for transformation of eukaryotes comprising the isolated genes; and
- methods for controlling the transcription of a gene of interest by the use of metallothionein promoters.

The use of particular plant metallothionein promoters for transcription of genes in a tissue of preference is the subject of several patents such as US patent 6 410 828 and PCT patent application WO 00/537763 by Dow Agrosciences. An isolated DNA sequence of a maize metallothionein promoter documents having embryo-specific and root-specific expression is disclosed in each document, respectively. Expression vectors containing the metallothionein promoter and methods for expressing a heterologous gene sequence in a transformed plant are also part of the inventions. Whether the promoters are inducible by metals is not part of the disclosed and claimed invention. For this reason, the inventions are not further discussed in this paper. Nevertheless use of the maize metallothionein promoter or the invention regardless of inducibility would be potentially covered by the claims. To view or download the United States patent and the PCT application as PDF files, click on US 6 410 828 (kb) and WO 00/537763(kb)

Patents on Yeast Copper-Responsive Promoter

Genentech has a granted United States patent related to inducible transcription control sequences isolated from a yeast metallothionein gene. These transcriptional control sequences are also known as yeast copper chelatin promoter.

Specific Patent Information

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 4 940 661</td>
<td>Title - Metallothionein transcription control sequences and use thereof</td>
<td>Genentech</td>
</tr>
<tr>
<td></td>
<td>The transcriptional control sequences of the invention include the transcriptional promoter and the metal ion regulatory region. As well, in a vector to be replicated in suitable host, a desired gene is under the control of a yeast chelatin promoter, a yeast chelatin transcriptional control sequence, or a metal ion regulatory region from yeast chelatin.</td>
<td></td>
</tr>
</tbody>
</table>
The yeast chelatin transcriptional control sequence contains the chelatin promoter, including transcription initiation and ribosome binding sites. This is a useful promoter in its own right, but to be induced by metal ions, the metal ion regulatory region is needed. This last region is also useful when ligated to other, non-metallothionein promoters, which are recognized by the desired host.

An isolated DNA sequence of a yeast chelatin metal ion regulatory region is also part of the protected invention.

**Patent on a mouse metallothionein promoter**

The granted United States patent of University Patents Inc. (now Competitive Technologies Inc.) relates to the metal-responsive promoter/regulator sequences of the mouse metallothionein-I gene.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 4 579 821</td>
<td>Title - Control of DNA sequence transcription</td>
<td>University Patents Inc. (now Competitive Technologies)</td>
</tr>
<tr>
<td></td>
<td>The mouse metallothionein–I gene promoter of the invention is operatively linked to a DNA sequence of interest, which is expressed in response to variations in the concentration of metal ions in the environment. The host organism, which stably incorporates the DNA sequence of interest, is a mammalian host cell.</td>
<td></td>
</tr>
</tbody>
</table>

**Remarks**


**Patent on a human metallothionein promoter**

The mammalian metallothionein promoter system claimed by the University of California (Berkeley) in a granted United States patent relates to a human metallothionein II transcriptional regulatory system, which allows for regulated control of expression by heavy metals and glucocorticoids.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title - Mammalian metallothionein promoter system</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 4 601 978</td>
<td>The claimed invention comprises:</td>
<td>University of California (Berkeley)</td>
</tr>
<tr>
<td></td>
<td>• A DNA sequence encoding the human metallothionein II transcriptional regulatory system, which includes the promoter region, the transcriptional initiation sequence (CAP site) and the regulatory sequences responsible for inducible transcription.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• A DNA construct where the human metallothionein II transcriptional regulatory sequences are combined with an extra chromosomal replication system that is recognized by a given mammalian host. The combined system regulates the expression of an inserted gene in the mammalian host.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• A vector containing a replicon from a bovine papilloma virus and a human metallothionein II transcriptional regulatory sequences and a restriction site downstream of the regulatory sequences for the insertion of a gene of interest to be expressed in a mammalian host. The bovine papilloma virus</td>
<td></td>
</tr>
</tbody>
</table>
Pathogenesis–related (PR) promoter

Scientific aspects

Pathogenesis–related (PR) proteins are a heterogeneous group of proteins induced in plants by pathogen infection and exogenous chemicals. PR proteins take part in the systemic acquired resistance (SAR) that develops in a resistant plant upon infection with a pathogen. As these proteins are responsive not only to pathogens but also to chemicals, the promoter sequences of these proteins have become attractive as inducible expression systems in plants. For this purpose, promoter sequences from diverse PR proteins have been isolated from plants such as Arabidopsis and maize. Recently, these systems have been developed for human cells as well.

Chemicals such as salicylic acid, ethylene, thiamine, benzol (1,2,3) thiadiazole–7–cabothonic acid S–methyl ester (BTH) have been identified as inducers of PR proteins. One of the best studied promoters is the PR–1a promoter from tobacco. The expression of the beta–glucuronidase (gus) gene, (when driven by the PR–1a promoter), increased 5–10 fold after 1–3 days of induction with salicylic acid. The same chemical induced GUS expression levels 10–fold after 8 days of spraying in field conditions. The PR–1a promoter has also been used to induce the expression of Bacillus thuringiinesis delta–endotoxin in transgenic plants.

Salicylic acid is a potent inducer but there are crop tolerance problems associated with its use. In contrast, BTH does not have a phytotoxic effect and has a longer–lasting induction response of the PR–1a promoter is achieved when compared with salicylic acid. The compounds also differ in their mechanism of action: salicylic acid induces expression only in the treated tissue, whereas BTH moves systemically through the plant.

Some of the drawbacks of PR gene promoters are their inducibility by common environmental stimuli such as UV–B, ozone, and also oxidative stress. This feature might complicate the control of gene expression by PR promoters in non–laboratory conditions.

IP issues

The patents covered in this section relate to DNA sequences of isolated PR–plant gene promoters, methods for inducing the transcription of a gene of interest in a plant under the control of a PR promoter, and methods for finding inducers of PR promoters. Novartis and Pioneer Hi–Bred are the main entities having patents related to these aspects.

Broad and in force patents or patent applications directed to any PR promoter or its use as inducible promoters in transformed cells were not found.

- Novartis

A large portfolio comprising two patent families filed by and granted to Novartis cover:

- DNA sequences of PR promoters isolated from Arabidopsis,
tobacco and cucumber;
- methods for inducing the transcription of a gene of interest in a plant under the control of a PR promoter; and
- methods of inducing specific tobacco or Arabidopsis PR promoters. The claims of these patents are however, narrow because specific plant PR-1 promoters are covered.

Novartis' portfolio also includes patents granted on isolated PR proteins, methods for isolating chemically-regulatable sequences and methods for screening agrochemicals that have the ability to induce SAR response. Because these areas are outside the scope of this paper, they are not included in the present analysis.

- **Pioneer Hi-Bred**

Pioneer's United States granted patent and European patent application are directed to isolated DNA sequences of maize PR-1 gene promoters, methods of using the promoters, and transformed plants. The isolated promoters include both inducible and constitutive promoters. Some of the stimuli that induce the maize PR-1 promoters are pathogen invasion, externally applied chemicals, and environmental stresses. Plant cells and stably transformed plants containing the promoters are also part of the invention.

See table next page for summary of the related patents.

The following table presents some bibliographic information on the patents and patent applications and a summary of the claimed inventions.

<table>
<thead>
<tr>
<th>Assigned to Novartis</th>
<th>Issued Patents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patent No.</td>
<td>Summary of the claims</td>
</tr>
<tr>
<td>Issue/Publication date</td>
<td></td>
</tr>
<tr>
<td>US 5 654 414</td>
<td>Isolated promoter region of a <em>cucumber chitinase/lysozyme gene</em> that is inducible by application of benzo 1,2,3,-thiadiazoles (BTH). Chitinases are pathogenesis–related enzymes induced in the SAR response. View Independent Claims</td>
</tr>
<tr>
<td>August 5, 1997</td>
<td></td>
</tr>
<tr>
<td>US 5 689 044</td>
<td>An isolated region of at least 603 bp that constitutes the <em>tobacco PR-1a gene promoter</em>. The promoter is inducible by application of salicylic acid, BTH and 2,6–dichloroisonicotinic acid (INA). The isolated promoter region of an <em>Arabidopsis PR-1 gene</em> is also claimed. This promoter is inducible by the same compounds as the tobacco PR-1a gene promoter. View Independent Claims</td>
</tr>
<tr>
<td>November 18, 1997</td>
<td></td>
</tr>
<tr>
<td>US 5 789 214</td>
<td>Methods for inducing gene transcription in a plant or a plant tissue by transforming such plant with a chimeric gene containing the <em>tobacco PR-1a gene promoter</em> or the <em>Arabidopsis PR-1 gene promoter</em> linked to a gene of interest and applying an inducer to the transformed plant. The inducers are selected from salicylic acid, BTH and isonicotinic acid (INA). View Independent Claims</td>
</tr>
<tr>
<td>August 4, 1998</td>
<td></td>
</tr>
<tr>
<td>AU 708 850 B2</td>
<td>Isolated full–length of the chemically–inducible <em>Arabidopsis PR-1 promoter</em> and isolated shorter portions of the promoter that are required for induction of gene expression by chemicals such as salicylic acid, BTH and 2,6–dichloroisonicotinic acid (INA). Isolated motifs in the promoter that when mutated alter the inducible activity of the promoter are also part of the invention. View Independent Claims</td>
</tr>
<tr>
<td>August 12, 1999</td>
<td></td>
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</tbody>
</table>
Remarks
Related applications also filed in Canada (CA 2232741 AA) and Japan (JP 11513897 T2). The claims as filed of the Canadian application are the same as the claims of the Australian granted patent. The related European application EP 868 426 A1 was withdrawn on January 2, 2003.

Assigned to Pioneer Hi-Bred

Issued Patent

<table>
<thead>
<tr>
<th>Patent No.</th>
<th>Issue/Publication date</th>
<th>Summary of the claims</th>
</tr>
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<tbody>
<tr>
<td>US 6 429 362</td>
<td>August 6, 2002</td>
<td>Two isolated DNA sequences of inducible maize PR–1 gene promoters. A method to drive the expression of a heterologous gene in a plant by using the claimed maize PR–1 promoters, a plant cell transformed with a DNA construct containing either of the two promoters and a plant stably transformed with such a construct are part of the claimed invention.</td>
</tr>
</tbody>
</table>

View Independent Claims

<table>
<thead>
<tr>
<th>EP 1 056 862 A1</th>
<th>December 6, 2000</th>
<th>The claims as filed of the European application are very similar to the granted claims of the related United States patent. However, unlike the United States patent, the claims as filed of the European application include:</th>
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<tbody>
<tr>
<td></td>
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<td>• DNA sequences that hybridize under stringent conditions to the promoter sequences;</td>
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<td>• a DNA sequence of a constitutive maize PR–1 gene promoter;</td>
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<td></td>
<td></td>
<td>• methods for inducing the expression of a gene of interest or constitutively expressing the gene of interest according to the promoter used;</td>
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<td></td>
<td></td>
<td>• the nucleotide sequences of the maize PR–1 genes;</td>
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<td></td>
<td></td>
<td>• methods for enhancing disease resistance in a plant by using the claimed PR–1 sequences; and</td>
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<tr>
<td></td>
<td></td>
<td>• plant cells and stably transformed plants with such PR–1 genes.</td>
</tr>
</tbody>
</table>

View Independent Claims

Remarks
Related applications have also been filed in Australia (AU 26737/99 A1), Canada (CA 2315549 AA) and South Africa (ZA 9901526 A).

Physically–regulated promoters

Summary

Promoters induced by environmental factors such as water or salt stress, anaerobiosis, temperature, illumination and wounding have potential for use in the development of plants resistant to various stress conditions. These promoters contain regulatory elements that respond to such environmental stimuli.

Temperature–induced promoters include cold– and heat–shock–induced promoters. In many cases, these
promoters are able to operate under normal temperature conditions, which vary according to the organism, but when either cold or heat is applied, the promoters maintain activity. In addition, expression can be enhanced by the application of higher or lower temperature as compared to the normal temperature conditions. One of the best studied eukaryotic heat–shock systems is the one found in Drosophila (fruit fly).

In plants, light–regulated promoters are critical in regulating plant growth and development through the modulation of expression of light–responsive genes. Light–responsive elements from genes such as the small subunit of ribulose–1,5–bisphosphate carboxylase–oxygenase (rbcS) gene, the chlorophyll a/b binding protein, and the chalcone synthase have been widely studied. A molecular dissection of their sequences has shown multiple cis–acting elements for light–dependent gene expression. The light–regulated elements in these promoters exhibit some properties of enhancer–like elements. It is unlikely that a single cis element is sufficient to confer light responsiveness to a non–light regulated promoter. In addition, light–regulated gene expression is under the influence of multiple environmental factors.

The patents presented in this section relate to promoters responsive to temperature and light. The promoters are derived from genes from multiple organisms including bacteria and plants. Methods to use promoters responsive to heat, cold, light or darkness in general terms are part of the inventions discussed here. In some cases, such as in patents related to promoters responsive to cold and light stimuli, particular DNA sequences of promoters are claimed.

The selected analyzed patents are categorized as follows:

- **Temperature–regulated promoters**
- **Light–regulated promoters**

### Temperature–regulated promoters – Heat

**Mycogen Plant Sciences**, **The United States Department of Health and Human Services and The General Hospital Corporation** have granted patents and patent applications that relate in general to DNA sequences of heat shock promoters and methods for expressing a gene of interest under the control of such promoters. Some of the inventions relate to the use of the heat shock promoters in transformed plants, while others do not specify the organism to be transformed.

#### Specific Patent Information

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Summary of Claims and Independent Claims</th>
<th>Assignee</th>
</tr>
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<tbody>
<tr>
<td>US 5 447 858</td>
<td><strong>Title – Heat shock promoter and gene</strong></td>
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<tr>
<td></td>
<td>The patents granted to Mycogen in the United States, Europe and Canada are directed to:</td>
<td></td>
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<td></td>
<td>- consensus DNA sequences of plant heat shock promoters;</td>
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<td>- bacterial strains containing a vector with a consensus heat shock promoter and a gene of interest under its control for expression in plants;</td>
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<tr>
<td></td>
<td>- recombinant Agrobacterium Ti–plasmids comprising a heat shock promoter;</td>
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<td>- methods for expressing a heterologous gene under the control of a heat shock promoter in plant cells; and</td>
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<td></td>
<td>- methods for identifying transformed plants.</td>
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<tr>
<td></td>
<td>In addition, the European patent claims in particular the use of a soybean heat shock promoter to drive the expression of a structural gene in a plant. The soybean heat shock promoter and the structural gene are engineered in a plasmid carried by an Agrobacterium strain used to infect the plant to be transformed.</td>
<td></td>
</tr>
<tr>
<td>EP 159 884 B1</td>
<td><strong>Title – Heat shock promoter and gene</strong></td>
<td>Mycogen Plant Sciences</td>
</tr>
<tr>
<td></td>
<td>The patents granted to Mycogen in the United States, Europe and Canada are directed to:</td>
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<td>- consensus DNA sequences of plant heat shock promoters;</td>
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<td>- bacterial strains containing a vector with a consensus heat shock promoter and a gene of interest under its control for expression in plants;</td>
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<td></td>
<td>- methods for identifying transformed plants.</td>
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<tr>
<td></td>
<td>In addition, the European patent claims in particular the use of a soybean heat shock promoter to drive the expression of a structural gene in a plant. The soybean heat shock promoter and the structural gene are engineered in a plasmid carried by an Agrobacterium strain used to infect the plant to be transformed.</td>
<td></td>
</tr>
<tr>
<td>CA 1338010 A1</td>
<td><strong>Title – Heat shock promoter and gene</strong></td>
<td></td>
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<tr>
<td></td>
<td>The patents granted to Mycogen in the United States, Europe and Canada are directed to:</td>
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<td>- consensus DNA sequences of plant heat shock promoters;</td>
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<tr>
<td></td>
<td>- bacterial strains containing a vector with a consensus heat shock promoter and a gene of interest under its control for expression in plants;</td>
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<tr>
<td></td>
<td>- recombinant Agrobacterium Ti–plasmids comprising a heat shock promoter;</td>
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<tr>
<td></td>
<td>- methods for expressing a heterologous gene under the control of a heat shock promoter in plant cells; and</td>
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</tr>
<tr>
<td></td>
<td>- methods for identifying transformed plants.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In addition, the European patent claims in particular the use of a soybean heat shock promoter to drive the expression of a structural gene in a plant. The soybean heat shock promoter and the structural gene are engineered in a plasmid carried by an Agrobacterium strain used to infect the plant to be transformed.</td>
<td></td>
</tr>
</tbody>
</table>
### AU 732 872 B2
- **Title**: Spatial and temporal control of gene expression using a heat shock protein promoter in combination with local heat

The granted Australian patent and the European application are related to methods for the controlled expression in a gene of interest by a heat-inducible promoter in a spatial and temporal fashion.

One of the methods of the invention is not directed to a particular organism or cell, but to a cell mass or multicellular organism. In another method, animal cells are selected for the expression of a therapeutic protein under the control of a heat shock promoter.

### CA 1324097 A1
- **Title**: Inducible heat shock and amplification system

The Canadian patent claims:

- A method for the production of a polypeptide or protein whose structural gene is under the control of an inducible heat shock promoter.

The structural gene and the heat inducible promoter are transformed in a host cell, which is not limited to a particular host organism.

A gene amplification system under the control of a constitutive promoter is used to increase the copy number of the structural gene.

- A host cell co-transformed with a gene amplification system as mentioned before and a structural gene driven by an inducible heat shock promoter.

- A method for increasing the production of a protein in a dihydrofolate reductase deficient mammalian cell. The structural gene of the protein of interest is placed under the control of a heat shock promoter and a dihydrofolate reductase gene is under the control of a constitutive promoter.

Dihydrofolate reductase is a small enzyme that plays a supporting role in the building of DNA and other processes. It manages the concentration of folate, an organic molecule that shuttles carbon atoms to enzymes that need them in their reactions. Dihydrofolate reductase recycles folate after the release of its carbon atoms.

The dihydrofolate reductase gene is used by the inventors as a gene amplification system. Dihydrofolate reductase deficient mammalian cells transformed with this gene are able to grow in the presence of methotrexate, which is an inhibitor of dihydrofolate reductase. Growing in increasing concentrations of methotrexate leads the cells to overproduce dihydrofolate.
reductase. If a structural gene controlled by an inducible heat shock promoter is co-transformed with the dihydrofolate reductase gene system, both genes are amplified together under the selection pressure.

**Remarks**

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### B. The United States Department of Health and Human Services' patents

The granted **Australian patent** and the **European application** are related to methods for the controlled expression in a gene of interest by a heat-inducible promoter in a spatial and temporal fashion.

One of the methods of the invention is not directed to a particular organism or cell, but to a **cell mass or multicellular organism**. In another method, **animal cells** are selected for the expression of a therapeutic protein under the control of a heat shock promoter.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AU 732872 B2</strong></td>
<td><strong>Title</strong> – Spatial and temporal control of gene expression using a heat shock protein promoter in combination with local heat</td>
<td><strong>The United States Department of Health and Human Services</strong></td>
</tr>
<tr>
<td><strong>Claim 1</strong></td>
<td>A method for the spatial and temporal control of the expression of a gene of interest within a preselected discrete region of a cell mass or multicellular organism, <strong>comprising</strong>: selectively heating a preselected discrete region of a cell mass or multicellular organism that includes cells that contain a genetically engineered gene of interest operably linked to a heat-inducible promoter, thereby inducing the expression of said gene of interest in the cells that are selectively heated.</td>
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</tr>
<tr>
<td><strong>Claim 9</strong></td>
<td>A method of providing a therapeutic protein to selected cells in an animal, <strong>comprising</strong> the steps of: introducing into cells of an animal a DNA molecule <strong>having</strong> a heat shock promoter sequence operably linked to and exerting regulatory control over a sequence encoding a therapeutic protein, and activating said heat shock promoter sequence through the application of a focused ultrasound so that said DNA segment expresses a therapeutically effective amount of said therapeutic protein.</td>
<td></td>
</tr>
<tr>
<td>Patent</td>
<td>Title and Claims</td>
<td>Details</td>
</tr>
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<tr>
<td>EP 922110 A2</td>
<td>The title and the claims are the same as AU 732872.</td>
<td>Earliest priority – 15 August 1996, Filed – 14 August 1997, Granted – Pending, Expected expiry – N/A</td>
</tr>
<tr>
<td>US 2002/165191</td>
<td>The title and the claims are the same as AU 732872.</td>
<td>Earliest priority – 15 August 1996, Filed – 11 March 2002, Granted – Pending, Expected expiry – N/A</td>
</tr>
<tr>
<td>US 2005/059623</td>
<td>This is a continuation of US 2002/165191 (see above) with claims 1–9 canceled</td>
<td>Earliest priority – 15 August 1996, Filed – 9 June 2004, Granted – Pending, Expected expiry – N/A</td>
</tr>
</tbody>
</table>

**Claim 1**
A method for the spatial and temporal control of the expression of a gene of interest within a preselected discrete region of a cell mass or multicellular organism, comprising: selectively heating a preselected discrete region of a cell mass or multicellular organism that includes cells that contain a genetically engineered gene of interest operably linked to a heat-inducible promoter, thereby inducing the expression of said gene of interest in the cells that are selectively heated.

**Claim 9**
A method of providing a therapeutic protein to selected cells in an animal, comprising the steps of: introducing into cells of an animal a DNA molecule having a heat shock promoter sequence operably linked to and exerting regulatory control over a sequence encoding a therapeutic protein, and activating said heat shock promoter sequence through the application of a focused ultrasound so that said DNA segment expresses a therapeutically effective amount of said therapeutic protein.

**Claim 10**
A method for the spatial and temporal control of the expression of a genetically engineered gene of interest operably linked to a heat shock promoter in host cells within a preselected region of a mammal, consisting: selectively heating the region to non-lethal supraphysiological temperatures for a period of time by applying electromagnetic radiation to the region, thereby spatially and temporally controlling the expression of the gene of interest.

**Claim 18**
A method of treating mammal, the method comprising: introducing a genetically engineered gene operably linked to a heat shock promoter into host cells of a region of the mammal; selectively heating the region to non–lethal supraphysiological temperatures by applying electromagnetic radiation for a period of time to the region, thereby spatially and temporally controlling the expression of the gene and amount of the protein produced in the region.
C. The General Hospital Corporation's patents

The General Hospital Corporation has granted patents in Canada and Australia and filed an application in Europe. However, this patent family was obviously abandoned.

The disclosed inventions are mainly related to:

- A method for the production of a polypeptide or protein whose structural gene is under the control of an inducible heat shock promoter.

  The structural gene and the heat inducible promoter are transformed in a host cell, which is not limited to a particular host organism.

  A gene amplification system under the control of a constitutive promoter is used to increase the copy number of the structural gene.

- A method for increasing the production of a protein in a dihydrofolate reductase deficient mammalian cell. The structural gene of the protein of interest is placed under the control of a heat shock promoter and a dihydrofolate reductase gene is under the control of a constitutive promoter.

- A host cell co-transformed with a gene amplification system as mentioned before and a structural gene driven by an inducible heat shock promoter.

Dihydrofolate reductase is a small enzyme that plays a supporting role in the building of DNA and other processes. It manages the concentration of folate, an organic molecule that shuttles carbon atoms to enzymes that need them in their reactions. Dihydrofolate reductase recycles folate after the release of its carbon atoms.

The dihydrofolate reductase gene is used by the inventors as a gene amplification system. Dihydrofolate reductase deficient mammalian cells transformed with this gene are able to grow in the presence of methotrexate, which is an inhibitor of dihydrofolate reductase. Growing in increasing concentrations of methotrexate leads the cells to overproduce dihydrofolate reductase. If a structural gene controlled by an inducible heat shock promoter is co-transformed with the dihydrofolate reductase gene system, both genes are amplified together under the selection pressure.

<table>
<thead>
<tr>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CA 1324097</strong></td>
<td>General Hospital Corporation</td>
</tr>
<tr>
<td>Date</td>
<td>Title</td>
</tr>
<tr>
<td>Earliest priority</td>
<td>Inducible heat shock and amplification system</td>
</tr>
<tr>
<td>– 6 February 1986</td>
<td></td>
</tr>
<tr>
<td>Filed</td>
<td></td>
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<tr>
<td>– 4 February 1987</td>
<td></td>
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<tr>
<td>Granted</td>
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<tr>
<td>– 9 November 1993</td>
<td></td>
</tr>
<tr>
<td>Lapsed</td>
<td></td>
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<tr>
<td>– 11 May 1996</td>
<td></td>
</tr>
<tr>
<td><strong>Claim 1</strong></td>
<td></td>
</tr>
<tr>
<td>A method for high level production of a polypeptide or protein in a transformed host cell comprising:</td>
<td></td>
</tr>
<tr>
<td>(a) transforming a host cell with a structural gene encoding for a polypeptide or protein, under the control of an inducible heat shock promoter;</td>
<td></td>
</tr>
<tr>
<td>(b) amplifying the copy number of said structural gene in said transformed host cells using an amplification system under the control of a promoter other than an inducible heat shock promoter;</td>
<td></td>
</tr>
<tr>
<td>(c) inducing said inducible promoter by heat shock to said transformed host cells at a temperature and for a time sufficient to transcribe said structural gene;</td>
<td></td>
</tr>
<tr>
<td>(d) allowing said heat shocked cells to recover at a lower temperature than said heat shock temperature and for a time sufficient to translate said transcribed structural gene, producing said polypeptide or protein.</td>
<td></td>
</tr>
<tr>
<td><strong>Claim 10</strong></td>
<td></td>
</tr>
<tr>
<td>A method for increasing the production of a structural polypeptide or protein in a transformed mammalian cell</td>
<td></td>
</tr>
</tbody>
</table>
comprising the steps of:
(a) culturing a dihydrofolate reductase (DHFR) deficient mammalian cell transformed with
  (i) a DHFR gene under the control of a constitutive promoter. and
  (ii) a structural gene encoding for a polypeptide or protein under the control of an inducible heat shock promoter, in a culture medium containing methotrexate, for a time sufficient to permit growth of said transformed mammalian cells;
(b) inducing said heat shock promoter at a temperature and for a time sufficient to transcribe said structural gene; and
(c) allowing said heat shocked cells to recover from said heat shock at a lower temperature and for a time sufficient to translate said transcribed structural gene, producing said polypeptide or protein.

Claim 17
A host cell cotransformed with:
(a) a gene amplification system under the control of a constitutive promoter, and
(b) a structural gene encoding for a polypeptide or protein under control of an inducible heat shock promoter.

Remarks

2. Cold-inducible promoters
Promoters responsive to low or cold temperatures (equal to or below 10°C) are the subject matter of patents and patent applications filed by the Japanese companies Takara Shuzo Co., Ltd and Japan Tobacco Inc., The University of Quebec in Montreal and Danisco.

Most of the cited institutes claim in their patents a particular "cold" promoter and its variant sequences.

A. Takara Shuzo Co., Ltd's patents and patent applications
The United States granted patents and the European and Canadian patent applications relate to a vector useful for the expression of a protein under low temperature conditions. The vector of the invention contains:

1. a promoter regulatory region; and
2. a 5'-untranslated region derived from a cold-shock protein gene.

The promoter region and 5' untranslated region of the vector are not limited to a particular promoter. In addition a promoter and a 5'-untranslated region are derived from the cold-shock protein (csp) A of *Escherichia coli*. The nucleotide sequence of such promoter is also claimed by the inventors.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6479260</td>
<td>Title – Low-temperature inducible expression vector</td>
<td>Takara Shuzo Co., Ltd (now Takara Holdings Inc.)</td>
</tr>
</tbody>
</table>
|               | Claim 1
|               | A vector which is characterized in containing each of the following elements:
<p>|               | (1) a promoter which shows its action in the host to be used; |</p>
<table>
<thead>
<tr>
<th>Claim 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>An isolated promoter consisting of a base sequence as shown in <strong>SEQ ID NO: 5</strong> in the Sequence Listing.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>An isolated promoter containing a base sequence as shown in <strong>SEQ ID NO: 5</strong> in the Sequence Listing and consisting of a base sequence having 135 or less bases, wherein the promoter does not contain the region which is ascribed to mRNA.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Title – Low-temperature inducible expression vector</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Claim 1</strong></td>
</tr>
<tr>
<td>A vector which is characterized in containing each of the following elements:</td>
</tr>
<tr>
<td>(1) a promoter which is derived from cold-shock protein gene and which shows its action in the host to be used;</td>
</tr>
<tr>
<td>(2) regulatory region for regulating the action of the promoter of (1), wherein said regulatory region is located downstream of the promoter of (1); and</td>
</tr>
<tr>
<td>(3) a region which codes for the 5'-untranslated region derived from cold-shock protein gene mRNA or a region which codes for the region where substitution, deletion, insertion, or addition of at least one base is applied to said untranslated region.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A method for the expression of the desired protein which is characterized in containing the following steps:</td>
</tr>
<tr>
<td>1) a step where a host is transformed with a vector containing:</td>
</tr>
<tr>
<td>a) a promoter which is derived from cold-shock protein gene and which shows its action in the host to be used;</td>
</tr>
<tr>
<td>b) a regulatory region for regulating the action of the promoter of a), wherein said regulatory region; and</td>
</tr>
<tr>
<td>c) a region which codes for the 5'-untranslated region derived from cold-shock protein gene mRNA or a region which codes for the region where substitution, deletion, insertion, or addition of at least one base is applied to the untranslated region wherein a gene coding for the desired protein is integrated;</td>
</tr>
<tr>
<td>2) a step where the resulting transformant is incubated; and</td>
</tr>
<tr>
<td>3) a step where action of promoter is induced via a function of a regulatory region and, at the same time, incubating temperature is made lower than the ordinary temperature to express the desired protein.</td>
</tr>
</tbody>
</table>

This patent is a Continuation of **US 6479260**.
elements:
(1) a promoter which shows its action in the host to be used;
(2) regulatory region for regulating the action of the promoter of (1);
and
(3) a region which codes for the 5'-untranslated region derived from
cold-shock protein gene mRNA or a region which codes for the region
where substitution, deletion, insertion or addition of at least one base
is applied to the said untranslated region.

**Preferred claim 14**

A promoter containing a base sequence as shown in SEQ ID NO:5 in
the Sequence Listing and consisting of a base sequence having 135 or
less bases.

### CA 2309600 AA

**Title** – Cold-inducible expression vector

The claims of this Canadian patent application are the same as EP
1033408 (above).

**Remarks**
The related Australian application AU 10546/99 lapsed on August 3,
2000.

Note: Patent information was last updated on 10 May 2006.

Search terms: "promoter" in abstract and "Takara Shuzo" in applicant.


### B. Patents on particular cold-temperature promoters

As mentioned above, the patents filed by most institutes relate to a specific low temperature–responsive
promoter.

The following table summarizes some bibliographic data of the patents and patent applications filed by the
cited entities and a brief description of the promoter claimed.

<table>
<thead>
<tr>
<th>Patent number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>US 6084089</strong></td>
<td>Title – Cold-inducible promoter sequences</td>
<td>Japan Tobacco</td>
</tr>
<tr>
<td></td>
<td><strong>Claim 1</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A DNA sequence comprising a nucleotide sequence from the first to the 3546th nucleotide in the nucleotide sequence shown in SEQ ID NO:1.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Claim 2</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A DNA sequence comprising a nucleotide sequence from the 2418th to the 3541st nucleotide in the nucleotide sequence shown in SEQ ID NO:1.</td>
<td></td>
</tr>
</tbody>
</table>
Claim 3
A DNA sequence comprising a nucleotide sequence from the first to the 4120th nucleotide in the nucleotide sequence shown in SEQ ID NO:2.

DNA sequences of a promoter isolated from potato. Partial and modified sequences of the promoter with cold-inducible promoter activity are also claimed.

According to the inventors, "cold-inducible" promoter means induced at a temperature not higher than 6°C and expression of the gene controlled by such promoter maintained for not less than 5 months.

Claim 1
A DNA sequence having a nucleotide sequence from first to 3546th nucleotide in the nucleotide sequence shown in SEQ ID NO:1, or a part thereof having a cold-inducible promoter activity, or a DNA sequence having the same nucleotide sequence as said DNA sequences except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted or added, which DNA sequence has a cold-inducible promoter activity.

Claim 3
A DNA sequence having a nucleotide sequence from 2418th to 3541st nucleotide in the nucleotide sequence shown in SEQ ID NO:1, or a part thereof having a cold-inducible promoter activity, or a DNA sequence having the same nucleotide sequence as the said DNA sequences except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted or added, which DNA sequence has a cold-inducible promoter activity.

Claim 4
A cold-inducible promoter sequence having a nucleotide sequence from 2418th to 3541st nucleotide in the nucleotide sequence shown in SEQ ID NO:1, or a DNA sequence having the same nucleotide sequence as said DNA sequence except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted or added, which DNA sequence has a cold-inducible promoter activity.

Claim 5
A DNA sequence having a nucleotide sequence from first to 4120th nucleotide in the nucleotide sequence shown in SEQ ID NO:2, or a part thereof having a cold-inducible promoter activity, or a DNA sequence having the same nucleotide sequence as the said DNA sequences except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted or added, which DNA sequence has a cold-inducible promoter activity.

Claim 7
A probe comprising a DNA fragment having at least 18 consecutive nucleotides in the region from 45th to 839th nucleotide in the sequence shown in SEQ ID NO:3 in the Sequence Listing or a sequence complementary thereto.

Title – Cold-inducible promoter sequences
Claim 1
A DNA sequence having a nucleotide sequence from first to 3546th nucleotide in the nucleotide sequence shown in SEQ ID. No. 1, or a part thereof having a cold-inducible promoter activity.

Claim 2
A DNA sequence having a nucleotide sequence from 2418th to 3541st nucleotide in the nucleotide sequence shown in SEQ ID. No. 1, or a part thereof having a cold-inducible promoter activity.

Claim 3
A cold-inducible promoter sequence having a nucleotide sequence from 2418th to 3541st nucleotide in the nucleotide sequence shown in SEQ ID. No. 1.

Claim 4
A DNA sequence having a nucleotide sequence from first to 4120th nucleotide in the nucleotide sequence shown in SEQ ID. No. 2, or a part thereof having a cold-inducible promoter activity.

Claim 5
Use of a DNA fragment having at least 15 consecutive nucleotides in the region from 45th to 839th nucleotide in the sequence shown in SEQ ID. No. 3 in the Sequence Listing or a sequence complementary thereto, for the preparation of a probe for screening cold-inducible promoters.

Remarks
The related patents were also granted in Japan (JP 3469902), China (CN 96192162.5) and Canada (CA 2213991).

**US 6184443**

Title – Cold-inducible promoter and tuber-specific promoter sequence from potato alpha amylase gene

Claim 1
An isolated alpha-amylase promoter having cold-sensitive promoter activity, having a sequence comprising the 5.5 Kb EcoRI DNA fragment of *Solanum tuberosum* from the transformed E. coli strain, DH5alpha-gPAm 351 (NCIMB Accession Number 40682).

Claim 12
An isolated alpha-amylase promoter having tuber-specific activity and having a sequence comprising SEQ ID NO: 1.

DNA sequences (variant, homologue and fragments) of an isolated alpha-amylase promoter from potato (*Solanum tuberosum*) having cold-sensitive activity. The promoter has tuber tissue specific activity.

Remarks
Related patent applications in Europe (EP 787194 A1) and Canada (CA 2202896 AA) were withdrawn and Lapsed, respectively. Related Australian (AU 27881/95) application has also lapsed.

**US 5847102**

Title – Cold-induced promoter from winter *Brassica napus*

Claim 1
An isolated DNA molecule comprising a 5' regulatory region of a low temperature-responsive gene BN115 from *Brassica napus*, comprising nucleotides 961–1210 of SEQ ID NO: 1.
Claim 7
An isolated cold inducible promoter, or a functional fragment thereof capable of regulating the expression of a gene in response to a change in temperature, said cold inducible promoter comprising nucleotides 1–1271 of SEQ ID NO:1.

Claim 8
An isolated DNA molecule comprising a sequence of at least 15 contiguous nucleotides of a 5' regulatory region of a low temperature responsive gene BN115 from Brassica napus, as defined by nucleotides 1–1271 of SEQ ID NO:1.

Claim 13
An isolated enhancer, or a functional fragment thereof each capable of mediating the expression of a gene under the control of a promoter and said enhancer or fragment, said enhancer obtained from the regulatory region of BN115 from Brassica napus.

Claim 16
An isolated negative regulatory element capable of repressing gene expression at 22° C., obtained from the regulatory region of BN115 from Brassica napus and comprising nucleotides 461–623 of SEQ ID NO:1.

Isolated DNA sequences comprising a promoter and 5' regulatory region of the low temperature responsive gene BN115 from Brassica napus. Sequences that hybridize to the promoter sequence and exhibit low temperature activity are also part of the claimed invention. An enhancer and a negative regulatory element present in the regulatory region are also claimed.

Title – Cold-induced promoter from winter Brassica napus
The claims are the same as US 5847102.
individual claims of this patent are discussed in detail below.

The other patents presented in this section relate to light-regulated promoters isolated from genes of specific organisms. The University of Warwick in UK, Suntory LTD in Japan and Mycogen Plant Sciences in the USA have filed patents on the use of promoters whose expression is induced by light, such as a promoter isolated from myxobacterium and promoters whose expression is inhibited by light exposure, such as a promoter isolated from a pea gene.

The following tables contains some bibliographic information about the patents and patent applications filed by the above mentioned entities and a brief summary of the most important aspects of the protected invention in the case of granted patents and of the claims as filed for patent applications.

The patents are classified according to whether the promoters are Light-inducible or Light-repressible.

A. Light-inducible promoters

The United States patent US 5750385 assigned to Calgene Inc, does not specifically claim a single, individual light-inducible promoter. Rather, the patent claims the use of a promoter activated by light in order to control expression of a gene introduced into plants by transformation. In at least some cases, the phenotype of the tissue expressing the gene is modified.

The specification of the patent discloses promoters such as fruit-specific and seed specific. Related patents (US 5420034, US 5753475 and US 6281410) have claims directed to these aspects. The claims in the instant patent are directed only to induced gene expression or modification of the phenotypic property in transformed plants as a result of light induced processes in chloroplasts.

The patent specification makes particular reference to the promoter from the soybean SSU gene (small subunit SSU) of ribulose–1,5–bisphosphate–carboxylase, although the claims are not so limited. Through the use of the soybean SSU promoter, the expression of the gene under its control can be light–induced. Thus in the presence of light, its expression is increased, while its expression is substantially reduced in the absence of light. Moreover, the vector construct may also include enhancers, operators, activators, or other regions involved with transcriptional regulation.

The patent claims methods for obtaining a transformed plant with a modified phenotype or for altering the phenotype of transformed plant tissues containing chloroplasts using a vector construct comprising:

- a promoter region from a gene that is light-inducible in a chloroplast-containing tissue,
- DNA sequence of interest, and
- a transcription termination region.

In dependent claims, the promoter is from a soybean SSU gene, and the plant is soybean, tomato, or rapeseed. Because the claims do not limit the source of the light-inducible promoter, the Calgene assigned patent has the broadest scope in the patent landscape surrounding light-inducible promoters.

Calgene Inc. was bought by Monsanto in 1996 and therefore its patents are probably now controlled by Monsanto (http://www.monsanto.com/monsanto/layout/about_us/contactus.asp)

Specific Patent Information

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5750385</td>
<td>Title – Methods and compositions for regulated transcription and expression of heterologous genes.</td>
<td>Calgene Inc.</td>
</tr>
<tr>
<td></td>
<td>Claim 1</td>
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<tr>
<td></td>
<td>A method for obtaining a plant having a modified phenotype, said method comprising:</td>
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<td></td>
<td>transforming a host plant cell with a DNA construct under genomic integration conditions, wherein said construct comprises as operably linked components in the direction of transcription,</td>
<td></td>
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</tbody>
</table>
a promoter region obtainable from a gene, wherein transcription of said gene is light-inducible in a plant chloroplast containing tissue, a DNA sequence of interest other than the native coding sequence of said gene, and a transcription termination region, wherein said components are functional in a plant cell, whereby said DNA construct becomes integrated into a genome of said plant cell;

regenerating a plant from said transformed plant cell, and
growing said plant under conditions whereby said DNA sequence of interest is expressed and a plant having said modified phenotype is obtained.

Claim 2

A method for altering the phenotype of chloroplast containing plant tissue as distinct from other plant tissue, said method comprising:
growing a plant, wherein said plant comprises cells containing a DNA construct integrated into their genome, said DNA construct comprising, in the 5' to 3' direction of transcription,

a transcriptional initiation region from a gene, wherein transcription of said gene is light-inducible in a plant chloroplast containing tissue, a DNA sequence of interest other than the coding sequence native to said transcriptional initiation region, and a transcriptional termination region, whereby said DNA sequence of interest is transcribed under transcriptional control of said transcriptional initiation region and a plant having an altered phenotype is obtained.

The claims are generally directed to methods for transforming plants for a modified phenotype using a DNA construct. The construct comprises a promoter that provides for light-inducible transcription in either a specific plant tissue or a plant part. Transcription may be induced during a particular stage of growth or in response to an external stimulus.


**University of Warwick** had granted patents in Europe (EP 310619) and Canada (CA 1321563) regarding a light inducible promoter. The claims are generally related to a DNA sequence comprising a promoter isolated from myxobacterium. The promoter is linked to a sequence further containing a restriction site for the insertion of a desired sequence. A method of producing a polypeptide under the control of the myxobacterium promoter is also claimed but limited to a transformed bacterial host.

However, the fact that both the European and the Canadian patents lapsed and the related Australian patent application (AU 75421/87) also lapsed suggested that this patent family had been abandoned.

**B. Light-repressible promoters**

**Suntory Ltd.** ([http://www.suntory.com/group/suntory_ltd.html](http://www.suntory.com/group/suntory_ltd.html)) has patents granted in Australia and New Zealand and pending applications in Europe and Canada. This patent family mainly claims for a promoter of the *pra2* gene from pea. **Mycogen Plant Sciences** ([http://www.dowagro.com/mycogen/contact/index.htm](http://www.dowagro.com/mycogen/contact/index.htm)) has two granted United States patents regarding methods for the expression and enhancing the level of expression of a structural gene in plants by using a dark- and light-active maize Cab promoter/regulatory system.

**Specific Patent Information**

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
</table>

114 of 191
Title – Photoinhibitory promoter

Claim 1
A DNA fragment containing the sequence of SEQ ID NO: 1 as a core sequence, whereby expression of a gene placed downstream of said DNA fragment is repressed in the presence of light.

Claim 4
A promoter containing the sequence of SEQ ID NO: 1 as a core sequence, whereby expression of a gene placed downstream of said promoter is promoted in the dark but repressed in the presence of light.

The claims are drawn to a promoter sequence or a fragment of the promoter sequence whose ability of controlling the expression of a gene placed downstream is promoted in the dark but repressed by light.

The promoter was isolated from a small G protein gene pra2 from pea. The pra2 gene was thought to be involved in the elongation of stems, at the epicotyl, during germination in the dark.

Title – Light-repressible promoters

The claims are exactly the same as AU 765413.

Title – Dark and light regulated chlorophyll A/B binding protein promoter–regulatory system

Claim 1
A method for the expression of a structural gene in a plant cell under conditions of darkness comprising the steps of:

a. transforming said plant cell with a recombinant molecule comprising a plant, dark- and light-active maize Cab promoter/regulatory system, wherein said promoter/regulatory system is the promoter/regulatory system of Cab AB1084 or cross-hybridizes with the promoter/regulatory system of Cab AB1084 under stringent conditions, and a heterologous plant-expressible structural gene that is under the regulatory control of said plant Cab promoter/regulatory system in said plant cell, and

b. maintaining conditions of darkness for expression of said structural gene in said transformed plant cell.

Claim 8
A method for enhancing the level of expression obtained in the dark of a plant-expressible gene in a plant cell by exposing said plant cell to conditions of illumination comprising the steps of:
a. transforming said plant cell with a recombinant molecule comprising a plant, dark- and light-active maize Cab promoter/regulatory system, wherein said promoter/regulatory system is the promoter/regulatory system of Cab AB1084 or cross-hybridizes with the promoter/regulatory system of Cab AB1084 under stringent conditions, and a heterologous plant-expressible structural gene that is under the regulatory control of said plant Cab promoter/regulatory system in said plant cell, and

b. maintaining conditions of darkness for expression of said structural gene in said transformed plant cell, and

c. applying conditions of illumination to said transformed plant cell such that enhancement by a factor of about 3- to 6-fold in expression of said structural gene is obtained.

Claim 15

A plant grown from a plant cell, wherein said plant cell is transformed with a recombinant molecule comprising a plant, dark- and light-active maize Cab promoter/regulatory system, wherein said promoter/regulatory system is the promoter/regulatory system of Cab AB1084 or cross-hybridizes with the promoter/regulatory system of Cab AB1084 under stringent conditions, and a heterologous plant-expressible structural gene that is under the regulatory control of said plant Cab promoter/regulatory system in said plant cell.

This patent mainly claims methods for the expression of a structural gene in a plant under dark conditions by placing the gene under the control of a dark and light-active maize chlorophyll a/b (Cab) promoter/regulatory system. The promoter/regulatory system used is either the Cab AB1084 or cross-hybridizes under stringent conditions. The expression of the gene under control of the system is further enhanced by a subsequent period of light. A plant grown from a plant cell transformed with such promoter/regulatory system is also part of the claimed invention.

**US 5656496**

- Earliest priority – 5 January 1989
- Filed – 5 June 1995
- granted 12 August 1997
- Expected expiry – 12 August 2014

**Title** – Dark and light regulated chlorophyll A/B binding protein promoter–regulatory system

**Claim 1**

A recombinant DNA molecule comprising:

(a) a dark- and light-active Cab promoter/regulatory system which cross-hybridizes with the Cab AB1084 promoter/regulatory system under stringent conditions, and wherein said promoter/regulatory system

(i) functions to direct the expression of a structural gene under its control during conditions of darkness and

(ii) is stimulated to direct enhanced expression of said structural gene under light conditions, and

(b) a heterologous plant–expressible structural gene wherein said structural gene is placed under the regulatory control of said plant promoter/regulatory system.

This US patent is a division of US 5639952. It is directed to a dark and light–active Cab promoter/regulatory system, which cross–hybridizes with the Cab AB1084. The promoter system operates the same as described in US 5639952.

Note: Patent information was last updated on 12 May 2006.

Search terms for Suntory patents: "promoter" in title or abstract and "Suntory" in applicant. Patent database: esp@cenet worldwide.
Chapter 4

Tissue-specific promoters

Summary

As mentioned in the Introduction, there are promoters controlling gene expression in a tissue–dependent manner and according to the developmental stage of the plant. The transgenes driven by these type of promoters will only be expressed in tissues where the transgene product is desired, leaving the rest of the tissues in the plant unmodified by transgene expression. Tissue-specific promoters may be induced by endogenous or exogenous factors, so they can be classified as inducible promoters as well.

Unlike constitutive expression of genes, tissue-specific expression is the result of several interacting levels of gene regulation. As such, it is then preferable to use promoters from homologous or closely related plant species to achieve efficient and reliable expression of transgenes in particular tissues. This is one of the main reasons for the large amount of tissue-specific promoters isolated from particular plants and tissues found in both scientific and patent literature. Patents claiming particular tissue promoters such as beta-amylase gene or barley hordein gene promoters (for seed gene expression), tomato pz7 and pz130 gene promoters (for ovary gene expression), tobacco RD2 gene promoter (for root gene expression), banana TRX promoter and melon actin promoter (for fruit gene expression) and so forth, are the most abundant in the patent literature.

The number of promoters “tailor-made” (isolated and identified) for the expression of transgenes in particular tissues and plants is in the hundreds. Very few patents are drawn to tissue-specific promoters (e.g. seed-specific or a root-specific promoter) in general terms.

The purpose of this section is to present those patents directed to plant tissue-specific promoters in broad terms. Patents related to particular promoters derived from specific genes and plants are beyond the scope of this paper.

The analysed patents are categorised according to tissue where the promoter controls the transcription of a gene of interest. These are:

- **Root promoters**: Pioneer Hi-Bred has filed several patent applications directed to root promoters that enhance or suppress the expression of a linked gene in root cells. In addition, the invention comprises methods for the identification and isolation of plant tissue-specific promoters in general.

- **Fruit promoters**: Calgene's large portfolio of patents on tissue-specific promoters includes fruit specific promoters that control the expression of genes in mature ovary tissue of a fruit and in the receptacle tissue of accessory fruits such as strawberry, apple and pear. The genes driven by the promoters of Calgene's inventions influence fruit development and ripening.

- **Seed promoters**: Calgene, Sapporo Breweries and the University of California have granted patents and patent applications drawn to seed-specific promoters in broad terms. Transcription cassettes having a seed-specific promoter and recombinant molecules containing a seed-maturation promoter are part of their inventions.

### Root Promoters

**Summary**

Pioneer Hi-Bred had filed patent applications related to plant promoters containing elements that drive the expression of genes of interest in root cells and tissues.

The claims as filed of the patent applications also describe very broad methods for the isolation and characterization of tissue-specific plant promoters in general. If the claims as filed are granted without change they would potentially cover processes for the identification of tissue-specific promoters regardless of the tissue where the expression of nucleotide sequences is sought.
Pioneer Hi-Bred has now obviously abandoned this patent, although it is still pending in Australia.

The Invention

Pioneer Hi-Bred’s patent applications in Australia, the U.S. and Europe cover the following aspects:

- Methods for identifying and isolating tissue-preferred plant promoter elements in general.
- The elements are not restricted to any particular tissue in the claims as filed.
- Plant promoters containing root-preferred promoter elements that enhance or suppress the expression of a linked sequence in root cells.
- Specific sequences of root-promoter elements are spelled out in the claims as filed. Some plant promoters of the invention have multiple root-preferred promoter elements.
- Methods for root-preferred expression of genes in plants by transforming a plant with an expression cassette having a promoter with elements for root expression; and
- Plant cells stably transformed with DNA construct containing root-preferred expression elements.

The term "root-preferred" means that the expression driven by a plant promoter of the invention is selectively enhanced or suppressed in roots in comparison to other tissues. Root cells and tissues include any part of the roots, and cover primary, lateral and adventitious roots.

Plants to be transformed with the constructs of the invention are not limited to any in particular in the claims as filed.

[Do we still need to keep this page like it is because this patent family is obviously aband oned ]

<table>
<thead>
<tr>
<th>Patent No</th>
<th>Title, Independent Claims and Summary</th>
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<tbody>
<tr>
<td>AU 2001/32896</td>
<td>Novel root-preferred promoter elements and method of use</td>
<td>Pioneer Hi-Bred</td>
</tr>
</tbody>
</table>

Title – Novel root-preferred promoter elements and method of use

Claim 1

A plant promoter comprising at least one tissue-preferred plant promoter element, said element identified by:

a) providing a first mixture of oligonucleotides each comprising a 5' flanking sequence, a central random sequence, and a 3' flanking sequence;

b) contacting said first mixture with a second mixture comprising nuclear proteins from a preferred plant tissue under binding conditions promoting complex formation between said oligonucleotides and said proteins;

c) separating said formed complexes electrophoretically;

d) isolating said separated complexes in ranges of electrophoretic mobility;

b) amplifying oligonucleotides of said isolated complexes by polymerase chain reaction utilizing primers to said flanking sequences;

f) providing said amplified oligonucleotides from step e) as the first mixture for a repetition of step a);

g) performing at least a second cycle of steps b–e with said provided oligonucleotides of step f);

h) assessing for a particular range of electrophoretic mobility and quantity of complex formation in progressive cycles of step g);

i) isolating oligonucleotides of a particular range of electrophoretic mobility wherein said range has increased complex formation in step h);

j) operably linking individual oligonucleotides of step i) to a promoter that drives expression in a plant cell, said promoter operably linked to a coding sequence in an expression cassette;

k) assessing tissue-preferred expression of said coding sequence; and
<table>
<thead>
<tr>
<th>Claim</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>A plant promoter comprising at least one root-preferred plant promoter element comprising a nucleotide sequence selected from the group consisting of: &lt;br&gt; a) a nucleotide sequence of SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.3, SEQ ID NO.4, SEQ ID NO.5, SEQ ID NO.6, SEQ ID NO.7, or SEQ ID NO.8; &lt;br&gt; b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a); and &lt;br&gt; c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a), wherein said contiguous nucleotides maintain function of the nucleotide sequence of a).</td>
</tr>
<tr>
<td>10</td>
<td>A plant promoter comprising at least one multimeric root-preferred promoter element comprising at least two root-preferred promoter elements further comprising a nucleotide sequence selected from the group consisting of: &lt;br&gt; a) a nucleotide sequence of SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.3, SEQ ID NO.4, SEQ ID NO.5, SEQ ID NO.6, SEQ ID NO.7, or SEQ ID NO.8; &lt;br&gt; b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a); and &lt;br&gt; c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a), wherein said contiguous nucleotides maintain function of the nucleotide sequence of a).</td>
</tr>
<tr>
<td>11</td>
<td>A plant promoter comprising at least one root-preferred plant promoter element that enhances expression of a coding sequence operably linked to said promoter, wherein said element comprises a nucleotide sequence selected from the group consisting of: &lt;br&gt; a) a nucleotide sequence of SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.3, SEQ ID NO.4, SEQ ID NO.5, SEQ ID NO.6, SEQ ID NO.7, or SEQ ID NO.8; &lt;br&gt; b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a); and &lt;br&gt; c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a), wherein said contiguous nucleotides maintain function of the nucleotide sequence of a).</td>
</tr>
<tr>
<td>12</td>
<td>A plant promoter comprising at least one root-preferred plant promoter element that suppresses expression of a coding sequence operably linked to said promoter, wherein said element comprises a nucleotide sequence selected from the group consisting of: &lt;br&gt; a) a nucleotide sequence of SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.3, SEQ ID NO.4, SEQ ID NO.5, SEQ ID NO.6, SEQ ID NO.7, or SEQ ID NO.8; &lt;br&gt; b) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a); and &lt;br&gt; c) a nucleotide sequence comprising at least 7 contiguous nucleotides of a sequence of a), wherein said contiguous nucleotides maintain function of the nucleotide sequence of a).</td>
</tr>
<tr>
<td>13</td>
<td>A transformed plant, or its parts, having stably incorporated into its genome a DNA construct comprising a plant promoter operably linked to a coding sequence, said plant promoter comprising at least one synthetic root-preferred plant promoter element.</td>
</tr>
</tbody>
</table>
Claim 19

A transformed plant cell, said plant cell having stably incorporated into its genome a DNA construct comprising a plant promoter operably linked to a coding sequence, said plant promoter comprising at least one synthetic root-preferred plant promoter element.

Claim 20

A method for root-preferred expression of a nucleotide coding sequence in a plant, said method comprising transforming a plant cell with a transformation vector comprising an expression cassette, said expression cassette comprising a plant promoter operably linked to said nucleotide coding sequence, said plant promoter comprising at least one synthetic root-preferred plant promoter element.

Claim 22

A method for identifying and isolating tissue-preferred promoter elements, said method comprising the steps of:
a) providing a first mixture of oligonucleotides each comprising a 5' flanking sequence, a central random sequence, and a 3' flanking sequence;
b) contacting said first mixture with a second mixture comprising nuclear proteins from a preferred plant tissue under binding conditions promoting complex formation between said oligonucleotides and said proteins;
c) separating said formed complexes electrophoretically;
d) isolating said separated complexes in ranges of electrophoretic mobility;
e) amplifying oligonucleotides of said isolated complexes by polymerase chain reaction utilizing primers to said flanking sequences;
f) providing said amplified oligonucleotides from step e) as the first mixture for a repetition of step a);
g) performing at least a second cycle of steps b–e with said provided oligonucleotides of step f);
h) assessing for a particular range of electrophoretic mobility and quantity of complex formation in progressive cycles of step g);
i) isolating by cloning, individual oligonucleotides of a particular range of electrophoretic mobility wherein said range has increased complex formation in step h);
j) simultaneous with step i) or as an individual step, operably linking isolated individual oligonucleotides of step i) to a promoter that drives expression in a plant cell, said promoter operably linked to a coding sequence in an expression cassette;
k) assessing tissue-preferred expression of said coding sequence; and
l) determining sequence of an oligonucleotide having tissue preferred expression in step k).

Remarks

Related application in the United States (US 2001/047525 A1) has been expressly abandoned. Applications in Europe (EP 1248850 A2) and Canada (CA 2390819) are also lapsed.

Fruit Promoters

Summary

Among the large patent portfolio of Calgene on tissue–specific promoters, there are three main patent families containing granted patents directed to fruit–specific regulatory regions. Patents and patent applications that were assigned to Calgene may now be held by Monsanto, to which any inquiries about licensing should be directed (http://www.monsanto.com/monsanto/layout/about_us/contactus.asp).
Patents of one of the patent families are drawn to DNA constructs containing promoters "preferentially expressed in fruit tissues". Although the basic patents in the patent family are involved in the expression of genes regulating fruit ripening and drive the expression of genes of interest in mature ovaries, the definitions are broad, and examples in which seed-specific expression is cited suggest that tissues such as seed, fruit integument, cotton fibers and so on would be construed as "fruit tissues". In addition, general methods to regulate the fruit phenotype are part of the patented inventions.

In the other two patent families, the definitions are more specific. One relates primarily to cotton fiber production, although it contains some broader claims. In the second, the promoters of the invention are expressed in receptacle tissue, a flower part that makes most of the fleshy tissue in accessory fruits such as strawberry, apple and pear. The genes driven by the promoter influence fruit development, maturation and ripening. Some analysis of this patent family is provided below.

**Broadest patent family**

The claims of the United States patent are drawn to methods for altering the phenotype of the fruit tissue of a plant transformed with DNA constructs comprising:

- a promoter preferentially expressed in a fruit tissue (note that this does not mean "fruit-specific");
- a DNA sequence of interest different from the native gene of the promoter (note that this need not be a "gene"—could be antisense or RNAi); and
- a transcriptional termination region.

Unlike the European patent, the genes from which the fruit-specific promoters are obtained are not limited to anthesis process or fruit maturation and ripening. The promoters are from genes preferentially transcribed in fruit tissue. Because there is no limitation in the fruit gene promoter used in the DNA construct to alter the phenotype of a fruit, the invention claimed in the United States patent, although directed to methods, is broader in scope than in the Canadian and European patents. Also, the claimed methods are described in very broad terms.

Calgene's European patent claims:

- A DNA construct comprising a fruit transcriptional initiation region from a gene that becomes active during anthesis (period during which a flower is fully open and functional), remains active until the ripe period and is transcribed in mature ovary tissue. The construct includes a gene of interest under the control of such transcriptional region and a transcriptional termination region.
- A DNA construct having a fruit promoter of a plant storage protein that becomes and remains active during the stages described above.
- A method for the modification of the phenotype of a tomato fruit. The sort of phenotypic modification is not specified in the claim.
- A tomato cell is transformed with a DNA construct as described above. A tomato plant is regenerated and grown from the transformed cell.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 4943674</td>
<td>Title – Fruit specific transcriptional factors</td>
<td>Calgene Inc.</td>
</tr>
<tr>
<td></td>
<td>Claim 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A DNA construct <strong>comprising</strong> in the direction of transcription,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a tomato 2All transcriptional initiation region joined to a DNA sequence of interest, wherein said DNA sequence of interest is other than the wild-type sequence and is under the transcriptional regulation of said 2All initiation region and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a transcriptional termination region.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A DNA construct <strong>comprising</strong> in the direction of transcription,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a tomato 2All transcriptional initiation region joined to a DNA sequence of interest, wherein said DNA sequence is other than the wild-type</td>
<td></td>
</tr>
</tbody>
</table>
sequence and **comprises** a unique restriction site for insertion of a second DNA sequence of interest to be under the transcriptional regulation of said 2All initiation region, and

- a transcriptional termination region.

**Claim 9**

A method for modifying the phenotype of tomato fruit said method **comprising**:

- transforming a tomato plant cell with a DNA construct under genomic integration conditions, wherein said DNA construct **comprises** in the direction of transcription,
  - a tomato 2All transcriptional initiation region jointed to a DNA sequence other than the wild-type sequence and capable of modifying the phenotype of fruit cells upon transcription, wherein said DNA sequence is under the transcriptional regulation of said initiation region and
  - a transcriptional termination region, whereby said DNA construct becomes integrated into the genome of said tomato plant cell;
- regenerating a plant from said transformed tomato plant cell; and
- growing said plant to produce tomato fruit of the modified phenotype.

**Claim 12**

Tomato fruit **comprising** a DNA construct **comprising** in the direction of transcription,

- a tomato 2All transcriptional initiation region jointed to a DNA sequence of interest, wherein said DNA sequence is other than the wild-type sequence and is under the transcriptional regulation of said 2All initiation region, and
- a transcriptional termination region.

**Claim 13**

A tomato plant **comprising**: a DNA construct **comprising** in the direction of transcription,

- a tomato 2All transcriptional initiation region jointed to a DNA sequence of interest, wherein said DNA sequence is other than the wild-type sequence and is under the transcriptional regulation of said 2All initiation region, and
- a transcriptional termination region.

**Claim 14**

A tomato plant **comprising**: a DNA construct **comprising** in the direction of transcription,

- a tomato 2All transcriptional initiation region jointed to a DNA sequence of interest, wherein said DNA sequence is other than the wild-type sequence and **comprises** a unique restriction site for insertion of a second DNA sequence of interest to be under the transcriptional regulation of said 2All initiation region, and
- a transcriptional termination region.

**Title** – Methods and compositions for regulated transcription and expression of heterologous genes

**Claim 1**

A method for obtaining a plant having a regulatable phenotype, said method **comprising**:

- transforming a host plant cell with a DNA construct under genomic integration conditions, wherein said construct **comprises** as operably linked components in the direction of transcription,
  - a promoter region obtainable from a gene, wherein transcription of said gene is preferentially regulated in a plant fruit tissue,
<table>
<thead>
<tr>
<th>Claim 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A method for altering the phenotype of fruit tissue as distinct from other plant tissue, said method comprising:</td>
</tr>
<tr>
<td>growing a plant, wherein said plant comprises cells containing a DNA construct integrated into their genome, said DNA construct comprising, in the 5' to 3' direction of transcription,</td>
</tr>
<tr>
<td>a transcriptional initiation region from a gene, wherein transcription of said gene is preferentially regulated in a plant fruit tissue,</td>
</tr>
<tr>
<td>a DNA sequence of interest other than the coding sequence native to said transcriptional initiation region, and</td>
</tr>
<tr>
<td>a transcriptional termination region, whereby said DNA sequence of interest is transcribed under transcriptional control of said transcriptional initiation region and a plant having an altered phenotype is obtained.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 5</th>
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</thead>
<tbody>
<tr>
<td>A method for modifying the genotype of a plant to impart a desired characteristic to fruit as distinct from other plant tissue, said method comprising:</td>
</tr>
<tr>
<td>transforming under genomic integration conditions, a host plant cell with a DNA construct comprising in the 5' to 3' direction of transcription,</td>
</tr>
<tr>
<td>a transcriptional initiation region from a gene, wherein transcription of said gene is preferentially regulated in a plant fruit tissue,</td>
</tr>
<tr>
<td>a DNA sequence of interest other than the native coding sequence of said gene, and</td>
</tr>
<tr>
<td>a transcriptional termination region, whereby said DNA construct becomes integrated into the genome of said plant cell;</td>
</tr>
<tr>
<td>regenerating a plant from said transformed host cell; and</td>
</tr>
<tr>
<td>growing said plant to produce fruit having a modified genotype.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>A method for modifying transcription in fruit tissue as distinct from other plant tissue, said method comprising:</td>
</tr>
<tr>
<td>growing a plant capable of developing fruit tissue under conditions to produce fruit, wherein said plant comprises cells containing a DNA construct integrated into their genome, said DNA construct comprising, in the 5' to 3' direction of transcription,</td>
</tr>
<tr>
<td>a fruit-specific transcriptional initiation region,</td>
</tr>
<tr>
<td>a DNA sequence of interest other than the coding sequence native to said transcriptional initiation region, and</td>
</tr>
<tr>
<td>a transcriptional termination region, whereby said DNA sequence of interest is transcribed under transcriptional control of said fruit-specific transcription initiation region.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A method to selectively express a heterologous DNA sequence of interest in fruit tissue as distinct from other plant tissue, said method comprising:</td>
</tr>
<tr>
<td>growing a plant capable of developing fruit tissue under conditions to produce fruit, wherein said plant comprises cells having a genomically integrated DNA construct comprising, as operably linked components in the 5' to 3' direction of transcription,</td>
</tr>
<tr>
<td>a fruit-specific transcriptional initiation region and a translational</td>
</tr>
</tbody>
</table>
initiation region,
a DNA sequence of interest other than the coding sequence native to
said transcriptional initiation region,
a transcriptional termination region downstream of said DNA
sequence of interest, whereby said DNA sequence of interest is expressed
under control of said fruit–specific transcriptional and translational
initiation region.

This patent is also a Continuation in part of US 5420034 (see the Seed
promoter section).

Claim 1
A DNA construct comprising in the direction of transcription,
a fruit–specific transcriptional initiation region from a gene which
becomes active at or immediately after anthesis and remains active at least
until the ripe period, and which is transcribed in mature ovary tissue,
joined to a DNA sequence of interest other than the wild–type sequence
associated with said initiation region, wherein said DNA sequence of
interest is under the transcriptional regulation of said initiation region and
a transcriptional termination region.

Claim 7
A DNA construct comprising in the direction of transcription,
a fruit–specific transcriptional initiation region of a plant storage protein
which becomes active at or immediately after anthesis and remains active
at least until the ripe period and which is transcribed in mature ovary
tissue, joined to a DNA sequence other than the wild–type sequence,
wherein said sequence comprises a unique restriction site for insertion of a
sequence of interest to be under the transcriptional regulation of said
initiation region, and
a transcriptional termination region.

Claim 12
A method for specifically modifying the phenotype of fruit substantially
distinct from other plant tissue, said method comprising:
transforming a plant cell with a DNA construct under genomic
integration conditions, wherein said DNA construct comprises in the
direction of transcription,
a fruit–specific transcriptional initiation region which becomes active
at or immediately after anthesis, and remains active at least until the ripe
period and which is transcribed preferentially in mature ovary tissue, joined
to a DNA sequence other than the wild–type sequence and capable of
modifying the phenotype of fruit cells upon transcription, wherein said
sequence is under the transcriptional regulation of said initiation region,
and
a transcriptional termination region, whereby said DNA construct
becomes integrated into the genome of said plant cell;
regenerating a plant from said transformed plant cell; and
-growing said plant to produce fruit of the modified phenotype.

Remarks
A related Canadian application (CA 1338827) has lapsed and a related
patent was granted in New Zealand (NZ 224787). There is also a related
patent in Australia. Related patent applications also filed in Israel (IL 86515
A0), and Japan (JP 2500163 T2). Related patent application in China (CN
1036305 A) was withdrawn 20 March 1991.

Note: Patent information was last updated on 15 May 2006. Search terms: “promoter” in abstract and

Cotton fiber patent family
As mentioned above, Cotton (*Gossypium hirsutum*) fiber can be construed as a type of "fruit tissue" in broader botanical terms. Calgene has a United States patent directed to a promoter from cotton expansin gene. The cotton expansin gene is expressed in developing fiber and, according to the specification, the promoter of the cotton expansin gene can be used to drive a gene of interest in developing cotton fiber for modifying cotton fiber phenotypes.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary of Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6566586</td>
<td>Title – Cotton expansin promoter sequence</td>
<td>Calgene LLC</td>
</tr>
</tbody>
</table>

- **Claim 1**
  
  An isolated DNA sequence comprising the sequence of SEQ ID NO: 1.

The only independent claim is drawn to an isolated DNA sequence comprising the 2614 bp cotton expansin promoter sequence. A recombinant DNA construct comprising the promoter sequence and a plant comprising a plant cell comprising the DNA construct are also claimed in the dependent claims.

**Remarks**

Related European application (EP 968292 A1) was withdraw and the application in Australia (AU 57322/98) also lapsed.


**Receptacle patent family**

A European patent application and a granted United States patent filed by Calgene are directed to fruit promoters in general driving the expression of genes in the receptacle tissue of a fruit.

**Specific Patent Information**

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Summary of Claims and Independent Claims</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP 973922</td>
<td>Title – Strawberry fruit promoters for gene expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The claims as filed of the European patent application are broader in this case, and recite:</td>
<td></td>
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<tr>
<td></td>
<td>- DNA constructs comprising a transcriptional factor driving the expression of a heterologous gene in the receptacle tissue of a fruit. The expression of the gene of interest is either:</td>
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<td></td>
<td>o increased during fruit ripening or</td>
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<td></td>
<td>o decreased during fruit development and maturation.</td>
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<tr>
<td></td>
<td>Calgene</td>
<td></td>
</tr>
<tr>
<td>US 6043410</td>
<td>Title – Strawberry fruit promoters for gene expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Claim 1</td>
<td></td>
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<tr>
<td></td>
<td>A DNA construct comprising the promoter sequence from RJ39, isolated from Fragaria, operably-linked to a heterologous DNA coding sequence of interest.</td>
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</tbody>
</table>
Seed Promoters

Summary

PLEASE NOTE that patent claims drawn to promoters that are active constitutively in plants, promoters that are active in "fruits" or in "reproductive parts" may be construed to cover seed-preferred promoters. Patent claims to particular sequences or to promoters that drive particular seed-specific genes in certain species (such as phaseolin or napin) may also be applicable, even though they may not mention the word "seed".

Thus, the list below mentioning a few patents claiming promoters that are broadly seed-specific is NOT a comprehensive list of promoters that are covered by patent claims.

Calgene, Sapporo Breweries and the University of California have filings drawn to seed-specific promoters in broad terms, listed below.

A patent family with several patents granted to Calgene, directed to a transcription cassette having a seed-specific promoter, is noted below, and see also the fruit-specific claims of Calgene elsewhere in this landscape, which may be construed to apply to seeds.

Patents and patent applications that were assigned to Calgene may now be held by Monsanto, to which any inquiries about licensing should be directed.

The granted United States patents [US 5420034](#) and [US 5608152](#) are directed to promoters isolated from specific seed genes (i.e. napin gene) and plants (i.e. *Brassica*). There are three other granted patents in this patent family, and patent applications in the same patent family may still be pending.

Note that patent claims are not granted the same way in every country, and this patent family presents a perfect example of that. The Australian granted patent claims are broad:

- A seed comprising a transcription cassette containing:
  - a seed-specific transcriptional initiation region;
  - a sequence of interest other than the native sequence regulated by the transcriptional region; and
  - a transcriptional termination region.

- A transcription construct comprising a polylinker with at least two restriction sites for the insertion of DNA sequences of interest under the control of a seed-specific promoter.

- A method to modify the genotype of a seed by the use of a transcription cassette as described above.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU 612326 B2</td>
<td>Title – Seed-specific transcriptional regulation</td>
<td>Calgene</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EP 255378 B2</th>
<th>Title – Seed-specific transcriptional regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Claim 1</strong></td>
<td>A DNA construct comprising in the 5' to 3' direction of transcription: a napin transcriptional initiation region, joined to a DNA sequence of interest other than (i) a DNA sequence encoding napin or (ii) a DNA sequence encoding a mammalian protein or peptide or mammalian viral pathogen peptide or protein; and a transcriptional termination region.</td>
</tr>
<tr>
<td><strong>Claim 6</strong></td>
<td>A method comprising the production of a DNA construct comprising in the 5' to 3' direction of transcription: a napin transcriptional initiation region wherein said napin transcriptional initiation region is free from the native DNA sequence under the regulatory control of said initiation region, joined to a cloning site, and a transcriptional termination region; provided that said construct does not comprise a DNA sequence encoding a mammalian peptide or protein or mammalian viral pathogen peptide or protein operably linked to said napin transcriptional initiation region.</td>
</tr>
<tr>
<td><strong>Claim 8</strong></td>
<td>A method of modifying the genotype of a plant to impart a desired characteristic to seed as distinct from other plant said method comprising: transforming a host plant cell under genomic integration conditions with a DNA construct comprising in the 5' to 3' direction of transcription: a seed specific napin transcriptional initiation region, joined to a DNA sequence of interest other than (a) a DNA sequence encoding a napin or (b) a DNA sequence encoding a mammalian peptide or protein or a mammalian viral pathogen peptide or protein; and a transcriptional termination region; and growing said plant to produce seed.</td>
</tr>
<tr>
<td><strong>Claim 9</strong></td>
<td>A method for specifically modifying the phenotype of seed as distinct from other plant tissue, said method comprising: (i) transforming a host plant cell under genomic integration conditions with a DNA construct comprising in the 5' to 3' direction of transcription: a seed specific napin transcriptional initiation region, joined to a DNA sequence of interest other than (a) a DNA sequence encoding napin or (b) a DNA sequence encoding a mammalian peptide or protein or a mammalian viral pathogen peptide or protein; and a transcriptional termination region; and (ii) growing a plant under conditions to produce seed, said plant being comprised of cells capable of developing seed tissue and said cells having integrated in their genome said DNA construct.</td>
</tr>
<tr>
<td><strong>Claim 10</strong></td>
<td>A method comprising the production of a DNA construct comprising in the 5' to 3' direction of transcription: a seed specific transcriptional initiation region which is from other than the bean phaseolin promoter; a DNA sequence other than the natural coding sequence joined to said initiation region, wherein said sequence encodes an acyl carrier protein; and a transcriptional termination region.</td>
</tr>
</tbody>
</table>
Claim 12
A method for modifying the genotype of a plant to impart a desired characteristic to seed as distinct from other plant tissues said method comprising:
- transforming a host plant cell under genomic integration conditions with a DNA construct comprising in the 5' to 3' direction of transcription:
  - a seed specific transcriptional initiation region;
  - a DNA sequence encoding an acyl carrier protein joined to said initiation region; and
  - a transcriptional termination region; and
- growing said plant to produce seed.

Claim 13
A method for specifically modifying the phenotype of seed as distinct from other plant tissue, said method comprising:
1. transforming a host plant cell under genomic integration conditions with a DNA construct comprising in the 5' to 3' direction of transcription:
   - a seed specific transcriptional initiation region;
   - a DNA sequence encoding an acyl carrier protein joined to said initiation region; and
   - a transcriptional termination region; and
2. growing a plant under conditions to produce seed, said plant being comprised of cells capable of developing seed tissue and said cells having integrated in their genome said DNA construct.

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**US 5420034**

Title – Seed-specific transcriptional regulation

**Claim 1**
A DNA construct comprising: in the 5' to 3' direction of transcription, a transcriptional initiation region from a gene which encodes a product preferentially expressed in a plant seed cell as compared with other plant cells, a DNA sequence of interest other than the native coding sequence of said gene, and a transcriptional termination region, wherein said gene is a napin gene, an acyl carrier protein gene or an EA9 gene.

**Claim 8**
An expression cassette comprising: in the 5'-3' direction of transcription, a seed-specific transcriptional initiation region wherein said transcriptional initiation region is free from the native DNA sequence under the regulatory control of said initiation region, a cloning site, and a transcriptional termination region, wherein said transcriptional initiation region is from a napin gene, an acyl carrier protein gene or an EA9 gene.

**Claim 9**
An expression cassette comprising: in the 5'-3' direction of transcription, a transcriptional initiation region and ribosome binding site from a gene expressed in a seed embryo or a seed coat cell or from a gene encoding a seed storage protein, a linker or polylinker having one or a plurality of restriction sites for insertion of a gene to be expressed under transcriptional control of said transcriptional initiation region, and a transcriptional termination region, wherein said transcriptional initiation region and said ribosome binding site are from a napin gene, an acyl carrier protein gene or an EA9 gene.

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**US 5608152**

Title – Seed-specific transcriptional regulation

**Claim 1**
A Brassica plant comprising: a DNA construct comprising, in the 5' to 3' direction of transcription, a transcriptional initiation region from a gene that encodes a product preferentially expressed in a plant seed cell as compared to other plant cells, a DNA sequence of interest other than the native coding...
sequence of said gene, and a transcriptional termination region, wherein said gene is a napin gene, an acyl carrier protein gene or an EA9 gene.

**Claim 8**

A Brassica seed comprising: a DNA construct comprising, in the 5' to 3' direction of transcription, a transcriptional initiation region from a gene that encodes a product preferentially expressed in a plant seed cell as compared to other plant cells, a DNA sequence of interest other than the native coding sequence of said gene, and a transcriptional termination region, wherein said gene is a napin gene, an acyl carrier protein gene or an EA9 gene.

This patent is a Division of [US 5420034](http://www.patentlens.net/daisy/promoters/ext/navaggregator/navaggr...).

### Remarks

Related patent granted in New Zealand (NZ 221259). Related application filed in China (CN 87106120) has been withdrawn.

## Sapporo Breweries' patents and applications

The granted US and Australian patents claim an isolated barley β-amylase promoter sequence. However, the independent claims as filed in the European and Canadian patent applications are very broad as they recite a promoter capable of expressing an introduced gene in plant seeds. There is no limitation in the gene source of the seed promoter. In dependent claims the promoter is derived from a beta-amylase gene from barley. It remains to be seen whether the independent claims will be granted as filed.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary</th>
<th>Assignee</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5952489</td>
<td>Title – Tissue-specific promoter</td>
<td>Sapporo Breweries Ltd.</td>
</tr>
<tr>
<td></td>
<td>Claim 1 An isolated barley β-amylase promoter comprising SEQ ID NO: 1.</td>
<td></td>
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<tr>
<td>AU 717055 B2</td>
<td>Title – Tissue-specific promoter</td>
<td></td>
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<tr>
<td></td>
<td>Claim 1 An isolated barley β-amylase promoter comprising a nucleic acid sequence of SEQ ID NO: 1, or a nucleic acid sequence of SEQ ID NO: 1 in which one or more bases are deleted, substituted or added to said sequence and which has promoter activity in plant seeds.</td>
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<tr>
<td>EP 781849</td>
<td>Title – Tissue-specific promoter</td>
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<tr>
<td></td>
<td>Claim 1 A promoter capable of expressing an introduced gene in plant seeds</td>
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</tbody>
</table>
University of California's patents and applications

Only independent claim 1 as filed of the European patent application is relevant for the present paper as it describes in general terms a recombinant nucleic acid molecule having a seed–maturation specific promoter. The promoter drives the expression of a protein in a subcellular compartment due to the presence of a signal peptide that targets the polypeptide to an intracellular body.

The other independent claims are more specific and describe promoter and signal sequences derived from barley hordein storage protein. It is important to note that claims from pending applications may still be granted.

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Title, Independent Claims and Summary</th>
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</tr>
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<tbody>
<tr>
<td>US 6642437</td>
<td>Title – Production of proteins in plant seeds</td>
<td>The Regents of the University of California</td>
</tr>
<tr>
<td></td>
<td><strong>Claim 1</strong></td>
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<td></td>
<td>A method for producing seeds containing a selected heterologous protein which is not a seed–storage protein, comprising the steps of: (a) stably transforming monocot plant cells with a chimeric gene having: (i) a transcriptional regulatory region from the gene of a maturation specific monocot storage protein selected from the group consisting of rice glutelins, oryzins, rice prolamines, barley hordeins, oat glutelins, and sorghum kafirins, millet pennisetins, and rye secalins, (ii) operably linked to said transcriptional regulatory region, a first DNA sequence encoding a monocot seed-specific N-terminal leader sequence capable of targeting a linked polypeptide to a protein storage body in monocot seeds, and (iii) a second DNA sequence encoding such selected non–seed–storage heterologous protein, and linked in translation frame with the first sequence, such that the first and second sequences encode a fusion protein composed of the selected heterologous non–seed–storage protein and an N–terminal leader sequence, (b) cultivating plants containing the transformed plant cells under seed–maturation conditions, wherein the expression of the non–seed–storage heterologous protein is at least twice that observed with an equivalent chimeric gene lacking the second DNA sequence encoding a monocot seed–specific N–terminal leader sequence, and (c) harvesting seeds from the cultivated plants.</td>
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</tr>
<tr>
<td>AU 746032 B2</td>
<td>Title – Production of proteins in plant seeds</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Claim 1</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A method of producing a selected heterologous protein which is not a seed–storage protein, comprising the steps of: (a) stably transforming a monocot plant cell with a chimeric gene comprising: (i) a transcriptional regulatory region from the gene of a maturation specific monocot storage protein selected from the group consisting of rice glutelins, oryzins, and prolamines, barley hordeins, wheat gliadins and glutenins, maize zeins and glutelins, oat glutelins, and sorghum kafirins, millet pennisetins, and rye secaliis, (ii) operably linked to said transcriptional regulatory region, a first DNA sequence encoding a monocot seed–specific leader sequence capable of targeting a linked polypeptide to a protein storage body in monocot seeds, and (iii) a second DNA sequence encoding the selected heterologous protein,</td>
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</table>
and linked in translation frame with the first sequence, such that the first and second sequences encode a fusion protein composed of the selected protein and an N-terminal leader sequence, and
(b) cultivating plants containing the cell under seed-maturation conditions to produce the selected heterologous protein.

Claim 5
A method of producing a transformed plant comprising the steps of:
(a) stably transforming a monocot plant cell with a chimeric gene comprising:
(i) a transcriptional regulatory region from the gene of a maturation specific monocot storage protein selected from the group consisting of rice glutelins, oryzins, and prolamines, barley hordeins, wheat gliadins and glutenins, maize zeins and glutelins, oat glutelins, and sorghum kafirins, millet pennisetins, and rye secalins,
(ii) operably linked to said transcriptional regulatory region, a first DNA sequence encoding a monocot seed-specific leader sequence capable of targeting a linked polypeptide to a protein storage body in monocot seeds,
(iii) a second DNA sequence encoding a selected heterologous protein which is not a seed-storage protein, and linked in translation frame with the first sequence, such that the first and second sequences encode a fusion protein composed of the selected protein and an N-terminal leader sequence; and
(b) cultivating a plant containing the cell.
Claim 35
A method of producing a stably transformed monocotyledenous plant expressing a selected polypeptide in seeds of the plant, comprising:
(a) placing an immature zygotic embryo of the plant on plant growth medium comprising maltose as a sugar source, an auxin at a concentration of about 0.1 mg/L to about 5 mg/L, a cytokinin at a concentration of 0 mg/L to about 5 mg/L and copper at a concentration of about 0.1 μM to about 50 μM, and incubating in dim light conditions so as to form green regenerative tissue;
(b) introducing a nucleic acid molecule into the tissue by to produce transformed tissue, wherein the nucleic acid molecule has a structure Ph−X, wherein Ph is a hordein promoter and X is a nucleic acid molecule encoding the selected polypeptide, and where Ph and X are operably linked;
(c) incubating the transformed tissue on the plant growth medium such that green structures are observed on the transformed material;
(d) regenerating at least one transformed plant from the green structures; and
(f) growing the transformed plant to produce seed.

Claim 39
A method of producing a stably transformed monocotyledenous plant expressing a selected polypeptide in seeds of the plant, comprising:
(a) placing an immature zygotic embryo of the plant on plant growth medium comprising maltose as a sugar source, an auxin at a concentration of about 0.1 mg/L to about 5 mg/L, a cytokinin at a concentration of 0 mg/L to about 5 mg/L and copper at a concentration of about 0.1 μM to about 50 μM, and incubating in dim light conditions so as to form green regenerative tissue;
(b) introducing a nucleic acid molecule into the tissue by to produce transformed tissue, wherein the nucleic acid molecule has a structure P−X or P−SS− wherein X is a nucleic acid molecule encoding a polypeptide, P is a seed maturation-specific promoter, and SS is a signal sequence that targets a linked polypeptide to an intracellular body;
(c) incubating the transformed tissue on the plant growth medium such that green structures are observed on the transformed material;
(d) regenerating at least one transformed plant from the green structures; and
(f) growing the transformed plant to produce seed.

Remarks
An application as a Continuation of US 6642437, which has exactly the same claims as (EP 1019517), is pending in the United States (US 2004/88754 A1). Related applications are also pending in Canada (CA 2305628) and Japan (JP 2001518305).

Note: Patent information on this page was last updated on 10 April 2006.

Appendix 1 & 2

Claims

Opine Promoters

The CaMv promoters: 35S and 19S

Plant Ubiquitin Promoter System

Maize alcohol dehydrogenase 1 (Adh−1) promoter

Synthetic promoters

Opine Promoters
1. Promoters and UAS from opine synthase genes

Patents granted to Biotechnology Research And Development Corporation & Purdue Research Foundation

Actual granted independent claims

| Claim 1 | A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising:
| a) a promoter derived from a mannopine synthase gene of \textit{Agrobacterium tumefaciens},
| b) an upstream activating sequence derived from a mannopine synthase gene of \textit{Agrobacterium tumefaciens}, and
| c) at least one upstream activating sequence derived from an octopine synthase gene of \textit{Agrobacterium tumefaciens}. |

| Claim 2 | A method for nematode inducible expression of a foreign gene in a plant, comprising:
| a) linking said foreign gene to a regulatory region comprising a promoter derived from a mannopine synthase gene of \textit{Agrobacterium tumefaciens} comprising 138 bases upstream of the transcription initiation site, and an upstream activating sequence derived from a mannopine synthase gene of \textit{Agrobacterium tumefaciens};
| b) inserting said foreign gene and said regulatory region in said plant, wherein expression is induced by nematode attack on the plant. |

| Claim 3 | A method for nematode inducible expression of a foreign gene in a plant, comprising:
| a) linking said foreign gene to a regulatory region comprising:
| i) a promoter derived from a mannopine synthase gene of \textit{Agrobacterium tumefaciens},
| ii) an upstream activating sequence derived from a mannopine synthase gene of \textit{Agrobacterium tumefaciens}, and
| iii) at least one upstream activating sequence derived from an octopine synthase gene of \textit{Agrobacterium tumefaciens};
| b) inserting said foreign gene and said regulatory region in said plant, wherein expression is induced by nematode attack on the plant. |

| Claim 4 | A chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from an \textit{Agrobacterium tumefaciens} octopine synthase gene operably linked to a promoter derived from an \textit{Agrobacterium tumefaciens} mannopine synthase gene. |

| Claim 5 | A cassette for expressing a foreign gene comprising the foreign gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from \textit{Agrobacterium tumefaciens} octopine synthase genes operably linked to a promoter derived from an \textit{Agrobacterium tumefaciens} mannopine synthase gene. |

| Claim 6 | A plasmid comprising a cassette comprising a foreign gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from \textit{Agrobacterium tumefaciens} octopine synthase genes operably linked to a promoter derived from an \textit{Agrobacterium tumefaciens} mannopine synthase gene. |

| Claim 7 | A method of expressing a foreign gene in a plant, comprising:
| a) linking said foreign gene to a chimeric regulatory region comprising at least three upstream activating sequences derived from an \textit{Agrobacterium tumefaciens} octopine synthase gene operably linked to a promoter derived from an \textit{Agrobacterium tumefaciens} mannopine synthase gene; and
b) inserting said foreign gene and said chimeric regulatory region into a plant, wherein said plant expresses said foreign gene.

Claim 8
A transgenic plant comprising a cassette comprising a foreign gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* octopine synthase genes operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

Claim 9
A chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* octopine synthase gene operably linked to an upstream activating sequence derived from an *Agrobacterium tumefaciens* mannopine synthase gene that is operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

Claim 10
A cassette for expressing a foreign gene comprising the foreign gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* octopine synthase genes operably linked to an upstream activating sequence derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

Claim 11
A transgenic plant comprising a cassette for expressing genes in plants comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to an upstream activating sequence derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

Claim 12
A transgenic plant comprising a chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to an upstream activating sequence derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

AU 687 961 B

Claim 1
A chimeric regulatory region for expressing genes in plants comprising an upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

Claim 2
A cassette for expressing a gene comprising a gene operably linked to a chimeric regulatory region comprising an upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

Claim 4
A chimeric regulatory region for expressing genes in plants comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.

Claim 8
A cassette for expressing a gene comprising a gene operably linked to a chimeric regulatory region comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.

Claim 13
A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising:
a) a promoter derived from a mannopine synthase gene of *Agrobacterium tumefaciens* by deletion to nucleotide position -138, and
b) an upstream activating sequence derived from a mannopine synthase gene of *Agrobacterium tumefaciens*.

**Claim 15**
A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising:

a) a promoter derived from a mannopine synthase gene of *Agrobacterium tumefaciens*,

b) an upstream activating sequence derived from a mannopine synthase gene of *Agrobacterium tumefaciens*, and

c) an upstream activating sequence derived from an octopine synthase gene of *Agrobacterium tumefaciens*.

**Claim 16**
A method for expressing a gene in a plant, comprising the steps of:

a) linking said gene to a chimeric regulatory region comprising an upstream activating sequence derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene;

b) inserting said gene and said chimeric regulatory region into a plant; and

c) allowing said plant to express said gene.

**Claim 17**
A method for expressing a gene in a plant, comprising the steps of:

a) linking said gene to a chimeric regulatory region comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from a different opine synthase gene than said promoter;

b) inserting said gene and said chimeric regulatory region into a plant; and

c) allowing said plant to express said gene.

**Claim 21**
A method of inducible expression of a foreign gene in a plant, comprising:

a) linking said foreign gene to a regulatory region comprising:

i. a promoter derived from a mannopine synthase gene of *Agrobacterium tumefaciens* by deletion to nucleotide position -138, and

ii. an upstream activating sequence derived from a mannopine synthase gene of *Agrobacterium tumefaciens*;

b) inserting said foreign gene and said regulatory region in said plant; and

c) inducing expression of said foreign gene.

**Claim 23**
A method for inducible expression of a foreign gene in a plant, comprising:

a) linking said foreign gene to a regulatory region comprising:

i. a promoter derived from a mannopine synthase gene of *Agrobacterium tumefaciens*;

ii. an upstream activating sequence derived from a mannopine synthase gene of *Agrobacterium tumefaciens*; and

iii. an upstream activating sequence derived from an octopine synthase gene of *Agrobacterium tumefaciens*;

b) inserting said foreign gene and said regulatory region in said plant; and

c) inducing expression of said foreign gene.

**Claim 32**
A chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a
promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 33**
A cassette for expressing a gene comprising a gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* octopine synthase genes operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene.

**Claim 34**
A method of expressing a gene in a plant, comprising the steps of:

a) linking said gene to a chimeric regulatory region comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase;
b) inserting said gene and said chimeric regulatory region into a plant; and
c) allowing said plant to express said gene.

**Claim 37**
A chimeric regulatory region for expressing a gene in a plant comprising at least three upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.

**Claim 39**
A cassette for expressing a gene in a plant comprising a gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from *Agrobacterium tumefaciens* opine synthase genes operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.

**Claim 40**
A chimeric regulatory region for expressing a gene in a plant comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene.

**Claim 41**
A chimeric regulatory region for expressing a gene in a plant comprising at least two upstream activating sequences derived from an *Agrobacterium tumefaciens* opine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* opine synthase gene.

**Patent applications filed by Biotechnology Research and Development Corporation & Purdue Research Foundation**

**Actual pending independent claims**

**EP 729 514 A1**

**Claim 1**
A chimeric regulatory region for expressing genes in plants comprising an upstream activating sequence derived from a first Agrobacterium tumefaciens opine synthase gene operably linked to a promoter derived from a second Agrobacterium tumefaciens opine synthase gene that is different from said first Agrobacterium tumefaciens opine synthase gene.

**Claim 4**
A cassette for expressing a gene comprising a gene operably linked to chimeric regulatory region comprising an upstream activating sequence derived from a first Agrobacterium tumefaciens opine synthase gene operably linked to a promoter derived from a second Agrobacterium tumefaciens opine synthase gene that is different from said first Agrobacterium tumefaciens opine synthase gene.

**Claim 8**
A chimeric regulatory region for expressing genes in plants comprising at least two upstream activating sequences derived from an Agrobacterium tumefaciens opine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens opine synthase gene.

**Claim 12**
A cassette for expressing a gene comprising a gene operably linked to a chimeric regulatory region comprising at least two upstream activating sequences derived from Agrobacterium tumefaciens opine
synthase genes operably linked to a promoter derived from a Agrobacterium tumefaciens opine synthase gene.

Claim 17
A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising a promoter derived from a mannopine synthase gene of Agrobacterium tumefaciens by deletion to nucleotide position–138 and an upstream activating sequence derived from a mannopine synthase gene of Agrobacterium tumefaciens.

Claim 19
A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising a promoter derived from a mannopine synthase gene of Agrobacterium tumefaciens, an upstream activating sequence derived from a mannopine synthase gene of Agrobacterium tumefaciens, and an upstream activating sequence derived from an octopine synthase gene of Agrobacterium tumefaciens.

Claim 20
A method of expressing a gene in a plant, comprising the steps of: linking said gene to a chimeric regulatory region comprising an upstream activating sequence derived from a first Agrobacterium tumefaciens opine synthase gene operably linked to a promoter derived from a second Agrobacterium tumefaciens opine synthase gene that is different from said first Agrobacterium tumefaciens opine synthase gene; inserting said gene and said chimeric regulatory region into a plant; and allowing said plant to express said gene.

Claim 23
A method of expressing a gene in a plant, comprising the steps of: linking said gene to a chimeric regulatory region comprising at least two upstream activating sequences derived from an Agrobacterium tumefaciens opine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens opine synthase; inserting said gene and said chimeric regulatory region into a plant; and allowing said plant to express said gene.

Claim 27
A method of inducible expressing a foreign gene in a plant, comprising: linking said foreign gene to a regulatory region comprising a promoter derived from a mannopine synthase gene of Agrobacterium tumefaciens by deletion to nucleotide position–138 and an upstream activating sequence derived from a mannopine synthase gene of Agrobacterium tumefaciens; inserting said foreign gene and said regulatory region in said plant; and inducing expression of said foreign gene.

Claim 29
A method for inducible expression of a foreign gene in a plant, comprising: linking said foreign gene to a regulatory region comprising a promoter derived from a mannopine synthase gene of Agrobacterium tumefaciens, an upstream activating sequence derived from a mannopine synthase gene of Agrobacterium tumefaciens, and an upstream activating sequence derived from an octopine synthase gene of Agrobacterium tumefaciens; inserting said foreign gene and said regulatory region in said plant; and inducing expression of said foreign gene.

CA 2174954

Claim 1
A chimeric regulatory region for expressing genes in plants comprising an upstream activating sequence derived from an Agrobacterium tumefaciens octopine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens mannopine synthase gene.

Claim 2
A cassette for expressing a gene comprising a gene operably linked to chimeric regulatory region comprising an upstream activating sequence derived from an Agrobacterium tumefaciens octopine synthase gene operably linked to a promoter derived from a Agrobacterium tumefaciens mannopine synthase gene.

Claim 4
A chimeric regulatory region for expressing genes in plants comprising at least two upstream activating sequences derived from an Agrobacterium tumefaciens opine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens opine synthase gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.

Claim 8
A cassette for expressing a gene comprising a gene operably linked to a chimeric regulatory region comprising at least two upstream activating sequences derived from Agrobacterium tumefaciens opine synthase genes operably linked to a promoter derived from a Agrobacterium tumefaciens opine synthase
gene, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter.

Claim 13
A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising a promoter derived from a mannopine synthase gene of Agrobacterium tumefaciens by deletion to nucleotide position –138 and an upstream activating sequence derived from a mannopine synthase gene of Agrobacterium tumefaciens.

Claim 15
A cassette for inducible expression of a foreign gene comprising said foreign gene operably linked to a regulatory region comprising a promoter derived from a mannopine synthase gene of Agrobacterium tumefaciens, an upstream activating sequence derived from a mannopine synthase gene of Agrobacterium tumefaciens, and an upstream activating sequence derived from an octopine synthase gene of Agrobacterium tumefaciens.

Claim 16
A method of expressing a gene in a plant, comprising the steps of: linking said gene to a chimeric regulatory region comprising an upstream activating sequence derived from a Agrobacterium tumefaciens octopine synthase gene operably linked to a promoter derived from a Agrobacterium tumefaciens mannopine synthase gene; inserting said gene and said chimeric regulatory region into a plant; and allowing said plant to express said gene.

Claim 17
A method of expressing a gene in a plant, comprising the steps of: linking said gene to a chimeric regulatory region comprising at least two upstream activating sequences derived from an Agrobacterium tumefaciens opine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens opine synthase, wherein at least one of said upstream activating elements are derived from a different opine synthase gene than said promoter; inserting said gene and said chimeric regulatory region into a plant; and allowing said plant to express said gene.

Claim 21
A method of inducible expressing a foreign gene in a plant, comprising: linking said foreign gene to a regulatory region comprising a promoter derived from a mannopine synthase gene of Agrobacterium tumefaciens by deletion to nucleotide position –138 and an upstream activating sequence derived from a mannopine synthase gene of Agrobacterium tumefaciens; inserting said foreign gene and said regulatory region in said plant; and inducing expression of said foreign gene.

Claim 23
A method for inducible expression of a foreign gene in a plant, comprising: linking said foreign gene to a regulatory region comprising a promoter derived from a mannopine synthase gene of Agrobacterium tumefaciens, an upstream activating sequence derived from a mannopine synthase gene of Agrobacterium tumefaciens, and an upstream activating sequence derived from an octopine synthase gene of Agrobacterium tumefaciens; inserting said foreign gene and said regulatory region in said plant; and inducing expression of said foreign gene.

Claim 32
A chimeric regulatory region for expressing genes in plants comprising at least three upstream activating sequences derived from an Agrobacterium tumefaciens octopine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens mannopine synthase gene.

Claim 33
A cassette for expressing a gene comprising a gene operably linked to a chimeric regulatory region comprising at least three upstream activating sequences derived from Agrobacterium tumefaciens octopine synthase genes operably linked to a promoter derived from a Agrobacterium tumefaciens mannopine synthase gene.

Claim 34
A method of expressing a gene in a plant, comprising the steps of: linking said gene to a chimeric regulatory region comprising at least three upstream activating sequences derived from an Agrobacterium tumefaciens octopine synthase gene operably linked to a promoter derived from an Agrobacterium tumefaciens mannopine synthase; inserting said gene and said chimeric regulatory region into a plant; and allowing said plant to express said gene.

The CaMV promoters: 35S and 19S

Actual granted independent claims
US 5 352 605
(view in patent database)

Claim 1
A chimeric gene which is expressed in plant cells comprising:

(i) a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV (35S) promoter isolated from CaMV protein-encoding DNA sequences and a CaMV (19S) promoter isolated from CaMV protein-encoding DNA sequences, and
(ii) a structural sequence which is heterologous with respect to the promoter.

Claim 4
A plant cell which comprises a chimeric gene that contains:

(i) a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV (35S) promoter and a CaMV (19S) promoter, wherein said promoter is isolated from CaMV protein-encoding DNA sequences, and
(ii) a structural sequence which is heterologous with respect to the promoter.

Claim 7
An intermediate plant transformation plasmid which comprises:

(i) a region of homology to an Agrobacterium tumefaciens vector,
(ii) a T–DNA border region from Agrobacterium tumefaciens and
(iii) a chimeric gene, wherein the chimeric gene is located between the T–DNA border and the region of homology,
said chimeric gene comprising:

(i) a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and
(ii) a structural sequence which is heterologous with respect to the promoter.

Claim 8
A plant transformation vector which comprises:

(i) a disarmed plant tumor inducing plasmid of Agrobacterium tumefaciens and
(ii) a chimeric gene, wherein the chimeric gene contains a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and
(iii) a structural sequence which is heterologous with respect to the promoter.

Claim 13
A DNA construct comprising:

(i) a CaMV promoter selected from the group consisting of
(a) a CaMV 35S promoter isolated from CaMV protein–encoding DNA sequences and
(b) a CaMV 19S promoter isolated from CaMV protein–encoding DNA sequences, and
(ii) a DNA sequence of interest heterologous to (i), wherein (ii) is under the regulatory control of (i) when said construct is transcribed in a plant cell.

Claim 14
A chimeric gene which is transcribed and translated in plant cells, said chimeric gene comprising:

(i) a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of:
(a) a CaMV 35S promoter region free of CaMV protein–encoding DNA sequences and
(b) a CaMV 19S promoter region free of CaMV protein–encoding DNA sequences, and
(ii) a DNA sequence which is heterologous with respect to the promoter.

Claim 15
A chimeric gene which is expressed in plants cells comprising:
(i) a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of

(a) a CaMV(35S) promoter region free of CaMV protein–encoding DNA sequences and
(b) a CaMV(19S) promoter region free of CaMV protein–encoding DNA sequences, and

(ii) a DNA sequence which is heterologous with respect to the promoter.

**Claim 16**
A chimeric gene which is transcribed in plants cells comprising:

(i) a promoter from a cauliflower mosaic virus, said promoter selected from the group consisting of

(a) a CaMV(35S) promoter free of CaMV protein–encoding DNA sequences and
(b) a CaMV(19S) promoter free of CaMV protein–encoding DNA sequences,

(ii) a DNA sequence which is heterologous with respect to the promoter and
(iii) a 3’ non–translated polyadenylation signal sequence.

**Claim 17**
A plant cell which comprises a chimeric gene where said chimeric gene comprises:

(i) a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of

a CaMV(35S) promoter and a CaMV(19S) promoter, wherein said promoter is free of CaMV protein–encoding DNA sequences, and

(ii) a DNA sequence which is heterologous with respect to the promoter and
(iii) a 3’ non–translated polyadenylation signal sequence.

**Patents on entire 35S and 19S promoters granted to Monsanto**

**Actual granted independent claims**

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5 530 196</td>
<td>A differentiated dicotyledonous plant comprising plant cells containing a chimeric gene which comprises:</td>
</tr>
<tr>
<td></td>
<td>a) a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of</td>
</tr>
<tr>
<td></td>
<td>1. a CaMV(35S) promoter free of CaMV protein–encoding DNA sequences and</td>
</tr>
<tr>
<td></td>
<td>2. a CaMV(19S) promoter free of protein–encoding DNA sequences, and</td>
</tr>
<tr>
<td></td>
<td>b) a structural sequence which is heterologous with respect to the promoter.</td>
</tr>
<tr>
<td>US 5 858 742</td>
<td>A differentiated dicotyledonous plant comprising plant cells containing in the plant genome a chimeric gene which comprises:</td>
</tr>
<tr>
<td></td>
<td>1. a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a</td>
</tr>
<tr>
<td></td>
<td>CaMV(35S) promoter and a CaMV(19S) promoter, and</td>
</tr>
<tr>
<td></td>
<td>2. a DNA sequence which is heterologous with respect to the promoter.</td>
</tr>
</tbody>
</table>

**Claim 5**
A differentiated dicotyledonous plant regenerated from plant cells, said plant cells containing a chimeric gene which comprises:

i. a promoter from cauliflower mosaic virus, said promoter selected from the group consisting of a CaMV(35S) promoter and a CaMV(19S) promoter, and

ii. a DNA sequence which is heterologous with respect to the promoter.
Claim 1
A method for transforming a plant cell which comprises transforming a plant cell with a chimeric DNA construct containing:

a) a promoter isolated from cauliflower mosaic virus (CaMV), said promoter selected from the group consisting of
   1. a CaMV(19S) promoter derived from the CaMV(19S) gene and
   2. a CaMV(35S) promoter derived from the CaMV(35S) gene, and
b) a DNA sequence which is heterologous with respect to the promoter; wherein the promoter regulates the transcription of the DNA sequence.

Claim 1
A chimeric gene which is expressed in plant cells comprising:

a) a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to
b) a DNA sequence which is heterologous with respect to the promoter, wherein:
   1. the promoter regulates the transcription of the DNA sequence, and
   2. the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to a plant or plant cell containing the DNA sequence relative to a wild-type plant or plant cell.

Claim 3
A plant cell comprising a chimeric gene which comprises:

a) a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to
b) a DNA sequence which is heterologous with respect to the promoter, wherein:
   1. the promoter regulates the transcription of the DNA sequence, and
   2. the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to the plant cell relative to a wild-type plant cell.

Claim 6
An intermediate plant transformation plasmid which comprises:

a) a region of homology to an A. tumefaciens vector;
b) a T-DNA border from A. tumefaciens, and
c) a chimeric gene,

wherein the chimeric gene is located between the T-DNA border and the region of homology, said chimeric gene comprising a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to a DNA sequence which is heterologous with respect to the promoter, wherein:

a) the promoter regulates the transcription of the DNA sequence, and
b) the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to a plant or plant cell containing the DNA sequence relative to a wild-type plant or plant cell.

Claim 9
A plant transformation vector which comprises a modified plant tumor inducing plasmid of A. tumefaciens which is capable of inserting a chimeric gene into susceptible plant cells, wherein the chimeric gene comprises a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to a DNA sequence which is heterologous with respect.
to the promoter, wherein:

a) the promoter regulates the transcription of the DNA sequence, and
b) the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to a plant or plant cell containing the DNA sequence relative to a wild-type plant or plant cell.

Claim 12
A differentiated dicotyledonous plant comprising plant cells containing a chimeric gene which comprises a promoter from cauliflower mosaic virus (CaMV), wherein said promoter is the CaMV(19S) promoter or the CaMV(35S) promoter, operably linked to a DNA sequence encoding said polypeptide which is heterologous with respect to the promoter, wherein:

a) the promoter regulates the transcription of the DNA sequence, and
b) the DNA sequence encodes a polypeptide conferring increased antibiotic resistance to the plant relative to a wild-type plant.

Claim 4
A chimeric gene capable of expressing a polypeptide in plant cells comprising in sequence:

a) a full-length transcript promoter region isolated from cauliflower mosaic virus
b) a 5' non-translated region
c) a structural coding sequence
d) a 3' non-translated region of a gene naturally expressed in plants, said region encoding a signal sequence for polyadenylation of mRNA, said structural coding sequence being heterologous with respect to said promoter region.

Patents granted to the Rockefeller University

Actual granted independent claims

US 5 097 025

Claim 1
An isolated DNA segment consisting of the nucleotide sequence:
5'-CGACCAGCAT CGTGGAAAA GAAGACGTTC CAACCACGTC TTCAAAGC-3'*.

*sequence of subdomain B2 of 35S CaMV promoter.

Claim 2
A DNA sequence consisting of:
a) a first nucleotide sequence 5'-CGAGGAGCAT CGTGGAAAA GAAGACGTTC CAACCACGTC TTCAAAGC-3'* and
b) a second nucleotide sequence corresponding to domain A of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B2 of 35S CaMV promoter.

Claim 3
A DNA sequence consisting of:
a) a first nucleotide sequence 5'–CATCGTTGAAG ATGCCTCTGC CGACAGTGGT CCCAAAGATG GACCCCCACC CAC-3'* and
b) a second nucleotide sequence corresponding to domain A of the CaMV35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B3 of 35S CaMV promoter.

Claim 4
A DNA sequence consisting of:
a) a first nucleotide sequence 5'–ATTCC ATTGCCC AGCTATCTGT CACTTTATTG TGAAGATAGT GGAAAAGGAA GGTGGCTCCT ACAAATGCCA TCATTGCGAT AAAGGAAAGG CC-3'* and
b) a second nucleotide sequence corresponding to domain A of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B4 of 35S CaMV promoter.

Claim 5
A DNA sequence consisting of:
a) a first nucleotide sequence 5′-TGAGACTTTT CAACAAAGGG TAATATCCGG AAACCTCCTC GGATT-3′* and
b) a second nucleotide sequence corresponding to domain A of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B5 of 35S CaMV promoter.

Claim 6
An isolated DNA segment consisting of the nucleotide sequence:
5′-CATCGTTGAAG ATGCCTCTGC CGACAGTGGT CCCAAAGATG GACCCCCACC CAC-3′*.

*sequence of subdomain B3 of 35S CaMV promoter.

Claim 7
An isolated DNA segment consisting of the nucleotide sequence:
5′-ATTCC ATTGCCC AGCTATCTGT CACTTTATTG TGAAGATAGT GAAAAAGGAA GGTGGCTCCT ACAATGCCA TCATTGCCGATT AAAGGAAAGG CC-3′*.

*sequence of subdomain B4 of 35S CaMV promoter.

Claim 8
An isolated DNA segment consisting of the nucleotide sequence:
5′-TGAGACTTTT CAACAAAGGG TAATATCCGG AAACCTCCTC GGATT-3′*.

*sequence of subdomain B5 of 35S CaMV promoter.

Claim 9
A DNA sequence consisting of:
a) a first nucleotide sequence 5′-CGAGGAGCAT CGTGGAAAAA GAAGACGTTC CAACCACGTC TTCAAAGC-3′* and
b) a second nucleotide sequence corresponding to the minimal promoter region of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B2 of 35S CaMV promoter.

Claim 10
A DNA sequence consisting of:
a) a first nucleotide sequence 5′-CATCGTTGAAG ATGCCTCTGC CGACAGTGGT CCCAAAGATG GACCCCCACC CAC-3′* and
b) a second nucleotide sequence corresponding to the minimal promoter region of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B3 of 35S CaMV promoter.

Claim 11
A DNA sequence consisting of:
a) a first nucleotide sequence 5′-ATTCC ATTGCCC AGCTATCTGT CACTTTATTG TGAAGATAGT GAAAAAGGAA GGTGGCTCCT ACAATGCCA TCATTGCCGATT AAAGGAAAGG CC-3′* and
b) a second nucleotide sequence corresponding to the minimal promoter region of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B4 of 35S CaMV promoter.

Claim 12
A DNA sequence consisting of:
a) a first nucleotide sequence 5′-TGAGACTTTT CAACAAAGGG TAATATCCGG AAACCTCCTC GGATT-3′* and
b) a second nucleotide sequence corresponding to the minimal promoter region of the CaMV 35S promoter coupled to said first nucleotide sequence by means of a synthetic multilinker.

*sequence of subdomain B5 of 35S CaMV promoter.
Patent granted to the Rockefeller University

Actual granted independent claims

**US 5 110 732**

(view in patent database)

**Claim 1**

In a method for the expression of a chimeric plant gene, the improvement which comprises the use of a tissue-specific promoter fragment which causes tissue-specific expression in leaves, stems, cotyledons, and vascular tissue of the hypocotyl while causing detectable levels of expression in root vascular tissue when operably coupled directly to a DNA segment corresponding to the -72 to +8 promoter fragment of the Cauliflower Mosaic Virus 35S gene, said tissue-specific promoter fragment having the sequence:

5'-TGAGACTTTT CAACAAAGGG TAATATCCCG AAACCTCCTC GGATTCCATT GCCCCAGCTAT CTGTCACTTT ATTGTGAAAA TAGTGAAAAA GGAAGGTTGC TCCTAAAAT GCCATCATGG CGATAAAGGA AAGGCCATCG TGAAGATGC CTCGGCCGAC AGTGGTCCCA AAGATGGACC CCCACCCAC GAGGAGCATC GTGAAAAAG AAGACGTTCC AACCACGTCT TCAAGGCAAG TGGATTGTG TGATA-3'*

*sequence corresponds to the complete domain B -from -343 to -90 nucleotides- of the 35S CaMV promoter.

**Claim 2**

A chimeric plant gene comprising in sequence in the 5' to 3' direction a tissue-specific promoter fragment consisting essentially of the sequence:

5'-TGAGACTTTT CAACAAAGGG TAATATCCCG AAACCTCCTC GGATTCCATT GCCCCAGCTAT CTGTCACTTT ATTGTGAAAA TAGTGAAAAA GGAAGGTTGC TCCTAAAAT GCCATCATGG CGATAAAGGA AAGGCCATCG TGAAGATGC CTCGGCCGAC AGTGGTCCCA AAGATGGACC CCCACCCAC GAGGAGCATC GTGAAAAAG AAGACGTTCC AACCACGTCT TCAAGGCAAG TGGATTGTG TGATA-3'*,

operably coupled directly to the -72 to +8 promoter fragment of the CaMV 35S gene, said -72 to +8 promoter fragment operably coupled to a structural gene.

*sequence corresponds to the complete domain B from -343 to -90 nucleotides of the 35S CaMV promoter.

**Claim 5**

A tissue-specific promoter fragment which functions in plants to cause tissue-specific expression in the leaves, stems, cotyledons and the vascular tissue of the hypocotyl and detachable levels of expression in root vascular tissue operably coupled directly to a DNA segment corresponding to the -72 to +8 promoter fragment of the Cauliflower Mosaic Virus 35S gene, said tissue-specific promoter fragment having the sequence from its 5' to 3' termini:

5'-TGAGACTTTT CAACAAAGGG TAATATCCCG AAACCTCCTC GGATTCCATT GCCCCAGCTAT CTGTCACTTT ATTGTGAAAA TAGTGAAAAA GGAAGGTTGC TCCTAAAAT GCCATCATGG CGATAAAGGA AAGGCCATCG TGAAGATGC CTCGGCCGAC AGTGGTCCCA AAGATGGACC CCCACCCAC GAGGAGCATC GTGAAAAAG AAGACGTTCC AACCACGTCT TCAAGGCAAG TGGATTGTG TGATA-3'*

*sequence corresponds to the complete domain B -from -343 to -90 nucleotides- of the 35S CaMV promoter.

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**Plant Ubiquitin Promoter System**

Patent application filed by Monsanto

Actual pending independent claims

**EP 1 210 446**

**Claim 1**

A DNA sequence comprising an ubiquitin regulatory system lacking heatshock elements.

**Claim 2**

A DNA sequence comprising an ubiquitin regulatory system that is not heat inducible.
Patents granted to Mycogen

Actual granted independent claims

<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5510474</td>
<td>Claim 1: An isolated DNA fragment, useful in effecting expression in both monocots and dicots of coding sequences placed 3’ to said fragment, wherein said DNA is approximately 2 kb in length, and said fragment further comprises, in the following order beginning with the 5’ most element and proceeding toward the 3’ terminus of said DNA fragment: a) two heat shock elements, which overlap; b) a promoter comprising a transcription start site; c) an intron of about 1 kb in length; d) and a translation start site; wherein said DNA fragment comprising said elements (i)–(iv) regulates gene expression in both dicots and monocots, and wherein DNA fragment comprises the nucleotide sequence shown from position –899 to 1092 of the maize ubiquitin sequence listed in FIG. 2.</td>
</tr>
<tr>
<td>US 5614399</td>
<td>Claim 1: A method for selective heat shock induced enhancement of the constitutive expression of a structural gene in a plant cell comprising the steps of: a) transforming said plant cell with a DNA construct comprising an approximately 2 kb plant ubiquitin regulatory region operably joined to a DNA sequence of interest, wherein said plant ubiquitin regulatory region is from a plant ubiquitin gene and comprises: 1. at least one heat shock element, 2. a promoter, 3. a transcription start site, and 4. an intron; and b) selectively applying stress conditions of high temperature to said transformed plant cell thereby inducing enhancement in expression of said DNA sequence of interest.</td>
</tr>
<tr>
<td>US 6020190</td>
<td>Claim 1: A DNA construct comprising: a) a DNA sequence no larger than 2 kb, said DNA sequence comprising a plant ubiquitin regulatory system, wherein said regulatory system contains: 1. a heat shock element, and 2. an intron, said intron being located at 3’ to said heat shock element, and b) a plant-expressible structural gene wherein said structural gene is placed under the regulatory control of said plant ubiquitin regulatory system.</td>
</tr>
<tr>
<td>US 6054574</td>
<td>(view in patent database)</td>
</tr>
</tbody>
</table>
Claim 1
A DNA fragment of approximately 2 kb in length, said DNA fragment comprising a plant ubiquitin regulatory system, wherein said regulatory system contains:

a) a promoter comprising a transcription start site,
b) one or more heat shock elements positioned 5' to said transcription start site, and
c) an intron positioned 3' to said transcription start site,

wherein said regulatory system is capable of regulating constitutive and inducible gene expression in both dicots and monocots such that the level of said constitutive gene expression in monocots is about one-third that obtained in said inducible gene expression in monocots.

Claim 9
A recombinant DNA construct comprising:

a) a DNA fragment of approximately 2 kb in length, said DNA fragment comprising a plant ubiquitin regulatory system, wherein said plant ubiquitin regulatory system contains:
   1. a promoter comprising a transcription start site,
   2. one or more heat shock elements positioned 5' to said transcription start site,
   3. a translational start site, and
   4. an intron positioned 3' to said transcription start site and 5' to said translational start site,

wherein said plant ubiquitin regulatory system is capable of regulating constitutive and inducible gene expression in both dicots and monocots such that said constitutive gene expression in monocots is at a level about one-third that obtained in said inducible gene expression in monocots, and

b) a plant-expressible heterologous structural gene positioned 3' to said plant ubiquitin regulatory system and
c) a polyadenylation signal positioned 3' to said structural gene,

wherein said heterologous gene is placed under the regulatory control of said plant ubiquitin regulatory system.

Claim 18
A DNA fragment, useful in effecting expression in both monocots and dicots of coding sequences placed 3' to said fragment, wherein said DNA is isolated or incorporated into a larger piece of DNA but in a position other than in the 5' sequence of a plant ubiquitin gene, is approximately 2 kb in length, and said DNA fragment further comprises, in the following order beginning with the 5' most element and proceeding toward the 3' terminus of said DNA fragment:

(a) one or more heat shock elements, which elements may or may not be overlapping;
(b) a promoter comprising a transcription start site; and
(c) an intron of about 1 kb in length;

and wherein said DNA fragment comprising said elements (a)–(c) is capable of regulating gene expression in both dicots and monocots.

wherein said DNA is isolated or incorporated into a larger piece of DNA but in a position other than in the 5' sequence of a plant ubiquitin gene, meaning of this?? Does not regulate the plant ubiquitin gene, is it that? or is not related to the 5'sequence of a plant ubiquitin gene at all??

CA 1339684

Claim 1
A DNA fragment approximately 2kb in length, said DNA fragment comprising a plant ubiquitin regulatory system, wherein said regulatory system contains:

a) overlapping heat shock elements and
b) an intron, and

wherein said regulatory system is capable of regulating constitutive and inducible gene expression in both dicots and monocots.

Claim 11
A recombinant DNA construct comprising:

a) a DNA fragment approximately 2kb in length, said DNA fragment comprising a plant ubiquitin regulatory system, wherein said regulatory system contains:
1. overlapping heat shock elements and
2. an intron; and
   wherein said regulatory system is capable of regulating constitutive and inducible gene
   expression in both dicots and monocots, and

b) a plant-expressible heterologous structural gene wherein said heterologous structural gene is placed
   under the regulatory control of said plant ubiquitin regulatory system.

Claim 23
A method for the constitutive expression of a structural gene and the selected stress-induced enhancement
in expression of said structural gene in a plant cell comprising the steps of:
a) transforming said plant cell with a DNA construct comprising an approximately 2 kb plant ubiquitin
   regulatory system, wherein is found a heat shock element and an intron, and
b) a plant-expressible structural gene that is under the regulatory control of said plant regulatory system,
   and
c) selectively applying stress conditions of high temperature to said transformed plant cell thereby inducing
   enhancement in expression of said structural gene.
Claim 1
A recombinant DNA molecule comprising: (a) an anaerobic regulatory element; (b) a plant-expressible promoter located 3' to said anaerobic regulatory element, and (c) a plant expressible structural gene located 3' to said plant-expressible promoter such that said structural gene is placed under the regulatory control of said promoter and said anaerobic regulatory element, wherein said structural gene is not in nature under the regulatory control of said anaerobic regulatory element.

Claim 25
A method for selective expression of a plant-expressible structural gene under anaerobic conditions in plant tissue which comprises the steps of:

a) constructing a recombinant DNA molecule which comprises
i. an anaerobic regulatory control element;
ii. a plant-expressible promoter located 3' to said anaerobic regulatory element, and
iii. a plant expressible structural gene located 3' to said plant expressible promoter such that said structural gene is placed under the regulatory control of said promoter and said anaerobic regulatory element;
b) transforming said plant tissue with said recombinant DNA molecule, and
c) placing said transformed plant cell under anaerobic conditions so that said plant-expressible structural gene is expressed.

Patent granted to Mycogen Plant Science Inc. and CSIRO

Claim 1
A recombinant DNA molecule comprising:
(a) an anaerobic regulatory element;
(b) a plant-expressible promoter located 3' to said anaerobic regulatory element, and
(c) a plant-expressible structural gene located 3' to said plant-expressible promoter such that said structural gene is placed under the regulatory control of said promoter and said anaerobic regulatory element
wherein said structural gene is not in nature under the regulatory control of said anaerobic regulatory element.

Claim 1
A method for selective expression of a plant-expressible structural gene under anaerobic conditions in plant tissue, which method comprises using as an anaerobic regulatory element a recombinant DNA molecule comprising a sequence selected from:
1) 5'-GCTGGTTTCT-3'
2) 5'-CGTGGTTTGCTTGCC-3', or a sequence having about 66% or greater homology thereto
3) 5'-CGAGCCTTTCTTCCC-3'
4) 5'-CTGCCTCCCTGGTTTCT-3', and
5) 5'-CTGCAGCCCCGGTTTCG-3', or a sequence having about 66% or greater homology thereto

Patent granted to Mycogen Plant Science Inc. and Lubrizol Enterprises

Claim 1
A recombinant DNA molecule comprising:
(a) an anaerobic regulatory element;
(b) a plant-expressible promoter located 3' to said anaerobic regulatory element, and
(c) a plant-expressible structural gene located 3' to said plant-expressible promoter such that said structural gene is placed under the regulatory control of said promoter and said anaerobic regulatory element
wherein said structural gene is not in nature under the regulatory control of said anaerobic regulatory element.

2. Patents on the recombinant promoter EMU

Patent granted to Lubrizol Genetics Inc.
<table>
<thead>
<tr>
<th>Patent Number</th>
<th>Claim 1</th>
<th>Patent Owner</th>
</tr>
</thead>
</table>
| AU 643 521 B2 | A recombinant promoter molecule for enhancing expression of a plant-expressible structural gene in a monocot plant cell comprising:  
a) a plurality of enhancer elements selected from the group consisting of the ARE and the OCS elements;  
b) a truncated, plant expressible promoter providing a TATA box region necessary to initiate transcription positioned 3' to said plurality of enhancer elements; and  
c) a nucleotide sequence naturally found as an intron positioned between the transcription start site and the translation start site in a plant-expressible gene; whereby a plant-expressible structural gene placed 3' to said recombinant promoter molecule is expressed in said monocot plant cell under regulatory control of said recombinant promoter molecule. | Patent granted to Mycogen Plant Science Inc. and CSIRO |
| EP 459 643 B1 | A recombinant promoter molecule for enhancing expression of a plant-expressible structural gene in a monocot plant cell comprising:  
a) a plurality of ARE enhancer elements;  
b) a truncated, plant expressible promoter providing a TATA box region necessary to initiate transcription positioned 3' to said plurality of enhancer elements; and  
c) a nucleotide sequence naturally found as an intron positioned between the transcription start site and the translation start site in a plant-expressible gene; whereby a plant-expressible structural gene placed 3' to said recombinant promoter molecule is expressed in said monocot plant cell under regulatory control of said recombinant promoter molecule. | Patent granted to CSIRO |
| US 5 290 924 | A recombinant promoter molecule, useful for enhancing expression of a plant-expressible structural gene in a monocot plant cell, said promoter molecule comprising:  
a) a plurality of enhancer elements selected from the group consisting of only ARE elements, only OCS elements, and combinations of ARE and OCS elements;  
b) a truncated, plant expressible promoter, providing a TATA box region and a transcription start site, said promoter selected from the group consisting of .DELTA.35S and .DELTA.ADH positioned 3' to said plurality of enhancer elements wherein said truncated promoter excludes the presence of enhancer sequences and wherein said truncated promoter is recombined with said plurality of enhancer elements positioned 5' to said truncated promoter; and  
c) a maize Adh1 intron positioned 3' to said transcription start site whereby a plant-expressible structural gene, placed 3' to said recombinant promoter molecule, is expressed in said monocot plant cell under regulatory control of said recombinant promoter molecule. | Synthetic promoters. Patents granted to Pioneer Hi-Bred International Inc. |
| US 6 072 050 | (view in patent database)                                                                 | Actual granted independent claims      |
Claim 1
A synthetic DNA promoter sequence functional in a plant cell, said promoter sequence comprising:

- a TATA motif,
- a transcription start site, and
- a region between said TATA motif and said start site that is at least 64% GC-rich;

wherein said region is not a region between a TATA motif and a transcription start site of native maize ubiquitin promoter, and

wherein said promoter sequence is set forth in SEQ ID NO:10.

Claim 2
A synthetic DNA promoter sequence functional in a plant cell, said promoter sequence comprising:

- a TATA motif,
- a transcription start site, and
- a region between said TATA motif and said start site that is at least 64% GC-rich;

wherein said region is not a region between a TATA motif and a transcription start site of native maize ubiquitin promoter, and

wherein said promoter sequence is set forth in SEQ ID NO:1.

Claim 3
An expression cassette comprising

- a synthetic promoter comprising:
  - a TATA motif,
  - a transcription start site and
  - a region between said TATA motif and said start site that is at least 64% GC rich,
  - a structural gene operatively linked to said promoter, and
  - a transcription end site polyadenylation signal;

wherein said region is not a region between a TATA motif and a transcription start site of native maize ubiquitin promoter, and

wherein sequence of said promoter is set forth in SEQ ID NO:1.

Claim 4
An expression cassette comprising

- a synthetic promoter comprising:
  - a TATA motif,
  - a transcription start site and
  - a region between said TATA motif and said start site that is at least 64% GC rich,
  - a structural gene operatively linked to said promoter, and
  - a transcription end site polyadenylation signal;

wherein said region is not a region between a TATA motif and a transcription start site of native maize ubiquitin promoter, and

wherein sequence of said promoter is set forth in SEQ ID NO:10.

Claim 5
An expression cassette comprising

- a synthetic promoter comprising:
  - a TATA motif,
  - a transcription start site and
  - a region between said TATA motif and said start site that is at least 64% GC rich,
  - a structural gene operatively linked to said promoter,
  - a transcription end site polyadenylation signal, and
  - an upstream element operatively linked to said promoter so that transcription is enhanced;

wherein said region is not a region between a TATA motif and a transcription start site of native maize ubiquitin promoter; and

wherein sequence of said upstream element is set forth in SEQ ID NO:2.

Claim 7
A synthetic upstream element having a sequence set forth in SEQ ID NO:2.

Claim 8
An expression cassette comprising:
a promoter sequence;
a structural gene operatively linked to said promoter sequence;
a polyadenylation signal; and
a synthetic upstream element comprising SEQ ID NO:2 operatively linked to said promoter so that
expression is enhanced.

Claim 13
An isolated nucleotide sequence comprising a DNA enhancer sequence comprising the nucleotide sequence set forth in SEQ ID No: 5.

Claim 14
A nucleotide sequence comprising a promoter construct, said construct comprising in operable linkage a core promoter sequence and a Ubi-1 UAR, wherein said Ubi-1 UAR is a maize Ubi UAR comprising the sequence set forth in SEQ ID No: 13.

Claim 15
An expression cassette comprising in operable linkage:

a core promoter sequence,
a Ubi UAR operably linked upstream to said core promoter to form a synthetic promoter construct,
a nucleotide sequence of interest operably linked to said synthetic promoter, and
a polyadenylation signal;
wherein said Ubi-1 UAR comprises the sequence set forth in SEQ ID No:13.

AU 729 929 B2
(view in patent database)

Claim 1
A synthetic DNA plant promoter sequence, said sequence comprising:

a TATA motif,
a transcription start site, and
a region between said TATA motif and said start site that is at least 64% GC-rich.

Claim 3
An expression cassette comprising

a synthetic promoter comprising
a TATA motif,
a transcription start site and
a region there between that is at least 65% GC rich,
a structural gene operatively linked to said promoter, and
a transcription end site polyadenylation signal.

Claim 17
A synthetic upstream element having a sequence of SEQ ID NO:2.

Claim 18
An expression cassette comprising:

a promoter sequence;
a structural gene operatively linked to said promoter sequence;
a polyadenylation signal; and
a synthetic upstream element comprising SEQ ID NO:2 operatively linked to said promoter sequence so that
expression is enhanced.

Claim 23
A synthetic DNA plant promoter sequence functional in a plant cell, said sequence comprising:

a TATA motif,
a transcription start site, and
a region between said TATA motif and said start site that is at least 64% GC-rich; wherein said promoter sequence is less than 1000 bp.
Claim 1
A synthetic DNA plant promoter sequence, said sequence comprising:

- a TATA motif;
- a transcription start site; and
- a region between said TATA motif and said start site that is at least about 64% GC-rich.

Claim 4
An expression cassette comprising:

- a synthetic promoter comprising:
  - a TATA motif;
  - a transcription start site and a region there between that is at least about 64% GC rich;
  - a structural gene operatively linked to said promoter; and
  - a transcription end site polyadenylation signal.

Claim 19
A synthetic upstream element having a sequence of SEQ ID NO: 2.

Claim 20
An expression cassette comprising:

- a promoter sequence;
- a structural gene operatively linked to said promoter sequence;
- a polyadenylation signal; and
- a synthetic upstream element homologous to SEQ ID NO: 2 operatively linked to said promoter so that expression is enhanced.

Claim 25
A DNA sequence comprising:

- a promoter construct, said construct comprising in operable linkage:
  - a core synthetic promoter sequence comprising a TATA motif,
  - a transcription start site, and
  - a region between said TATA motif and said start site that is at least 64% GC-rich; and
  - an upstream activating region operably linked to said core synthetic promoter.

Claim 32
An expression cassette comprising:

- a core synthetic promoter sequence comprising a TATA motif,
- a transcription start site, and
- a region between said TATA motif and said start site that is at least 64% GC-rich;
- an upstream activating region operably linked to said synthetic promoter to enhance transcription;
- a structural gene operably linked to said synthetic promoter; and
- a polyadenylation signal.

Claim 38
A method for controlling the level of expression of a transgenic nucleotide sequence in a plant cell said method comprising transforming with an expression cassette comprising a promoter having at least one Ubi–1 UAR.

Claim 40
A nucleotide sequence comprising:

- a promoter construct, said construct comprising in operable linkage:
  - a core promoter sequence; and
  - a Ubi–1 UAR.

Claim 46
An expression cassette comprising in operable linkage:

- a core promoter sequence;
- a Ubi UAR operably linked to said core promoter to form a synthetic promoter construct;
- a nucleotide sequence of interest operably linked to said synthetic promoter; and
- a polyadenylation signal.
Claim 1
A synthetic DNA plant promoter sequence, said sequence comprising:

- a TATA motif;
- a transcription start site; and
- a region between said TATA motif and said start site that is at least 64% GC rich.

Claim 4
An expression cassette comprising:

- a synthetic promoter comprising:
  - a TATA motif;
  - a transcription start site and
  - a region there between that is at least 64% GC rich;
  - a structural gene operatively linked to said promoter; and
  - a transcription end site polyadenylation signal.

Claim 19
A synthetic upstream element having a sequence of SEQ ID NO:2.

Claim 20
An expression cassette comprising:

- a promoter sequence;
- a structural gene operatively linked to said promoter sequence;
- a polyadenylation signal; and
- a synthetic upstream element comprising SEQ ID NO:2 operatively linked to said promoter so that expression is enhanced.

Claim 25
A synthetic DNA promoter sequence functional in plant cells, wherein said DNA sequence is synthetically created and non-naturally occurring, consisting of:

- a TATA motif;
- a transcriptional start site; and
- a region between said TATA motif and said start site that is at least 64% GC rich.

Claim 26
A synthetic DNA plant promoter sequence functional in plant cells, said sequence comprising:

- a TATA motif;
- a transcription start site;
- a region between said TATA motif and said start site that is at least 64% GC rich;
- wherein said promoter sequence is less than 1900 bp.

US 2001/0047092 A1 and WO 01/53476 A2

Claim 1
A plant promoter comprising at least one synthetic multimeric promoter element region having a nucleotide sequence selected from the group consisting of:

- a nucleotide sequence comprising six DRE 1 (SEQ ID NO.: 59), two ABRE1 (SEQ ID NO.: 2), three As-1 (SEQ ID NO.: 7), one GT-2 (SEQ ID NO.: 24), and two PCNA IIA (SEQ ID NO.: 45) promoter elements;
- a nucleotide sequence comprising three DRE 1 (SEQ ID NO.: 59), three ABRE1 (SEQ ID NO.: 2), one As-1 (SEQ ID NO.: 7), two GT-2 (SEQ ID NO.: 24), and two PCNA IIA (SEQ ID NO.: 45) promoter elements;
- a nucleotide sequence comprising five DRE 1 (SEQ ID NO.: 59), three ABRE1 (SEQ ID NO.: 2), two As-1 (SEQ ID NO.: 7), and five GT-2 (SEQ ID NO.: 24) promoter elements;
- a nucleotide sequence comprising four DRE 1 (SEQ ID NO.: 59), three ABRE1 (SEQ ID NO.: 2), three GT-2 (SEQ ID NO.: 24), and one PCNA IIA (SEQ ID NO.: 45) promoter elements;
- a nucleotide sequence comprising two DRE 1 (SEQ ID NO.: 59), one ABRE1 (SEQ ID NO.: 2), five As-1 (SEQ ID NO.: 7), one GT-2 (SEQ ID NO.: 24), and three PCNA IIA (SEQ ID NO.: 45) promoter elements;
- a nucleotide sequence comprising five DRE 1 (SEQ ID NO.: 59), two ABRE1 (SEQ ID NO.: 2), one As-1 (SEQ ID NO.: 7), one GT-2 (SEQ ID NO.: 24), and two PCNA IIA (SEQ ID NO.: 45) promoter elements;
- a nucleotide sequence comprising one DRE 1 (SEQ ID NO.: 59), two ABRE1 (SEQ ID NO.: 2), two As-1 (SEQ ID NO.: 7), and one GT-2 (SEQ ID NO.: 24) promoter elements;
- a nucleotide sequence comprising two DRE 1, one ABRE 1 (SEQ ID NO.: 2), three As-1 (SEQ ID NO.: 7),
and one GT-2 (SEQ ID NO.: 24) promoter elements; and

i) a nucleotide sequence that hybridizes under stringent conditions to any of the nucleotide sequences of a),
b), c), d), e), f), g), and h).

Claim 7
A plant, or its parts, having stably incorporated into its genome a DNA construct comprising a plant promoter operably linked to a coding sequence, said plant promoter comprising at least one synthetic multimeric promoter element region (SMPER) that enhances expression of said coding sequence.

Claim 8
A plant, or its parts, having stably incorporated into its genome a DNA construct comprising a plant promoter operably linked to a coding sequences, said plant promoter comprising at least one synthetic multimeric promoter element region having a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence comprising promoter elements DRE1, ABRE1, DRE1, As-1, ABRE1, DRE1, GT-2, As-1, DRE1, PCNA IIA, PCNA IIA, DRE1, As-1, and DRE1 sequentially (SEQ ID NO.: 66);
b) a nucleotide sequence comprising promoter elements DRE1, DRE1, As-1, PCNA IIA, ABRE1, PCNA IIA, ABRE1, DRE1, GT-2, GT-2, and ABRE1 sequentially (SEQ ID NO.: 67);
c) a nucleotide sequence comprising promoter elements GT-2, ABRE1, ABRE1, GT-2, As-1, GT-2, GT-2, DRE1, GT-2, DRE1, As-1, DRE1, DRE1, and ABRE1 sequentially (SEQ ID NO.: 65);
d) a nucleotide sequence comprising promoter elements ABRE1, ABRE1, GT-2, GT-2, GT-2, DRE1, DRE1, DRE1, ABRE1, and PCNA IIA sequentially (SEQ ID NO.: 68);
e) a nucleotide sequence comprising promoter elements PCNA IIA, As-1, GT-2, As-1, DRE1, As-1, As-1, PCNA IIA, As-1, PCNA IIA, DRE1, and ABRE1 sequentially (SEQ ID NO.: 69);
f) a nucleotide sequence comprising promoter elements As-1, GT-2, DRE1, DRE1, ABRE1, PCNA IIA, DRE1, PCNA IIA, ABRE1, DRE1, and DRE1 sequentially (SEQ ID NO.: 71);
g) a nucleotide sequence comprising promoter elements As-1, ABRE1, GT-2, As-1, ABRE1, and DRE1 sequentially (SEQ ID NO.: 72);
h) a nucleotide sequence comprising promoter elements DRE1, ABRE1, GT-2, DRE1, As-1, As-1, and As-1 sequentially (SEQ ID NO.: 70);
i) a nucleotide sequence set forth in FIGS. 7, 8, 9, 10, 11, 12, 13, or 14 (SEQ ID NOS.: 65-72); and
j) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j).

Claim 12
A plant cell having stably incorporated into its genome a DNA construct comprising a plant promoter operably linked to a coding sequence, said plant promoter comprising at least one synthetic multimeric promoter element region having a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence comprising promoter elements DRE1, ABRE1, DRE1, As-1, ABRE1, DRE1, GT-2, As-1, DRE1, PCNA IIA, PCNA IIA, DRE1, As-1, and DRE1 sequentially (SEQ ID NO.: 66);
b) a nucleotide sequence comprising promoter elements DRE1, DRE1, As-1, PCNA IIA, ABRE1, PCNA IIA, ABRE1, DRE1, GT-2, GT-2, and ABRE1 sequentially (SEQ ID NO.: 67);
c) a nucleotide sequence comprising promoter elements GT-2, ABRE1, ABRE1, GT-2, As-1, GT-2, GT-2, DRE1, GT-2, DRE1, As-1, DRE1, DRE1, and ABRE1 sequentially (SEQ ID NO.: 65);
d) a nucleotide sequence comprising promoter elements ABRE1, ABRE1, GT-2, GT-2, GT-2, DRE1, DRE1, DRE1, ABRE1 and PCNA IIA sequentially (SEQ ID NO.: 68);
e) a nucleotide sequence comprising promoter elements PCNA IIA, As-1, GT-2, As-1, DRE1, As-1, As-1, PCNA IIA, As-1, PCNA IIA, DRE1, and ABRE1 sequentially (SEQ ID NO.: 69);
f) a nucleotide sequence comprising promoter elements As-1, GT-2, DRE1, DRE1, ABRE1, PCNA IIA, DRE1, PCNA IIA, ABRE1, DRE1, and DRE1 sequentially (SEQ ID NO.: 71);
g) a nucleotide sequence comprising promoter elements As-1, ABRE1, GT-2, As-1, ABRE1, and DRE1 sequentially (SEQ ID NO.: 72);
h) a nucleotide sequence comprising promoter elements DRE1, ABRE1, GT-2, DRE1, As-1, As-1, and As-1 sequentially (SEQ ID NO.: 70);
i) a nucleotide sequence set forth in FIGS. 7, 8, 9, 10, 11, 12, 13, or 14 (SEQ ID NOS.: 65-72); and
j) a nucleotide sequence that comprises a variant of a nucleotide sequence set forth in FIGS. 7, 8, 9, 10, 11, 12, 13, or 14 (SEQ ID NOS.: 65-72); and
k) a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j).
Claim 17
A method of selecting promoter elements active in a tissue of interest, comprising:

a) isolating or synthesizing oligonucleotides representing known or putative promoter elements or transcription factor binding sites;
b) labeling said oligonucleotides;
c) pooling said oligonucleotides to create an array which facilitates screening;
d) hybridizing said oligonucleotides with nuclear extracts of said tissue of interest; and
e) selecting those oligonucleotides exhibiting preferential binding to said nuclear extracts.

Claim 18
A method of creating synthetic multimeric promoter element regions active in a tissue of interest, comprising:

a) selecting known or putative promoter elements or transcription factor binding sites which exhibit preferential binding to nuclear extract prepared from said tissue of interest;
b) combining said selected oligonucleotides in novel arrangements encompassing variation in number of copies, sequential order, orientation, and spacer regions; and
c) testing said novel arrangements for their effect on transcription and selecting those demonstrating enhancement or suppression of linked gene expression.

Appendix 3
Claims

- Alcohol-inducible promoters
- Tetracycline-regulated promoters
- Steroid-responsive promoters
- Metal-responsive promoters
- Pathogenesis-related (PR) promoters
- Physically-regulated promoters

Alcohol-inducible promoters

Patent granted to Syngenta

Actual granted independent claims

EP 637 339 B1

(view in patent database)

Claim 1
A chemically-inducible plant gene expression cassette comprising:

a first promoter operatively linked to the alcR regulator sequence obtainable from Aspergillus nidulans which encodes the AlcR regulator protein, and
an inducible promoter operatively linked to a target gene, the inducible promoter being activated by the regulator protein in the presence of an effective exogenous inducer whereby application of the inducer causes expression of the target gene.

Claim 11
A method for controlling plant gene expression comprising:

transforming a plant cell with a chemically-inducible plant gene expression cassette which has a first promoter operatively linked to the alcR regulator sequence obtainable from Aspergillus nidulans which encodes the AlcR regulator protein, and
an inducible promoter operatively linked to a target gene, the inducible promoter being activated by the regulator protein in the presence of an effective exogenous inducer whereby application of the inducer causes expression of the target gene.
A chimeric promoter comprising an upstream region containing a promoter regulatory sequence obtainable from the *alcA* gene promoter of *Aspergillus nidulans* and a downstream region containing a transcription initiation sequence, characterized in that said upstream and downstream regions are heterologous, the promoter is chemically-inducible and the transcription initiation sequence is obtainable from the core promoter region of a promoter which is active in plant cells.

**Tetracycline-regulated promoters**

**Patent granted to Yale University**

Actual granted independent claims

**US 5 851 796**

(view in patent database)

**Claim 1**
A polynucleotide comprising a nucleotide sequence encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eukaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence.

**Claim 23**
A method to inhibit expression of a heterologous protein in a eukaryotic cell comprising:

a) obtaining a eukaryotic cell comprising:

1. a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a procaryotic tet repressor and a eukaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence;

2. a second polynucleotide molecule encoding the heterologous protein, said second polynucleotide molecule being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and

b) cultivating the eukaryotic cell in a medium comprising tetracycline or a tetracycline analogue such that expression of the heterologous protein is inhibited.

**Claim 25**
A method to enhance the expression of a heterologous protein in a eukaryotic cell comprising:

a) obtaining a eukaryotic cell comprising:

1. a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a procaryotic tet repressor and a eukaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence;

2. a second polynucleotide molecule encoding the heterologous protein, said second polynucleotide molecule being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and

b) cultivating the eukaryotic cell in a medium lacking tetracycline or a tetracycline analogue such that expression of the heterologous protein is enhanced.
Claim 26
A method to activate the expression of a heterologous protein in a eucaryotic cell comprising:

a) obtaining a eucaryotic cell comprising:

1. a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence;

2. a second polynucleotide molecule encoding the heterologous protein, said second polynucleotide molecule being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and

b) cultivating the eucaryotic cell in a medium lacking tetracycline or a tetracycline analogue such that expression of the heterologous protein is activated.

Claim 27
A kit comprising a carrier means having in close confinement therein at least two container means, wherein

a) a first container means contains a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; and

b) a second container means contains a second polynucleotide molecule encoding said inducible minimal promoter, which promoter contains at least one tet operator sequence, which tet operator sequence is strategically positioned for being operably linked to a heterologous polynucleotide sequence encoding a polypeptide.

Claim 28
A kit comprising a carrier means having in close confinement therein at least two container means, wherein

a) a first container means contains a eucaryotic cell transfected with a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; and

b) a second container means contains a second polynucleotide molecule comprising an inducible minimal promoter, which promoter contains at least one tet operator sequence, which tet operator sequence is strategically positioned for being operably linked to a heterologous polynucleotide sequence encoding a heterologous polypeptide.

EP 832 254 A1

Claim 1
A composition of matter comprising a polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence.

Claim 11
A method to decrease or shut off expression of a heterologous protein comprising:

a) transforming a eucaryotic cell with

1. a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and a eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence;
2. a second polynucleotide molecule encoding the heterologous protein, said protein being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and

b) cultivating the eucaryotic cell in a medium comprising tetracycline or a tetracycline analogue.

Claim 13
A method to activate or enhance the expression of a heterologous protein comprising:

a) transforming a eucaryotic cell with

i. a first polynucleotide molecule encoding tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and an eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible promoter, which promoter contains at least one tet operator sequence;

ii. a second polynucleotide molecule encoding the heterologous protein, said protein being operably linked to an inducible minimal promoter, and said promoter containing at least one tet operator sequence; and

b) cultivating the eucaryotic cell in a medium lacking tetracycline or a tetracycline analogue.

Claim 17
A composition of matter consisting essentially of the plasmid pTet–Splice.

Claim 18
A composition of matter consisting essentially of the plasmid pTet–tTAK.

Claim 19
A kit comprising a carrier means having in close confinement therein at least two container means, wherein

a) a first container means contains a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and an eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; and

b) a second container means contains a second polynucleotide molecule encoding said inducible minimal promoter, which promoter contains at least one tet operator sequence, which tet operator sequence is strategically positioned for being operably linked to a heterologous polynucleotide sequence encoding a polypeptide.

Claim 20
A kit comprising a carrier means having in close confinement therein at least two container means, wherein

a) a first container means contains a eucaryotic cell transfected with a first polynucleotide molecule encoding a tetracycline transactivator fusion protein, said protein comprising a prokaryotic tet repressor and an eucaryotic transcriptional activator protein, and said polynucleotide molecule being operably linked to an inducible minimal promoter, which promoter contains at least one tet operator sequence; and

b) a second container means contains a second polynucleotide molecule comprising an inducible minimal promoter, which promoter contains at least one tet operator sequence, which tet operator sequence is strategically positioned for being operably linked to a heterologous polynucleotide sequence encoding a heterologous polypeptide.

Patents granted to BASF

Title: Tight control of gene expression in eucaryotic cells by tetracycline-responsive promoters

Actual granted independent claims

US 5 464 758

(view in patent database)
<table>
<thead>
<tr>
<th>Claim 1</th>
<th>A polynucleotide molecule coding for a transactivator fusion protein comprising:</th>
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<tbody>
<tr>
<td>a) a prokaryotic Tet repressor and</td>
<td></td>
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<tr>
<td>b) a eucaryotic transcriptional activator protein domain.</td>
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</tbody>
</table>

| Claim 10 | A polynucleotide molecule coding for a protein, wherein said polynucleotide is operably linked to a minimal promoter and at least one tet operator sequence. |

<table>
<thead>
<tr>
<th>Claim 16</th>
<th>A eucaryotic cell transfected with</th>
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<tr>
<td>a) a first polynucleotide molecule coding for a transactivator fusion protein comprising:</td>
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<td></td>
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<tr>
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</tr>
</tbody>
</table>

US 5 650 298
(view in patent database)

| Claim 1 | An isolated DNA molecule for integrating a polynucleotide sequence encoding a tetracycline-controllable transactivator (tTA) at a predetermined location in a second target DNA molecule, the tTA comprising a prokaryotic Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells, the DNA molecule comprising a polynucleotide sequence encoding the tTA flanked at 5' and 3' ends by additional polynucleotide sequences of sufficient length for homologous recombination between the DNA molecule and the second target DNA molecule at a predetermined location. |

| Claim 11 | An isolated DNA molecule for integrating a polynucleotide sequence encoding a tetracycline-controllable transactivator (tTA) and a tTA-responsive promoter within a predetermined gene of interest in a second target DNA molecule, the DNA molecule comprising: |
| a) a first polynucleotide sequence comprising a 5' flanking regulatory region of the gene of interest, operably linked to; |
| b) a second polynucleotide sequence encoding a tTA, the tTA comprising a prokaryotic Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells; and |
| c) a third polynucleotide sequence comprising a tTA-responsive promoter, operably linked to; |
| d) a fourth polynucleotide sequence comprising at least a portion of a coding region of the gene of interest; |
wherein the first and fourth polynucleotide sequences are of sufficient length for homologous recombination between the DNA molecule and the gene of interest in the second target DNA molecule such that expression of the tTA is controlled by 5' regulatory elements of the gene of interest and expression of the gene of interest is controlled by the tTA-responsive promoter.

CA 2165162

Claim 1
Worded the same as claim 1 of US 5 650 298.

Claim 11
Worded the same as claim 11 of US 5 650 298.

Claim 42
Use of tetracycline or a tetracycline analogue for the inhibition of a second transgene in a transgenic animal, said animal having:

a) a first transgene comprising a polynucleotide sequence encoding a tetracycline-controllable transactivator (tTA), the tTA comprising a prokaryotic Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells; and
b) the second transgene comprising a gene of interest operably linked to a tTA-responsive promoter.

Claim 43
The use of tetracycline or a tetracycline analogue for the inhibition of transcription of a second transgene in a transgenic animal, said animal having:

a) a polynucleotide sequence encoding a tetracycline-controllable transactivator (tTA), the tTA comprising a prokaryotic Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells, wherein the first transgene is integrated by homologous recombination at a predetermined location within a chromosome within cells of the animal; and
b) the second transgene comprising a gene of interest operably linked to a tTA-responsive promoter.

Claim 44
Use of tetracycline or a tetracycline analogue for inhibiting transcription of the gene of interest in a transgenic animal having a transgene comprising a polynucleotide sequence encoding a tetracycline-controllable transactivator (tTA) and a tTA-responsive promoter, wherein the transgene is integrated by homologous recombination at a predetermined location within a gene of interest within cells of the animal such that expression of the tTA is controlled by 5’ regulatory elements of the gene of interest and expression of the gene of interest is controlled by the tTA-responsive promoter.

Claim 48
A method for producing a non-human transgenic animal comprising:

a) introducing a DNA molecule encoding the tTA into a fertilized oocyte;
b) implanting the fertilized oocyte in a pseudopregnant foster mother; and
c) allowing the fertilized oocyte to develop into the non-human transgenic animal to thereby produce the non-human transgenic animal.

AU 684 524 B2

Claim 1
Worded the same as Claim 1 of Canadian patent and US 5 650 298.

Claim 11
Worded the same as Claim 11 of Canadian patent and US 5 650 298.

Claim 42
A non-human transgenic animal having a transgene comprising a polynucleotide sequence encoding a tetracycline-controllable transactivator (tTA), the tTA comprising a prokaryotic Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells.

Claim 52
A non-human transgenic animal having a transgene comprising a polynucleotide sequence encoding a tetracycline-controllable transactivator (tTA), the tTA comprising a prokaryotic Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells.
activates transcription in eucaryotic cells, wherein the transgene is integrated by homologous recombination at a predetermined location within a chromosome within cells of the animal.

Claim 55
A transgenic animal having a transgene comprising a polynucleotide sequence encoding a tetracycline–controllable transactivator (tTA) and a tTA–responsive promoter, wherein the transgene is integrated by homologous recombination at a predetermined location within a gene of interest within cells of the animal such that expression of the tTA is controlled by 5′ regulatory elements of the gene of interest and expression of the gene of interest is controlled by the tTA–responsive promoter.

US 5 589 362
(view in patent database)

Claim 1
An isolated nucleic acid molecule encoding a fusion protein which regulates transcription, the fusion protein comprising a Tet repressor having at least one amino acid mutation that confers on the fusion protein an ability to bind a class B tet operator sequence having a nucleotide substitution at position +4 or +6, operatively linked to a polypeptide which regulates transcription in eukaryotic cells.

Claim 13
A method for regulating transcription of a tet operator–linked gene in an isolated cell, comprising:

a) introducing into the isolated cell a nucleic acid molecule encoding a fusion protein which regulates transcription, the fusion protein comprising a Tet repressor having at least one amino acid mutation that confers on the fusion protein an ability to bind a class B tet operator sequence having a nucleotide substitution at position 14 or 16, operatively linked to a polypeptide which regulates transcription in eukaryotic cells; and
b) modulating the concentration of a tetracycline, or analogue thereof, in contact with the isolated cell.

US 5 654 168
(view in patent database)

Claim 1
An isolated nucleic acid encoding a fusion protein which activates transcription, the fusion protein comprising a first polypeptide which binds to a tet operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to a second polypeptide which activates transcription in eukaryotic cells.

Claim 26
A kit comprising a carrier means having in close confinement therein at least two container means comprising:

a) a first container means containing a first nucleic acid encoding a fusion protein which activates transcription, the fusion protein comprising a polypeptide which binds to a first class of tet operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to a polypeptide which activates transcription in eukaryotic cells; and
b) a second container means containing a second nucleic acid comprising a first cloning site for introduction of a first nucleotide sequence to be transcribed operatively linked to at least one tet operator sequence of a first class type.

US 5 789 156
(view in patent database)

Claim 1
An isolated nucleic acid encoding a fusion protein which inhibits transcription in eukaryotic cells, the fusion protein comprising a first polypeptide which binds to tet operator sequences, operatively linked to a heterologous second polypeptide which inhibits transcription in eukaryotic cells.
Claim 46
A kit comprising a carrier means having in close confinement therein at least two container means comprising:

a) a first container means containing a first nucleic acid encoding a fusion protein which inhibits transcription in eukaryotic cells, the fusion protein comprising

i. a first polypeptide which binds to tet operator sequences either

ii. in the presence but not the absence of tetracycline or a tetracycline analogue or

iii. in the absence but not the presence of tetracycline or a tetracycline analogue, operatively linked to a heterologous second polypeptide which inhibits transcription in eukaryotic cells, or

a eukaryotic cell line into which said first nucleic acid has been stably introduced; and

b) a second container means containing a second nucleic acid comprising a cloning site for introduction of a nucleotide sequence to be transcribed operatively linked to at least one tet operator sequence.

Patents entitled: "Methods for regulating gene expression"

US 5 814 618

Claim 1
A method for regulating expression of a tet operator-linked gene in a cell of a subject, comprising:

a) introducing into the cell a nucleic acid molecule encoding a fusion protein which inhibits transcription in eukaryotic cells, the fusion protein comprising

a first polypeptide which binds to a tet operator sequence, operatively linked to a heterologous second polypeptide which inhibits transcription in eukaryotic cells; and

b) modulating the concentration of a tetracycline, or analogue thereof, in the subject.

US 5 888 981

Claim 17
A method for regulating expression of a gene in a cell of a subject, comprising:

a) obtaining the cell from the subject;

b) introducing into the cell a first nucleic acid molecule which operatively links a gene to at least one tet operator sequence;

c) introducing into the cell a second nucleic acid molecule encoding a fusion protein which inhibits transcription, the fusion protein comprising

a first polypeptide which binds to a tet operator sequence, operatively linked to a second polypeptide which inhibits transcription in eukaryotic cells, to form a modified cell;

d) administering the modified cell to the subject; and

e) modulating the concentration of a tetracycline, or analogue thereof, in the subject.
transactivator (tTA), the tTA comprising a Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells, to form a modified cell;
d) administering the modified cell to the subject; and
e) modulating the concentration of a tetracycline, or analogue thereof, in the subject.

Claim 1
A method for regulating expression of a tet operator–linked gene in a cell of a subject, comprising:
a) introducing into the cell a nucleic acid molecule encoding a fusion protein which activates transcription, the fusion protein comprising
a first polypeptide which binds to a tet operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to
a second polypeptide which activates transcription in eukaryotic cells; and
b) modulating the concentration of a tetracycline, or analogue thereof, in the subject, such that expression of a tet operator–linked gene in a cell of the subject is regulated.

Claim 16
A method for regulating expression of a gene in a cell of a subject, comprising:
a) obtaining the cell from the subject;
b) introducing into the cell a first nucleic acid molecule which operatively links a gene to at least one tet operator sequence;
c) introducing into the cell a second nucleic acid molecule encoding a fusion protein which activates transcription, the fusion protein comprising
a first polypeptide which binds to a tet operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to
a second polypeptide which activates transcription in eukaryotic cells, to form a modified cell;
d) administering the modified cell to the subject; and
e) modulating the concentration of a tetracycline, or analogue thereof, in the subject such that expression of the gene which is operatively linked to at least one tet operator sequence is regulated in a cell of the subject.

Claim 28
An isolated recombinant vector for coordinate, bidirectional transcription of a first and a second nucleotide sequence, the vector comprising a nucleotide sequence comprising in a 5' to 3' direction:
a) a first cloning site for introduction of a first nucleotide sequence to be transcribed, operatively linked to
b) at least one tet operator sequence, operatively linked to
c) a second cloning site for introduction of a second nucleotide sequence transcribed,
the vector further comprising additional regulatory sequences such that the vector is sufficient for use in eukaryotic cells,
wherein transcription of a first and second nucleotide sequence introduced into the vector proceeds in opposite directions relative to the at least one tet operator sequence.

Claim 35
An isolated nucleic acid composition comprising at least one recombinant vector for independent regulation of transcription of a first and a second nucleotide sequence, the nucleic acid composition comprising nucleotide sequences comprising:
a) a first cloning site for introduction of a first nucleotide sequence to be transcribed, operatively linked to
b) at least one tet operator sequence of a first class type; and
b) a second cloning site for introduction of a second nucleotide sequence to be transcribed, operatively linked to at least one tet operator sequence of a second class type.

Transgenic mice with a tetracycline–responsive transcriptional regulator

Patents granted to BASF
Claim 1
A transgenic mouse having a transgene integrated into the genome of the mouse and also having a tet operator-linked gene in the genome of the mouse, wherein:

a) the transgene comprises a transcriptional regulatory element functional in cells of the mouse operatively linked to a polynucleotide sequence encoding a tetracycline-controllable transactivator fusion protein (tTA),
b) said fusion protein comprises a Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription of said tet operator-linked gene in eucaryotic cells,
c) said tet operator-linked gene confers a detectable and functional phenotype on the mouse when expressed in cells of the mouse,
d) said transgene is expressed in cells of the mouse at a level sufficient to produce amounts of said fusion protein that are sufficient to activate transcription of the tet operator-linked gene; and
e) in the absence of tetracycline or a tetracycline analogue in the mouse, said fusion protein binds to the tet operator-linked gene and activates transcription of the tet operator linked gene such that the tet operator-linked gene is expressed at a level sufficient to confer the detectable and functional phenotype on the mouse,

wherein the level of expression of tet operator-linked gene can be down modulated by administering tetracycline or a tetracycline analogue to the mouse.

Claim 13
A transgenic mouse having a transgene integrated into the genome of the mouse, wherein:

a) the transgene comprises a polynucleotide sequence encoding a tet racycline-controllable transactivator fusion protein (tTA) and a tTA-responsive promoter,
b) said fusion protein comprises a Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription of a gene of interest in eucaryotic cells,
c) the transgene is integrated by homologous recombination at a predetermined location within a said gene of interest within cells of the mouse such that expression of the fusion protein is controlled by 5' regulatory elements of the gene of interest and expression of the gene of interest is controlled by the tTA-responsive promoter, expression of the gene of interest confers a detectable and functional phenotype on the mouse,
d) said transgene is expressed in cells of the mouse at a level sufficient to produce amounts of said fusion protein that are sufficient to activate transcription of the gene of interest linked to the tTA-responsive promoter, and
e) in the absence of tetracycline or a tetracycline analogue in the mouse, said fusion protein binds to the tTA-responsive promoter and activates transcription of the gene of interest such that the gene of interest is expressed at a level sufficient to confer the detectable and functional phenotype on the mouse,
f) wherein the level of expression of the gene of interest can be down modulated by administering tetracycline or a tetracycline analogue to the mouse.

Claim 20
A transgenic mouse having a transgene integrated into the genome of the mouse, wherein:

a) the transgene comprises a transcriptional regulatory element functional in cells of the mouse operatively linked to a polynucleotide sequence encoding a tetracycline-controllable transactivator fusion protein (tTA),
b) said fusion protein comprises a Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription of a tet operator-linked gene in eucaryotic cells, and
c) said fusion protein is expressed in cells of the mouse.
d) said transgene is expressed in cells of the mouse at a level sufficient to produce amounts of said fusion protein that are sufficient to inhibit transcription of the tet operator–linked gene; and

e) in the absence of tetracycline or a tetracycline analogue in the mouse, said fusion protein binds to the tet operator–linked gene and inhibits transcription of the tet operator linked gene,

f) wherein the level of expression of the tet operator–linked gene can be upregulated by administering tetracycline or a tetracycline analogue to the mouse.

Claim 2
A transgenic mouse having a transgene integrated into the genome of the mouse and also having a tet operator–linked gene in the genome of the mouse, wherein:

a) the transgene comprises a transcriptional regulatory element functional in cells of the mouse operatively linked to a polynucleotide sequence encoding a fusion protein which inhibits transcription of said tet operator linked gene,

b) said fusion protein comprises a first polypeptide that is a mutated Tet repressor that binds to tet operator sequences in the presence, but not the absence, of tetracycline or a tetracycline analogue, operably linked to a heterologous second polypeptide which inhibits transcription of
c) said tet operator–linked gene in eucaryotic cells,

d) said tet operator–linked gene confers a detectable and functional phenotype on the mouse when expressed in cells of the mouse,

e) said transgene is expressed in cells of the mouse at a level sufficient to produce amounts of said fusion protein that are sufficient to inhibit transcription of the tet operator–linked gene; and

f) in the presence of tetracycline or a tetracycline analogue in the mouse, said fusion protein binds to the tet operator–linked gene and inhibits transcription of the tet operator linked gene,

g) wherein the level of expression of the tet operator–linked gene can be upregulated by depleting tetracycline or a tetracycline analogue from the mouse.

Claim 3
A transgenic mouse having a transgene integrated into the genome of the mouse, wherein:

a) the transgene comprises a transcriptional regulatory element functional in cells of the mouse operatively linked to a polynucleotide sequence encoding a fusion protein which inhibits transcription of a tet operator–linked gene,

b) the fusion protein comprising a first polypeptide that is a Tet repressor or, a mutated Tet repressor that binds to a tet operator sequence, operatively linked to a second polypeptide which inhibits transcription in eukaryotic cells, and
c) said fusion protein is expressed in cells of the mouse.
Claim 35
A transgenic mouse having a transgene integrated into the genome of the mouse, wherein:

a) the transgene comprises a transcriptional regulatory element functional in cells of the mouse operatively linked to a polynucleotide sequence encoding a fusion protein which activates transcription of a tet operator-linked gene,
b) the fusion protein comprising a first polypeptide which is a mutated Tet repressor that binds to a tet operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to a second polypeptide which activates transcription in eukaryotic cells, and
c) said fusion protein is expressed in cells of the mouse.

Claim 1
A method for producing a transgenic mouse, comprising:

a) introducing into a fertilized oocyte of a mouse a DNA molecule encoding a tetracycline-controllable transactivator (tTA), the tTA comprising a prokaryotic Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells;
b) implanting the fertilized oocyte in a pseudopregnant foster mother; and
c) allowing the fertilized oocyte to develop into a transgenic mouse to thereby produce the transgenic mouse, wherein said tTA is expressed in cells of the mouse at a level sufficient to transactivate a tet operator-linked gene.

Claim 2
A method for producing a transgenic mouse having a transgene encoding a tetracycline-controllable transactivator (tTA) integrated at a predetermined location within chromosomal DNA of cells of the mouse, comprising:

a) introducing into a population of embryonic stem cells of a mouse a DNA molecule encoding a tTA, the DNA molecule comprising:
   1. a first polynucleotide sequence comprising a 5' flanking regulatory region of the gene of interest, operably linked to;
   2. a second polynucleotide sequence encoding a tTA, the tTA comprising a prokaryotic Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells; and
   3. a third polynucleotide sequence comprising a tTA-responsive promoter, operably linked to;
   4. a fourth polynucleotide sequence comprising at least a portion of a coding region of the gene of interest
b) selecting an embryonic stem cell in which DNA encoding the tTA has integrated at a predetermined location within the chromosomal DNA of the cell;
c) implanting the embryonic stem cell into a blastocyst;
d) implanting the blastocyst into a pseudopregnant foster mother; and
e) allowing the blastocyst to develop into a transgenic mouse to thereby produce the transgenic mouse, wherein said tTA is expressed in cells of the mouse at a level sufficient to transactivate a tet operator-linked gene.

Claim 3
A method for producing a transgenic mouse having a transgene encoding a tetracycline-controllable transactivator (tTA) and a tTA-responsive promoter integrated at a predetermined location within a gene of interest in cells of the mouse, comprising:

a) introducing into a population of embryonic stem cells of a mouse a DNA molecule encoding a tTA, the DNA molecule comprising:
   1. a first polynucleotide sequence comprising a 5' flanking regulatory region of the gene of interest, operably linked to;
   2. a second polynucleotide sequence encoding a tTA, the tTA comprising a prokaryotic Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells; and
   3. a third polynucleotide sequence comprising a tTA-responsive promoter, operably linked to;
   4. a fourth polynucleotide sequence comprising at least a portion of a coding region of the gene of interest.
5. wherein the first and fourth polynucleotide sequences are of sufficient length for homologous recombination between the DNA molecule and the gene of interest such that expression of the tTA is controlled by 5' regulatory elements of the gene of interest and expression of the gene of interest is controlled by the tTA-responsive promoter, under conditions suitable for homologous recombination between the DNA molecule encoding the tTA and the gene of interest within the cell;

b) selecting an embryonic stem cell in which DNA encoding the tTA has integrated at a predetermined location within the gene of interest in the cell;

c) implanting the embryonic stem cell into a blastocyst;

d) implanting the blastocyst into a pseudopregnant foster mother; and

e) allowing the blastocyst to develop into a transgenic mouse to thereby produce the transgenic mouse, wherein said gene of interest confers a detectable and functional phenotype on the mouse when expressed in cells of the transgenic mouse, said tTA is expressed in cells of the transgenic mouse at a level sufficient to activate transcription of the gene of interest; and

in the absence of tetracycline or a tetracycline analogue in the mouse, said tTA binds to the tTA responsive promoter operably linked to the gene of interest and activates transcription of the gene of interest such that the gene of interest is expressed at a level sufficient to confer the detectable and functional phenotype on the mouse, wherein the level of expression of the gene of interest can be down modulated by administering tetracycline or a tetracycline analogue to the mouse.

**Claim 1**
A fusion protein which activates transcription comprising:

- a first polypeptide which binds to a tet operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to

- a second polypeptide which activates transcription in eukaryotic cells.

**Claim 1**
A fusion protein which inhibits transcription in eukaryotic cells, the fusion protein comprising:

- a first polypeptide which binds to tet operator sequences, operatively linked to

- a heterologous second polypeptide which inhibits transcription in eukaryotic cells.

Patents entitled: "Transgenic organisms having tetracycline-regulated transcriptional regulatory systems"
c) said transgene is expressed in cells of the plant at a level sufficient to produce amounts of said fusion protein that are sufficient to activate transcription of the \textit{tet} operator–linked gene; and
d) in the presence of tetracycline or a tetracycline analogue in the plant, said fusion protein binds to the \textit{tet} operator–linked gene and activates transcription of the \textit{tet} operator–linked gene such that the \textit{tet} operator–linked gene is expressed at a level sufficient to confer the detectable and functional phenotype on the plant,
wherein the level of expression of the \textit{tet} operator–linked gene can be down modulated by depleting tetracycline or a tetracycline analogue from the plant.

Claim 2

A transgenic plant having a transgene integrated into the genome of the plant, wherein:

a) the transgene comprises
a transcriptional regulatory element functional in cells of the plant operatively linked to a polynucleotide sequence encoding a fusion protein which activates transcription of a \textit{tet} operator linked gene,

the fusion protein comprising
a) a first polypeptide which is a mutated Tet repressor that binds to a \textit{tet} operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to
b) a second polypeptide which activates transcription in eukaryotic cells, and
said fusion protein is expressed in cells of the plant.

Claim 3

A transgenic plant having a transgene integrated into the genome of the plant and also having a \textit{tet} operator–linked gene in the genome of the plant, wherein:

the transgene comprises
a transcriptional regulatory element functional in cells of the plant operatively linked to a polynucleotide sequence encoding a fusion protein which inhibits transcription of said \textit{tet} operator linked gene,

the fusion protein comprises
a first polypeptide which is a mutated Tet repressor that binds to a \textit{tet} operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to
a second polypeptide which inhibits transcription in eukaryotic cells,
said \textit{tet} operator–linked gene confers a detectable and functional phenotype on the plant when expressed in cells of the plant,
said transgene is expressed in cells of the plant at a level sufficient to produce amounts of said fusion protein that are sufficient to inhibit transcription of the \textit{tet} operator–linked gene; and
in the presence of tetracycline or a tetracycline analogue in the plant, said fusion protein binds to the \textit{tet} operator–linked gene and inhibits transcription of the \textit{tet} operator–linked gene, wherein the level of expression of the \textit{tet} operator–linked gene can be upregulated by depleting tetracycline or a tetracycline analogue from the plant.

Claim 4

A transgenic plant having a transgene integrated into the genome of the plant, wherein: the transgene comprises a transcriptional regulatory element functional in cells of the plant operatively linked to a polynucleotide sequence encoding a fusion protein which inhibits transcription of a \textit{tet} operator linked gene, the fusion protein comprising a first polypeptide which is a mutated Tet repressor that binds to a \textit{tet} operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to a second polypeptide which inhibits transcription in eukaryotic cells, and said fusion protein is expressed in cells of the plant.

Claim 5

A transgenic plant having a transgene integrated into the genome of the plant and also having a \textit{tet} operator–linked gene in the genome of the plant, wherein: the transgene comprises a transcriptional regulatory element functional in cells of the plant operatively linked to a polynucleotide sequence encoding a fusion protein which inhibits transcription of a \textit{tet} operator linked gene, said fusion protein comprises a first polypeptide that is a Tet repressor, operably linked to a heterologous second polypeptide which inhibits transcription of said \textit{tet} operator–linked gene in eukaryotic cells, said tet operator–linked gene confers a detectable and functional phenotype on the plant when expressed in cells of the plant, said transgene is expressed in cells of the plant at a level sufficient to produce amounts of said fusion protein that are sufficient to inhibit transcription of the \textit{tet} operator–linked gene; and in the absence of tetracycline or a tetracycline analogue in the plant, said fusion protein binds to the \textit{tet} operator–linked gene and inhibits transcription of the \textit{tet} operator linked gene, wherein the level of expression of the \textit{tet} operator–linked gene can be upregulated by administering tetracycline or a tetracycline analogue to the...
plant.

**Claim 6**
A transgenic plant having a transgene integrated into the genome of the plant, wherein: the transgene comprises a transcriptional regulatory element functional in cells of the plant operatively linked to a polynucleotide sequence encoding a fusion protein which inhibits transcription of a tet operator linked gene, the fusion protein comprising a first polypeptide which is a Tet repressor, operatively linked to a second polypeptide which inhibits transcription in eukaryotic cells, and said fusion protein is expressed in cells of the plant.

**Claim 1**
A transgenic plant having a transgene integrated into the genome of the plant and also having a tet operator–linked gene in the genome of the plant, wherein: the transgene comprises a transcriptional regulatory element functional in cells of the plant operatively linked to a polynucleotide sequence encoding a fusion protein which activates transcription of said tet operator linked gene, the fusion protein comprises a first polypeptide which is a Tet repressor operatively linked to a second polypeptide which directly or indirectly activates transcription in plant cells, said tet operator–linked gene confers a detectable and functional phenotype on the plant when expressed in cells of the plant, said transgene is expressed in cells of the plant at a level sufficient to produce amounts of said fusion protein that are sufficient to activate transcription of the tet operator–linked gene; and in the absence of tetracycline or a tetracycline analogue in the plant, said fusion protein binds to the tet operator–linked gene and activates transcription of the tet operator linked gene such that the tet operator–linked gene is expressed at a level sufficient to confer the detectable and functional phenotype on the plant, wherein the level of expression of the tet operator–linked gene can be down modulated by administering tetracycline or a tetracycline analogue to the plant.

**Claim 7**
A transgenic plant having a transgene integrated into the genome of the plant, wherein: the transgene comprises a transcriptional regulatory element functional in cells of the plant operatively linked to a polynucleotide sequence encoding a fusion protein which activates transcription of a tet operator linked gene, the fusion protein comprising a first polypeptide which is a Tet repressor, operatively linked to a second polypeptide which inhibits transcription in eukaryotic cells, and said fusion protein is expressed in cells of the plant.

**Claim 1**
An isolated nucleic acid encoding a fusion protein which activates transcription, the fusion protein comprising a first polypeptide which binds to a tet operator sequence in the presence, but not the absence, of tetracycline or a tetracycline analogue operatively linked to a second polypeptide which activates transcription in eukaryotic cells.

**Claim 12**
A fusion protein which activates transcription comprising a first polypeptide which binds to a tet operator sequence in the presence, but not the absence, of tetracycline or a tetracycline analogue operatively linked to a second polypeptide which activates transcription in eukaryotic cells.

**Claim 35**
An isolated nucleic acid encoding a fusion protein which inhibits transcription in eukaryotic cells, the fusion protein comprising a first polypeptide which binds to a tet operator sequence operatively linked to a heterologous second polypeptide which inhibits transcription in eukaryotic cells.

**Claim 51**
A fusion protein which inhibits transcription in eukaryotic cells, comprising a first polypeptide which binds to a tet operator sequence operatively linked to a heterologous second polypeptide which inhibits transcription in eukaryotic cells.

**Claim 73**
A host cell comprising:

a) a first nucleic acid encoding a first fusion protein which activates transcription, the first fusion protein comprising a first polypeptide which binds to a tet operator sequence operatively linked to a second polypeptide which activates transcription in eukaryotic cells;

b) a second nucleic acid encoding a second fusion protein which inhibits transcription,
the second fusion protein comprising a third polypeptide which binds to a tet operator sequence operatively linked to a fourth polypeptide which inhibits transcription in eukaryotic cells; and
c) a third nucleic acid molecule comprising a nucleotide sequence to be transcribed operatively linked to at least one tet operator sequence.

Claim 76
A non-human transgenic organism comprising:
a) a first transgene encoding a first fusion protein which activates transcription, the first fusion protein comprising a first polypeptide which binds to a tet operator sequence operatively linked to a second polypeptide which activates transcription in eukaryotic cells;
b) a second transgene encoding a second fusion protein which inhibits transcription, the second fusion protein comprising a third polypeptide which binds to a tet operator sequence operatively linked to a fourth polypeptide which inhibits transcription in eukaryotic cells; and
c) a third transgene comprising a nucleotide sequence to be transcribed operatively linked to at least one tet operator sequence.

Claim 79
A recombinant vector for coordinate, bidirectional transcription of a first and a second nucleotide sequence to be transcribed, the vector comprising a nucleotide sequence comprising in a 5' to 3' direction:
a first cloning site for introduction of a first nucleotide sequence to be transcribed, which is operatively linked to at least one tet operator sequence, which is operatively linked to a second cloning site for introduction of a second nucleotide sequence to be transcribed, wherein transcription of the first and second nucleotide sequence introduced into the vector proceeds in opposite directions relative to the at least one tet operator sequence.

Claim 83
A composition of matter comprising at least one recombinant vector for independent regulation of transcription of a first and a second nucleotide sequence to be transcribed, the at least one vector comprising a nucleotide sequence comprising:
a) a first cloning site for introduction of a first nucleotide sequence to be transcribed, operatively linked to at least one tet operator sequence of a first class type; and
b) a second cloning site for introduction of a second nucleotide sequence to be transcribed, operatively linked to at least one tet operator sequence of a second class type.

Claim 87
A kit comprising a carrier means having in close confinement therein at least two container means comprising:
a) a first container means containing a first nucleic acid encoding a fusion protein which activates transcription, the fusion protein comprising a first polypeptide which binds to a tet operator sequence in the presence of tetracycline or a tetracycline analogue operatively linked to a second polypeptide which activates transcription in eukaryotic cells; and
b) a second container means containing a second nucleic acid comprising a cloning site for introduction of a nucleotide sequence to be transcribed operatively linked to at least one tet operator sequence.

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Claim 1
An isolated nucleic acid encoding a fusion protein which inhibits transcription in eukaryotic cells, the fusion protein comprising:
a) a first polypeptide which is a Tet repressor that binds to a tet operator sequence in the absence but not the presence of tetracycline or a tetracycline analogue operatively linked to
b) a heterologous second polypeptide which inhibits transcription in eukaryotic cells.

Steroid-responsive promoters

1. Promoters based on glucocorticoid receptor (GR)

Actual granted independent claims
Claim 1
An expression vector adapted for replication in an animal cell comprising a glucocorticoid responsive promoter, said promoter comprising:

a) a plurality of at least 5 glucocorticoid response elements (GREs),
b) a viral or mammalian TATA box, and
c) a viral or mammalian initiator element with a transcriptional initiator site located from 20 to 50 bases from said TATA box,

said promoter lacking upstream elements which bind nuclear factor I, and said vector further comprising a restriction endonuclease site downstream from said promoter for insertion of DNA to be expressed from said promoter; wherein said DNA is expressed from said vector in an animal cell.

Claim 10
A promoter consisting of:

a) a plurality of at least five glucocorticoid response elements (GREs),
b) a TATA box, and

c) an initiator site containing a transcriptional initiator site located from 20 to 50 bases from said TATA box,

said promoter lacking upstream elements which bind nuclear factor I, wherein said promoter is responsive to ligand-bound glucocorticoid, progesterone, androgen or mineralocorticoid receptor when transiently transfected into cells, when stably integrated within a genome, or when stably propagated in an episomal vector.

2. Promoters based on estrogen receptor (ER)

Patent applications filed by The Rockefeller University

Actual pending independent claims

Claim 1
A method for selecting transgenic plants comprising a silent selectable marker wherein said method comprises the steps of:

a) transforming a plant cell with a vector wherein said vector comprises DNA encoding a regulatory region of an estrogen receptor and further wherein said vector comprises a gene which promotes shoot formation, wherein said gene is under the control of an inducible promoter;
b) growing said plant cells in the absence of a plant hormone but in the presence of an inducer of said inducible promoter; and
c) excising shoots which develop, wherein said shoots can grow into transgenic plants when grown in the absence of said inducer.

Claim 14
A method for inducing plant somatic embryo formation comprising the steps of:

a) transforming a plant cell with a vector encoding a gene which promotes somatic embryogenesis, wherein said gene is under the control of an inducible promoter; and
b) growing said plant cells in the absence of a plant hormone but in the presence of an inducer of said inducible promoter, wherein somatic embryos will develop.

Claim 26
A method for selecting transgenic plants wherein said method comprises growing a transgenic plant, comprising an antibiotic resistance gene under the control of a promoter comprising DNA encoding a regulatory domain of an estrogen receptor inducible by 17-estradiol or 4-hydroxytamoxifen, in the presence of an antibiotic, wherein said antibiotic is one to which resistance is conferred by said antibiotic resistance gene, and in the presence of 17-estradiol or 4-hydroxytamoxifen.
| Claim 27 |
| A method for selecting transgenic plants wherein said method comprises growing a transgenic plant, comprising a herbicide resistance gene wherein said herbicide resistance gene is under the control of a promoter comprising DNA encoding a regulatory domain of an estrogen receptor inducible by 17-estradiol or 4-hydroxyl tamoxifen, in the presence of a herbicide, wherein said herbicide is one to which resistance is conferred by said herbicide resistance gene, and in the presence of 17-estradiol or 4-hydroxyl tamoxifen. |

| Claim 1 |
| A method for selecting transgenic lettuce plants comprising a silent selectable marker wherein said method comprises the steps of: a) transforming lettuce root cells with a vector wherein said vector comprises a gene selected from the group consisting of an ipt gene, a CKI1 gene, a gene from the knotted family, and a gene the expression of which is capable of promoting shoot regeneration, wherein said gene is under the control of an inducible promoter; b) growing said lettuce root cells to allow shoot development; and c) excising shoots which develop from plants having a shooty phenotype, wherein said shoots can grow into normal transgenic plants when grown in the absence of said inducer. |

| Claim 11 |
| A vector comprising a chemically inducible promoter wherein said vector comprises DNA encoding an estrogen receptor. |

| Claim 25 |
| A vector comprising: |
| i) a constitutive promoter, |
| ii) DNA encoding a DNA binding domain of bacterial repressor LexA, |
| iii) DNA encoding a transactivating domain of VP16, |
| iv) DNA encoding an estrogen receptor, and |
| v) one or more LexA binding sites. |

| Claim 26 |
| A nucleic acid comprising a chemically inducible promoter wherein said nucleic acid further comprises DNA encoding an estrogen receptor. |

| Claim 41 |
| A nucleic acid comprising i) a constitutive promoter, ii) DNA encoding a DNA binding domain of bacterial repressor LexA, iii) DNA encoding a transactivating domain of VP16, iv) DNA encoding an estrogen receptor, and v) one or more LexA binding sites. |

| Claim 42 |
| A transgenic lettuce plant or transgenic lettuce plant cell comprising a vector wherein said vector comprises a chemically inducible promoter. |

| Claim 43 |
| A transgenic plant or transgenic plant cell comprising a vector wherein said vector comprises a chemically inducible promoter which can be induced by an estrogen. |

| Claim 50 |
| A method for making a transgenic plant display a fluorescent design, a word or words wherein said method comprises the steps of: a) preparing a transgenic plant which comprises a luciferase gene under the control of a chemically inducible promoter which is controlled by an estrogen; and b) placing a chemical which induces said chemically inducible promoter onto said transgenic plant in the pattern of the design, word or words which are desired; whereby said plant will produce luciferase and will fluoresce in the pattern in which the chemically inducible promoter was placed onto said transgenic plant. |

| Claim 51 |
| A transgenic lettuce plant comprising an antibiotic resistance gene wherein said antibiotic resistance gene is under the control of an inducible promoter. |

| Claim 52 |
| A transgenic plant comprising an antibiotic resistance gene wherein said antibiotic resistance gene is under the control of an inducible promoter, wherein said inducible promoter comprises DNA encoding a regulatory domain of an estrogen receptor. |

| Claim 57 |
| A transgenic plant comprising a herbicide resistance gene wherein said herbicide resistance gene is under the control of an inducible promoter, wherein said inducible promoter comprises DNA encoding a |
regulatory domain of an estrogen receptor

Claim 61
An organism or a cell comprising a gene wherein a natural promoter of said gene is lacking or inoperative and said gene is under the control of a transgenic inducible promoter

Claim 74
A method to screen for mutations in a gene of an organism or cell comprising:

a) preparing an organism or a cell wherein a natural promoter of said gene is lacking or inoperative and said gene is under the control of a transgenic inducible promoter; and
b) growing said organism or cell

3. Promoters based on ecdysone receptor

Patents granted to Zeneca (now Sygenta)

US 6 379 945

Claim 1
An isolated or synthetic DNA sequence encoding a polypeptide selected from the group consisting of:

a) the *Heliothis virescens* ecdysone steroid receptor shown in SEQ ID NO: 5;
b) the transactivation domain of the *Heliothis virescens* ecdysone steroid receptor shown in amino acids 1–162 of SEQ ID NO: 5;
c) the DNA binding domain of the *Heliothis virescens* ecdysone steroid receptor shown in amino acids 163–228 of SEQ ID NO: 5;
d) the hinge domain of the *Heliothis virescens* ecdysone steroid receptor shown in amino acids 229–326 of SEQ ID NO: 5;
e) the ligand binding domain of the *Heliothis virescens* ecdysone steroid receptor shown in amino acids 327–545 of SEQ ID NO: 5;
f) the carboxy terminus of the *Heliothis virescens* ecdysone steroid receptor shown in amino acids 546–577 of SEQ ID NO: 5; and
g) the hinge and ligand binding domains of the *Spodoptera exigua* ecdysone steroid receptor shown in SEQ ID NO: 7.

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Claim 1
DNA comprising the sequence shown in Seq. ID No. 2, or a DNA sequence which hybridizes to said DNA sequence under high stringency conditions.

Claim 2
DNA comprising the sequence shown in Seq. ID No.3, or a DNA sequence which hybridizes to said DNA sequence under high stringency conditions.

Claim 3
DNA comprising the sequence shown in Seq. ID No. 4, or a DNA sequence which hybridizes to said DNA sequence under high stringency conditions

Claim 11
A polypeptide comprising the *Heliothis* ecdysone receptor or a functionally active fragment thereof, wherein the polypeptide is substantially free from other proteins with which is ordinarily associated, and which is coded for by the DNA of any one of the preceding claims

Claim 12
A polypeptide comprising the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof.

Claim 13
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor ligand binding domain.
Claim 14
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor DNA binding domain.

Claim 15
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor transactivation domain.

Claim 16
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor hinge domain.

Claim 17
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor carboxy terminal domain.

Claim 19
DNA comprising the sequence shown in Seq. ID. No. 6, or a DNA sequence which hybridizes to said DNA sequence under high stringency conditions.

Patent applications filed by Syngenta

EP 828 829 A1

Claim 1
DNA comprising the sequence shown in Seq ID No. 2.

Claim 2
DNA comprising the sequence shown in Seq ID No. 3.

Claim 3
DNA comprising the sequence shown in Seq ID No. 4.

Claim 4
DNA comprising a sequence which shows 60% or more homology with the sequence shown in Seq ID No 1, 2 or 3.

Claim 6
DNA which hybridizes to the sequence shown in Seq. ID No. 2, 3 or 4, and which codes for at least part of the *Heliothis* ecdysone receptor.

Claim 8
DNA comprising part of the sequence shown in Seq ID No. 2, and which codes for at least part of the *Heliothis* ecdysone receptor ligand binding domain.

Claim 9
DNA comprising part of the sequence shown in Seq ID No. 3, and which codes for at least part of the *Heliothis* ecdysone receptor ligand binding domain.

Claim 10
DNA comprising part of the sequence shown in Seq ID No. 4, and which codes for at least part of the *Heliothis* ecdysone receptor ligand binding domain.

Claim 15
DNA comprising part of the sequence shown in Seq ID No. 2, and which codes for at least part of the *Heliothis* ecdysone receptor DNA binding domain.

Claim 16
DNA comprising part of the sequence shown in Seq ID No. 3, and which codes for at least part of the *Heliothis* ecdysone receptor DNA binding domain.

Claim 17
DNA comprising part of the sequence shown in Seq ID No. 4, and which codes for at least part of the *Heliothis* ecdysone receptor DNA binding domain.

Claim 22
DNA comprising part of the sequence shown in Seq ID No. 2, and which codes for at least part of the *Heliothis* ecdysone receptor transactivation domain.
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<tbody>
<tr>
<td>23</td>
<td>DNA comprising part of the sequence shown in Seq ID No. 3, and which codes for at least part of the <em>Heliothis</em> ec dysone receptor transactivation domain.</td>
</tr>
<tr>
<td>24</td>
<td>DNA comprising part of the sequence shown in Seq ID No. 4, and which codes for at least part of the <em>Heliothis</em> ec dysone receptor transactivation domain.</td>
</tr>
<tr>
<td>29</td>
<td>DNA comprising part of the sequence shown in Seq ID No. 2, and which codes for at least part of the <em>Heliothis</em> ec dysone receptor hinge domain.</td>
</tr>
<tr>
<td>30</td>
<td>DNA comprising part of the sequence shown in Seq ID No. 3, and which codes for at least part of the <em>Heliothis</em> ec dysone receptor hinge domain.</td>
</tr>
<tr>
<td>31</td>
<td>DNA comprising part of the sequence shown in Seq ID No. 4, and which codes for at least part of the <em>Heliothis</em> ec dysone receptor hinge domain.</td>
</tr>
<tr>
<td>36</td>
<td>DNA having part of the sequence shown in Seq ID No. 2, and which codes for at least part of the <em>Heliothis</em> ec dysone receptor carboxy terminal region.</td>
</tr>
<tr>
<td>37</td>
<td>DNA having part of the sequence shown in Seq ID No. 3, and which codes for at least part of the <em>Heliothis</em> ec dysone receptor carboxy terminal region.</td>
</tr>
<tr>
<td>38</td>
<td>DNA having part of the sequence shown in Seq ID No. 4, and which codes for at least part of the <em>Heliothis</em> ec dysone receptor carboxy terminal region.</td>
</tr>
<tr>
<td>44</td>
<td>A polypeptide comprising the amino acid sequence shown in Seq ID No. 4 or any allelic variant or derivative thereof.</td>
</tr>
<tr>
<td>45</td>
<td>A polypeptide comprising part of the amino acid sequence shown in Seq ID No. 4 or any allelic variant or derivative thereof, which sequence provides the <em>Heliothis</em> ec dysone receptor ligand binding domain.</td>
</tr>
<tr>
<td>46</td>
<td>A polypeptide comprising part of the amino acid sequence shown in Seq ID No. 4 or any allelic variant or derivative thereof, which sequence provides the <em>Heliothis</em> ec dysone receptor DNA binding domain.</td>
</tr>
<tr>
<td>47</td>
<td>A polypeptide comprising part of the amino acid sequence shown in Seq ID No. 4 or any allelic variant or derivative thereof, which sequence provides the <em>Heliothis</em> ec dysone receptor transactivation domain.</td>
</tr>
<tr>
<td>48</td>
<td>A polypeptide comprising part of the amino acid sequence shown in Seq ID No. 4 or any allelic variant or derivative thereof, which sequence provides the <em>Heliothis</em> ec dysone receptor hinge domain.</td>
</tr>
<tr>
<td>49</td>
<td>A polypeptide comprising part of the amino acid sequence shown in Seq ID No. 4 or any allelic variant or derivative thereof, which sequence provides the <em>Heliothis</em> ec dysone receptor carboxy terminal region.</td>
</tr>
<tr>
<td>51</td>
<td>DNA comprising the sequence shown in Seq ID No. 6.</td>
</tr>
<tr>
<td>52</td>
<td>DNA comprising a sequence which shows 60% or more homology with the sequence shown in Seq ID No. 6.</td>
</tr>
<tr>
<td>54</td>
<td>DNA which hybridizes to the DNA sequence shown in Seq ID No. 6 and which codes for at least part of <em>Spodoptera</em> ec dysone receptor.</td>
</tr>
<tr>
<td>56</td>
<td>DNA comprising part of the sequence shown in Seq ID No. 6, and which codes for at least part of the <em>Spodoptera</em> ec dysone receptor ligand binding domain.</td>
</tr>
</tbody>
</table>
Claim 61
DNA comprising part of the sequence shown in Seq ID No. 6, and which codes for at least part of the *Spodoptera* ecdysone receptor hinge domain.

CA 2219121 AA

Claim 1
DNA comprising the sequence shown in Seq. ID No. 2, or a sequence which shows homology thereto, or a sequence which hybridizes to the sequence shown in Seq. ID No. 2.

Claim 2
DNA comprising the sequence shown in Seq. ID No. 3, or a sequence which shows homology thereto, or a sequence which hybridizes to the sequence shown in Seq. ID No. 3.

Claim 3
DNA comprising the sequence shown in Seq. ID No. 4, or a sequence which shows homology thereto, or a sequence which hybridizes to the sequence shown in Seq. ID No. 4.

Claim 14
A polypeptide comprising the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof.

Claim 15
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor ligand binding domain.

Claim 16
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor DNA binding domain.

Claim 17
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor transactivation domain.

Claim 18
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor hinge domain.

Claim 19
A polypeptide comprising part of the amino acid sequence shown in Seq. ID. No. 4 or any allelic variant or derivative thereof, which sequence provides the *Heliothis* ecdysone receptor carboxy terminal domain.

Claim 21
DNA comprising the sequence shown in Seq. ID. No. 6, or a sequence which shows homology thereto, or a sequence which hybridizes to the sequence shown in Seq. ID. No. 6.

Claim 23
DNA comprising a sequence which shows homology in the range of 65% to 99% to the sequence shown in Seq. ID. No. 6.

Patent application filed by Pioneer Hi-Bred

EP 1 112 360 A1

Claim 1
An isolated nucleic acid sequence that encodes an insect protein from a Pyralidae species, wherein said nucleotide sequence is selected from the group consisting of:

a) a nucleotide sequence comprising a sequence encoding an ecdysone receptor or Ultraspiracle;

b) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1 or 3;

c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2 or 4;

d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).

Claim 2
An isolated polypeptide from a Pyralidae species, wherein said polypeptide is selected from the group consisting of:

a) a polypeptide sequence comprising an Ecdysone receptor or Ultraspiracle;
b) a polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 2 or 4;
c) a polypeptide encoded by a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1 or 3;
d) a polypeptide encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1 or 3.

Claim 3
A method of selectively inducing gene expression of a protein of interest in a plant, said method comprising:
a) stably incorporating into the genome of said plant an expression cassette, said expression cassette comprising a promoter operably linked to a nucleotide sequence encoding an Ecdysone receptor, wherein said nucleotide sequence encoding the Ecdysone receptor is selected from the group consisting of:
   i) a nucleotide sequence from a Pyrilidae species comprising a sequence encoding an Ecdysone receptor;
   ii) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1;
   iii) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2;
   iv) a nucleotide sequence that hybridizes under stringent conditions to a sequence of i), ii), or iii);
b) further stably incorporating into the genome of said plant a second expression cassette, wherein said expression cassette comprises a transcriptional regulatory region operably linked to a nucleotide sequence encoding said protein of interest, and wherein said transcriptional regulatory region is activated by the ligand–receptor complex;
c) contacting said plant with a ligand which complexes with said receptor, wherein said receptor–ligand complex interacts with the transcription regulatory region and induces gene expression of the protein of interest.

Claim 23
An expression vector comprising a promoter operably linked to a nucleotide sequence encoding an Ecdysone receptor wherein said nucleotide sequence encoding the Ecdysone receptor is selected from the group consisting of:
a) a nucleotide sequence from a Pyrilidae species comprising a sequence encoding an Ecdysone receptor;
b) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1;
c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2;
d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).

Claim 25
An expression vector comprising a promoter operably linked to a nucleotide sequence encoding ultraspiracle, and said nucleotide sequence encoding ultraspiracle is selected from the group consisting of:
a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 3;
b) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 4;
c) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a) or b).

Metal–responsive promoters

Patents on yeast copper–responsive promoter

Patent granted to Genentech

US 4 940 661
(view in patent database)

Claim 1
A vector comprising a selection gene, yeast origin of replication or autonomously replicating sequence and DNA encoding a eukaryotic polypeptide other than yeast chelatin, said DNA encoding a eukaryotic polypeptide that is under the control of:
a) a yeast chelatin promoter,
b) a yeast chelatin transcription control sequence, or
 c) a metal ion regulatory region of the yeast chelatin transcription control sequence, which is free of the
yeast chelatin promoter,

whereby the vector is replicable in a suitable host.

**Claim 14**

An isolated DNA sequence comprising a yeast chelatin metal ion regulatory region free of the yeast chelatin promoter and free of DNA encoding yeast chelatin.

**Patent on a mouse metallothionein promoter**

Patent granted to University Patents Inc.

**US 4 579 821**

(visit patent database)

**Claim 1**

A process for subjecting the transcription of a selected DNA sequence to external control under given environmental conditions which comprises the steps of:

A) providing a selected isolated structural gene that is transcriptionally responsive to a mouse metallothionein-I promoter/regulator DNA sequence under the given environmental conditions; and

B) operatively fusing the selected structural gene with said promoter/regulator DNA sequence.

**Claim 2**

In the genetic engineering process for securing transcription and expression of a selected isolated structural gene sequence in a mammalian host cell wherein said selected structural gene is stably incorporated as a chromosomal or extrachromosomal constituent of the host, the improvement comprising the step of:

operatively fusing with said selected structural gene sequence a mouse metallothionein-I promoter/regulator DNA sequence, which is responsive to environmental variations within the host cell in the concentration of ions of metal.

**Claim 3**

A fusion gene product, suitable for use in genetic transformation of a mammalian host cell, said product comprising:

a non mouse metallothionein structural gene sequence to be incorporated in said host cell operatively fused with a mouse metallothionein-I promoter/regulator DNA sequence.

**Patent on a human metallothionein promoter**

Patent granted to the University of California (Berkeley)

**US 4 601 978**

(visit patent database)

**Claim 1**

A DNA sequence of less than 500 base pairs, said DNA sequence comprising the human MT-II transcriptional regulatory system further comprising the transcription initiation sequence.

**Claim 5**

A DNA construct capable of regulated expression of an inserted gene in a mammalian host, said construct comprising an extrachromosomal replication system recognized by a mammalian host, which replication system has been joined to a human MT-II gene inducible regulatory system in vitro, said construct having DNA sequence(s) defining at least one restriction site within the transcriptional control of the regulatory region for insertion of the gene.

**Claim 10**

A DNA construct useful for expression of a structural gene to produce a polypeptide in a mammalian host, said vector comprising (a) a replicon from bovine papilloma virus and (b) a regulatory system from a human MT-II gene comprising a promoter and a terminator and having at least one restriction site downstream from the promoter in the direction of transcription.

**Pathogenesis-related (PR) promoters**
### Patents granted to Novartis (now Syngenta)

#### US 5 654 414

**Claim 1**
A nucleic acid promoter fragment isolated from the 5' flanking region upstream of the coding region of a cucumber chitinase/lysozyme gene that is inducible by application of benzo-1,2,3-thiadiazoles.

#### US 5 689 044

**Claim 1**
A chemically inducible nucleic acid promoter fragment isolated from the 5' flanking region upstream of the coding region of a tobacco PR-1a gene, wherein said promoter fragment comprises a nucleotide fragment of at least 603-bp adjacent to the coding region of said tobacco PR-1a gene, wherein said promoter fragment is inducible by application of a benzo-1,2,3-thiadiazole, an isonicotinic acid compound, or a salicylic acid compound.

**Claim 3**
A chemically inducible nucleic acid promoter fragment isolated from the 5' flanking region upstream of the coding region of an Arabidopsis PR-1 gene, wherein the coding region of said Arabidopsis PR-1 gene comprises the DNA sequence set forth in SEQ ID NO:33 or a DNA sequence which would encode the protein encoded by SEQ ID NO:33, wherein said promoter fragment is inducible by application of a benzo-1,2,3-thiadiazole, an isonicotinic acid compound, or a salicylic acid compound.

#### US 5 789 214

**Claim 1**
A method of inducing gene transcription in a plant or plant tissue, comprising the steps of:

A) transforming said plant or plant tissue, each with a chimeric gene comprising:
   (i) a chemically inducible nucleic acid promoter fragment of at least 603-bp isolated from the 5' flanking region adjacent the coding region of a tobacco PR-1a gene, and
   (ii) a coding sequence of interest operatively linked to said promoter fragment; and
B) exposing said transgenic plant or plant tissue to a benzo-1,2,3-thiadiazole, an isonicotinic acid compound, or a salicylic acid compound, whereby transcription of said coding sequence of interest is induced in said plant or plant tissue.

**Claim 8**
A method of inducing gene transcription in a plant or plant tissue, comprising the steps of:

A) transforming said plant or plant tissue, each with a chimeric gene comprising:
   (i) a chemically inducible nucleic acid promoter fragment isolated from the 5' flanking region adjacent the coding region of an Arabidopsis PR-1 gene, wherein said Arabidopsis PR-1 gene comprises a DNA sequence that specifically hybridizes to SEQ ID NO:33 or wherein said Arabidopsis PR-1 gene comprises a DNA sequence that encodes the protein encoded by SEQ ID NO:33, and
   (ii) a coding sequence of interest operatively linked to said promoter fragment; and
B) exposing said transgenic plant or plant tissue to a benzo-1,2,3-thiadiazole, an isonicotinic acid compound, or a salicylic acid compound, whereby transcription of said coding sequence of interest is induced in said plant or plant tissue.

#### AU 708 850 B2

**Claim 1**
An isolated DNA molecule comprising a nucleotide sequence selected from the following group:

- a) a full–length chemically inducible promoter fragment comprising nucleotides 1 through 4258 of SEQ ID NO: 1;
- b) an 815–bp long chemically inducible promoter fragment comprising nucleotides 3444 through 4258 of
SEQ ID NO: 1; and
  c) a 698-bp long chemically inducible promoter fragment comprising nucleotides 3561 through 4258 of
  SEQ ID NO: 1.

Claim 14
An isolated DNA molecule involved in inducibility of a chemically inducible promoter selected from the
following group:
  a) LS4 comprising nucleotides 3584 through 3593 of SEQ ID NO: 1;
  b) LS7 comprising nucleotides 3614 through 3623 of SEQ ID NO: 1;
  c) LS 10 comprising nucleotides 3644 through 3653 of SEQ ID NO: 1; and
  d) a region spanning LS7–LS 1 0 and comprising nucleotides 3614 through 3653 of SEQ ID NO: 1.

Patent granted to Pioneer Hi-Bred

US 6 429 362
(view in patent database)

Claim 1
An isolated promoter comprising a nucleotide sequence that initiates transcription in a plant cell, wherein
said nucleotide sequence is selected from the group consisting of:
  a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 3 or 4; and
  b) a nucleotide sequence comprising the plant promoter sequence deposited in the plasmid designated as
     ATCC Accession No. 207139 or 207131.

Claim 5
A method for driving expression of a heterologous nucleotide sequence in a plant, said method comprising
the steps of:
  a) transforming a plant cell with an expression cassette comprising the heterologous nucleotide sequence
     operably linked to a promoter that initiates transcription in a plant cell, wherein said promoter is selected
     from the group consisting of:
     i) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 3 or 4; and
     ii) a nucleotide sequence comprising the plant promoter sequence deposited in the plasmid designated as
         ATCC Accession No. 207139 or 207131; and
  b) regenerating a stably transformed plant from said plant cell.

Claim 9
A plant cell transformed with a DNA construct comprising a heterologous nucleotide sequence operably
linked to a promoter that initiates transcription in said plant cell, wherein said promoter comprises a
nucleotide sequence selected from the group consisting of:
  a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 3 or 4; and
  b) a nucleotide sequence comprising the plant promoter sequence deposited in the plasmid designated as
     ATCC Accession No. 207139 or 207131.

Claim 13
A plant stably transformed with a DNA construct comprising a heterologous nucleotide sequence operably
linked to a promoter that initiates transcription in a plant cell, wherein said promoter comprises a
nucleotide sequence selected from the group consisting of:
  a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 3 or 4; and
  b) a nucleotide sequence comprising the plant promoter sequence deposited in the plasmid designated as
     ATCC Accession No. 207139 or 207131.

Patent application filed by Pioneer Hi-Bred

EP 1 056 862 A1

Claim 1
An isolated nucleic acid molecule having a nucleotide sequence for a promoter that is capable of initiating
transcription in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:
a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5;  
b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No.  
c) a nucleotide sequence comprising at least 40 contiguous 10 nucleotides of the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5; and  
d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).

Claim 5
A method for inducing expression of a heterologous nucleotide sequence in a plant, said method comprising:  
A) transforming a plant cell with a DNA construct comprising said heterologous nucleotide sequence operably linked to a promoter that is capable of initiating transcription in a plant cell in response to a stimulus,  
B) regenerating a stably transformed plant from said plant cell, and  
C) exposing said plant to said stimulus, wherein said promoter comprises a nucleotide sequence selected from the group consisting of:  
a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1, 2, 3, or 4;  
b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No.  
c) a nucleotide sequence comprising at least 40 contiguous nucleotides of the sequence set forth in SEQ ID NO: 1, 2, 3, or 4; and  
d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).

Claim 9
A method for constitutively expressing a heterologous nucleotide sequence in a plant, said method comprising:  
A) transforming a plant cell with a DNA construct comprising said heterologous nucleotide sequence operably linked to a promoter that is capable of initiating constitutive transcription in a plant cell and  
B) regenerating a stably transformed plant from said plant cell, wherein said promoter comprises a nucleotide sequence selected from the group consisting of:  
a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 5;  
b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No.  
c) a nucleotide sequence comprising at least 40 contiguous nucleotides of the sequence set forth in SEQ ID NO: 5; and  
d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).

Claim 13
A plant cell stably transformed with a DNA construct comprising a heterologous nucleotide sequence operably linked to a promoter that is capable of initiating transcription in said plant cell, wherein said promoter comprises a nucleotide sequence selected from the group consisting of:  
a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5;  
b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No.  
c) a nucleotide sequence comprising at least 40 contiguous nucleotides of the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5; and  
d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).

Claim 17
A plant stably transformed with a DNA construct comprising a heterologous nucleotide sequence operably linked to a promoter that is capable of initiating transcription in a plant cell, wherein said promoter comprises a nucleotide sequence selected from the group consisting of:  
a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5;  
b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No.  
c) a nucleotide sequence comprising at least 40 contiguous nucleotides of the sequence set forth in SEQ ID NO: 1, 2, 3, 4, or 5; and  
d) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), or c).
Claim 22
An isolated nucleic acid molecule having a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 6, 8, 10, or 14;
b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No._
c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 7, 9, 11, or 15;
d) a nucleotide sequence comprising at least 16 contiguous nucleotides of a sequence of a), b), or c); and
e) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), c), or d).

Claim 26
A method for creating or enhancing disease resistance in a plant, said method comprising:

A) transforming said plant with a DNA construct comprising a PR-1 sequence operably linked to a promoter that drives expression of a coding sequence in a plant cell and
B) regenerating stably transformed plants,
wherein said PR-1 sequence is selected from the group consisting of:

a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 6, 8, 10, or 14;
b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No._
c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 7, 9, 11, or 15;
d) a nucleotide sequence comprising at least 16 contiguous nucleotides of a sequence of a), b), or c); and
e) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), c), or d).

Claim 33
A plant cell stably transformed with a DNA construct comprising a PR-1 sequence operably linked to a promoter that drives expression of a coding sequence in a plant cell, wherein said PR-1 sequence is selected from the group consisting of:

a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 6, 8, 10, or 14;
b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No._
c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 7, 9, 11, or 15;
d) a nucleotide sequence comprising at least 16 contiguous nucleotides of a sequence of a), b), or c); and
e) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), c), or d).

Claim 34
A plant stably transformed with a DNA construct comprising a PR-1 sequence operably linked to a promoter that drives expression of a coding sequence in a plant cell, wherein said PR-1 sequence is selected from the group consisting of:

a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO: 6, 8, 10, or 14;
b) a nucleotide sequence selected from the group consisting of sequences deposited as ATCC Accession No._
c) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 7, 9, 11, or 15;
d) a nucleotide sequence comprising at least 16 contiguous nucleotides of a sequence of a), b), or c); and
e) a nucleotide sequence that hybridizes under stringent conditions to a sequence of a), b), c), or d).

Physically-regulated promoters
Heat-regulated

Actual granted independent claims

Patents granted to Mycogen Plant Science

US 5 447 858
(view in patent database)
### Claim 1
A recombinant heat shock gene comprising a plant heat shock promoter and a heterologous structural gene whose expression is controllable thereby, wherein said promoter comprises the consensus nucleotide sequence 5′-C-T-X-G-A-A-X-X-T-A-C-X-X-X-3′, where X is A, T, C or G.

### Claim 2
A recombinant heat shock gene comprising a plant heat shock promoter and a heterologous structural gene whose expression is controlled thereby, wherein said promoter comprises the consensus nucleotide sequence 5′-C-T-S-G-A-A-M-R-T-A-C-W-M-K-3′, where S is C or G; M is A or C; R is A or G; W is A or T; and K is T or G.

### Claim 8
A vector comprising a fragment of DNA capable of functioning as a plant heat shock promoter wherein said fragment of DNA comprises the consensus nucleotide sequence 5′-C-T-X-G-A-A-X-X-T-A-C-X-X-X-3′, where X is A, T, C or G.

### Claim 9
A vector comprising a fragment of DNA capable of functioning as a plant heat shock promoter wherein said fragment comprises the consensus nucleotide sequence of 5′-C-T-S-G-A-A-M-R-T-A-C-W-M-K-3′, where S is C or G; M is A or C; R is A or G; W is A or T; and K is T or G.

### Claim 19
A bacterial strain containing therein recombinant DNA comprising:

a) a fragment of DNA capable of controlling heat shock expression of a gene in a plant; and
b) a structural gene oriented with respect to said fragment of DNA so as to be expressible under the control thereof


### Claim 20
A bacterial strain containing therein recombinant DNA comprising:

a) a fragment of DNA capable of controlling heat shock expression of a gene in a plant; and
b) a structural gene oriented with respect to said fragment of DNA so as to be expressible under the control thereof

wherein the fragment of DNA capable of controlling heat shock expression of a gene in a plant comprises the consensus nucleotide sequence 5′-C-T-S-G-A-A-M-R-T-A-C-W-M-K-3′, where S is C or G; M is A or C; R is A or G; W is A or T; and K is T or G.

### Claim 34
A method for recognizing a plant cell containing recombinant DNA comprising the steps of:

1) transferring into said plant cell recombinant DNA comprising:
   b) a transformation recognition gene under the control of said heat shock promoter capable of causing an observable or detectable reaction when expressed;
2) applying a stress to said plant cell in which it is desired to recognize those containing recombinant DNA, which stress is capable of inducing a response in said heat shock promoter; and
3) observing or detecting the reaction caused by expression of said transformation recognition gene under the control of said heat shock promoter to recognize said plant cell containing recombinant DNA including said transformation recognition gene.

### Claim 36
A method for recognizing a plant cell containing recombinant DNA comprising the steps of:

1) transferring into said plant cell recombinant DNA comprising:
   a) a heat shock promoter which comprises the consensus sequence 5′-C-T-S-G-A-A-M-R-T-A-C-W-M-K-3′, where S is C or G; M is A or C; R is A or G; W is A or T; and K is T or G;
   b) a transformation recognition gene under the control of said heat shock promoter capable of causing an
observable or detectable reaction when expressed;
2) applying a stress to said plant cell in which it is desired to recognize those containing recombinant DNA, which stress is capable of inducing a response in said heat shock promoter; and
3) observing or detecting the reaction caused by expression of said transformation recognition gene under the control of said heat shock promoter to recognize said plant cell containing recombinant DNA including said transformation recognition gene.

Claim 1
A recombinant DNA plasmid comprising:

a) a vector,
b) a T–DNA fragment of a Ti-plasmid from an Agrobacterium strain, which T–DNA fragment is functional for insertion into a plant genome, and
c) a fragment of plant DNA which is capable of controlling gene expression in response to heat shock, and which fragment is inserted into said T–DNA fragment such that said T–DNA fragment retains functionality for insertion into a plant genome, wherein said fragment of plant DNA comprises a plant heat shock promoter comprising the consensus nucleotide sequence 5'–C–T–X–G–A–A–X–X–T–A–C–X–X–X–3', wherein X is A, T, C or G.

Claim 17
A method for modifying a plant genome so as to allow expression of a structural gene under control of a soybean heat shock gene promoter fragment, comprising the steps of:

a) isolating a soybean heat shock gene promoter fragment comprising the consensus nucleotide sequence 5'–C–T–X–G–A–A–X–X–T–A–C–X–X–X–3', wherein X is A, T, C or G,
b) cloning said soybean heat shock gene promoter fragment into a T–DNA shuttle vector producing a recombinant DNA plasmid,
c) isolating a DNA fragment carrying foreign structural genes or soybean genes not naturally expressed under the regulatory control of a soybean heat shock gene promoter, and inserting said DNA fragment into said recombinant DNA plasmid at a position on the X–side of said soybean heat shock gene promoter producing a heat shock expression plasmid,
wherein said DNA fragment is oriented with respect to said soybean heat shock gene promoter as to be expressible under control thereof,
d) transforming said heat shock expression plasmid into a first bacterial strain capable of supporting replication of said heat shock expression plasmid,
e) mixing said bacterial strain capable of supporting replication of said heat shock expression plasmid with a second bacterial strain carrying a helper plasmid capable of transporting said heat shock expression plasmid into an Agrobacterium strain incapable of supporting replication of said heat shock expression plasmid, said Agrobacterium strain carrying a resident plasmid,
f) selecting for recombination between said heat shock expression plasmid and said resident plasmid giving a recombinant resident plasmid,
g) infecting a plant or a plant cell culture with said Agrobacterium strain containing and replicating said recombinant resident plasmid,
h) selecting a plant or a plant cell culture comprising plant cells containing said foreign structural genes or said soybean genes under control of said soybean heat shock gene promoter transferred from said recombinant resident plasmid to said plant cells, said foreign structural genes or said soybean genes being expressed following heat shock treatment or other stress treatment.

CA 1338010
Claims 1, 8, and 19 are worded exactly the same as claims 1, 8 and 19 of the United States patent.

Claim 34
A method for obtaining a transient increase in expression level of a heterologous structural gene in a plant comprising:

1) inserting into said plant genome a DNA fragment comprising a promoter capable of inducing a heat shock response in a plant exposed to stress, which promoter comprises the consensus nucleotide sequence 5'–C–T–X–G–A–A–X–X–T–A–C–X–X–X–5', where X is A, T, C or G,
said promoter being combined with said structural gene such that said structural gene is expressed under the control of said promoter, followed by 2) exposing said plant to stress such that said promoter responds thereby obtaining a transient increase in expression level of said structural gene in said plant.

Claim 50
A method for controlling the expression of a structural gene in a plant comprising the steps of:

1) transforming said plant with a DNA fragment comprising a DNA promoter sequence capable of controlling heat shock expression in a plant and a structural gene, wherein said DNA promoter sequence comprises the consensus nucleotide sequence 5'-C-T-X-G-A-A-X-X-T-A-C-X-X-X-3', where X is A, T, C or G and said structural gene is oriented with respect to said DNA promoter sequence so as to be controllable thereby;
2) applying stress to said plant so as to activate said DNA promoter sequence and thereby cause the expression of said structural gene.

Claim 54
It is worded exactly the same as claim 34 of the United States patent.

Patent granted to the U.S. Department of Health and Human Services

AU 732 872 B2 (view in patent database)*

Claim 1
A method for the spatial and temporal control of the expression of a gene of interest within a preselected discrete region of a cell mass or multicellular organism, comprising selectively heating a preselected discrete region of a cell mass or multicellular organism that includes cells that contain a genetically engineered gene of interest operably linked to a heat-inducible promoter, thereby inducing the expression of said gene of interest in the cells that are selectively heated.

Claim 9
A method of providing a therapeutic protein to selected cells in an animal, comprising the steps of:

A) introducing into cells of an animal a DNA molecule having a heat shock promoter sequence operably linked to and exerting regulatory control over a sequence encoding a therapeutic protein, and
B) activating said heat shock promoter sequence through the application of a focused ultrasound so that said DNA segment expresses a therapeutically effective amount of said therapeutic protein.

* Independent claims as filed of the European patent application EP 922 110 A2 are the same as the independent, granted claims of the Australian patent.

Patent granted to The General Hospital Corporation

CA 1324097

Claim 1
A method for high level production of a polypeptide or protein in a transformed host cell comprising:

a) transforming a host cell with a structural gene encoding for a polypeptide or protein, under the control of an inducible heat shock promoter;

b) amplifying the copy number of said structural gene in said transformed host cells using an amplification system under the control of a promoter other than an inducible heat shock promoter;

c) inducing said inducible promoter by heat shock to said transformed host cells at a temperature and for a time sufficient to transcribe said structural gene;

d) allowing said heat shocked cells to recover at a lower temperature than said heat shock temperature and for a time sufficient to translate said transcribed structural gene, producing said polypeptide or protein.

Claim 10
A method for increasing the production of a structural polypeptide or protein in a transformed mammalian cell comprising the steps of:

a) culturing a dihydrofolate reductase (DHFR) deficient mammalian cell transformed with

(i) a DHFR gene under the control of a constitutive promoter, and

(ii) a structural gene encoding for a polypeptide or protein under the control of an inducible heat shock promoter, in a culture medium containing methotrexate, for a time sufficient to permit growth of said transformed mammalian cells;
b) inducing said heat shock promoter at a temperature and for a time sufficient to transcribe said structural
gene; and

c) allowing said heat shocked cells to recover from said heat shock at a lower temperature and for a time
sufficient to translate said transcribed structural gene, producing said polypeptide or protein.

Claim 17
A host cell cotransformed with:

a) a gene amplification system under the control of a constitutive promoter, and

b) a structural gene encoding for a polypeptide or protein under control of an inducible heat shock
promoter.

Cold-regulated promoters

Patents filed by Takara Shuzo Co.

Actual granted independent claims

<table>
<thead>
<tr>
<th>Claim 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A vector which is characterized in containing each of the following elements:</td>
</tr>
<tr>
<td>(1) a promoter which shows its action in the host to be used;</td>
</tr>
<tr>
<td>(2) regulatory region for regulating the action of the promoter of (1); and</td>
</tr>
<tr>
<td>(3) a region which codes for the 5'-untranslated region derived from cold-shock protein gene mRNA or a</td>
</tr>
<tr>
<td>region which codes for the region where substitution, deletion, insertion or addition of at least one base is</td>
</tr>
<tr>
<td>applied to the said untranslated region and</td>
</tr>
<tr>
<td>which contains a base sequence as shown in SEQ ID NO:1 in the Sequence Listing.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>An isolated promoter consisting of a base sequence as shown in SEQ ID NO: 5 in the Sequence Listing.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>An isolated promoter containing a base sequence as shown in SEQ ID NO: 5 in the Sequence Listing and</td>
</tr>
<tr>
<td>consisting of a base sequence having 135 or less bases,</td>
</tr>
<tr>
<td>wherein the promoter does not contain the region which is ascribed to mRNA.</td>
</tr>
</tbody>
</table>

Actual filed independent claims

<table>
<thead>
<tr>
<th>Claim 1</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>region which codes for the region where substitution, deletion, insertion or addition of at least one base is</td>
</tr>
<tr>
<td>applied to the said untranslated region.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>A promoter containing a base sequence as shown in SEQ ID NO:5 in the Sequence Listing and</td>
</tr>
<tr>
<td>consisting of a base sequence having 135 or less bases.</td>
</tr>
</tbody>
</table>

Patents filed by Japan Tobacco Inc.

Actual granted independent claims
<table>
<thead>
<tr>
<th>Claim 1</th>
<th>A DNA sequence comprising a nucleotide sequence from the first to the 3546th nucleotide in the nucleotide sequence shown in SEQ ID NO:1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claim 2</td>
<td>A DNA sequence comprising a nucleotide sequence from the 2418th to the 3541st nucleotide in the nucleotide sequence shown in SEQ ID NO:1.</td>
</tr>
<tr>
<td>Claim 3</td>
<td>A DNA sequence comprising a nucleotide sequence from the first to the 4120th nucleotide in the nucleotide sequence shown in SEQ ID NO:2.</td>
</tr>
</tbody>
</table>

**Claim 1**

A DNA sequence having a nucleotide sequence from first to 3546th nucleotide in the nucleotide sequence shown in SEQ ID. No. 1, or a part thereof having a cold-inducible promoter activity, or a DNA sequence having the same nucleotide sequence as said DNA sequences except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted or added, which DNA sequence has a cold-inducible promoter activity.

**Claim 3**

A DNA sequence having a nucleotide sequence from 2418th to 3541st nucleotide in the nucleotide sequence shown in SEQ ID. No. 1, or a part thereof having a cold-inducible promoter activity, or a DNA sequence having the same nucleotide sequence as the said DNA sequences except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted or added, which DNA sequence has a cold-inducible promoter activity.

**Claim 4**

A cold-inducible promoter sequence having a nucleotide sequence from 2418th to 3541st nucleotide in the nucleotide sequence shown in SEQ ID. No. 1, or a DNA sequence having the same nucleotide sequence as said DNA sequence except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted or added, which DNA sequence has a cold-inducible promoter activity.

**Claim 5**

A DNA sequence having a nucleotide sequence from first to 4120th nucleotide in the nucleotide sequence shown in SEQ ID. No. 2, or a part thereof having a cold-inducible promoter activity, or a DNA sequence having the same nucleotide sequence as the said DNA sequences except that one or more nucleotides are deleted or substituted, or one or more nucleotides are inserted or added, which DNA sequence has a cold-inducible promoter activity.

**Claim 7**

A probe comprising a DNA fragment having at least 18 consecutive nucleotides in the region from 45th to 839th nucleotide in the sequence shown in SEQ ID NO:3 in the Sequence Listing or a sequence complementary thereto.

*Independent claims as filed of the European application EP 812 917 A1 are the same as the granted claims of the Australian patent. The only difference is in Claim 7 where instead of "having at least 18 consecutive nucleotide", the probe recited in the European application has "at least 15 consecutive nucleotides".*
### Actual filed independent claims

<table>
<thead>
<tr>
<th>Claim</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claim 1</td>
<td>A promoter comprising a nucleotide sequence corresponding to the 5.5 Kb EcoR1 fragment isolated from <em>Solanum tuberosum</em> or a variant, homologue or fragment thereof.</td>
</tr>
<tr>
<td>Claim 2</td>
<td>A promoter comprising a nucleotide sequence corresponding to the 5.5 b EcoR1 fragment isolated from <em>Solanum tuberosum</em> or a variant, homologue or fragment thereof, but wherein at least a part of the promoter is inactivated.</td>
</tr>
<tr>
<td>Claim 3</td>
<td>A promoter comprising at least the nucleotide sequence shown as Seq.I.D. No. 1 or a variant, homologue or fragment thereof.</td>
</tr>
<tr>
<td>Claim 4</td>
<td>A promoter comprising the nucleotide sequence of any of one of the sequences shown as Seq.I.D.Nos. 4 – 17 or a variant, homologue or fragment thereof.</td>
</tr>
<tr>
<td>Claim 5</td>
<td>A promoter comprising a nucleotide sequence corresponding to the 5.5 Kb EcoR1 fragment isolated from <em>Solanum tuberosum</em> or a variant, homologue or fragment thereof, but wherein at least the nucleotide sequence shown as Seq.I.D. No. 1 is inactivated.</td>
</tr>
<tr>
<td>Claim 6</td>
<td>A promoter comprising a nucleotide sequence corresponding to the 5.5 Kb EcoR1 fragment isolated from <em>Solanum tuberosum</em> or a variant, homologue or fragment thereof, but wherein at least any of one of the sequences shown as Seq.I.D.Nos. 2–16 is inactivated.</td>
</tr>
</tbody>
</table>

### Patents granted to the Canadian Ministry of Agriculture

#### Actual granted independent claims

<table>
<thead>
<tr>
<th>Claim</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claim 1</td>
<td>An isolated DNA molecule comprising a 5’ regulatory region of a low temperature-responsive gene BN115 from <em>Brassica napus</em>, comprising nucleotides 961–1210 of SEQ ID NO:1.</td>
</tr>
<tr>
<td>Claim 7</td>
<td>An isolated cold inducible promoter, or a functional fragment thereof capable of regulating the expression of a gene in response to a change in temperature, said cold inducible promoter comprising nucleotides 1–1271 of SEQ ID NO:1.</td>
</tr>
<tr>
<td>Claim 8</td>
<td>An isolated DNA molecule comprising a sequence of at least 15 contiguous nucleotides of a 5’ regulatory region of a low temperature responsive gene BN115 from <em>Brassica napus</em>, as defined by nucleotides 1–1271 of SEQ ID NO:1.</td>
</tr>
<tr>
<td>Claim 13</td>
<td>An isolated enhancer, or a functional fragment thereof each capable of mediating the expression of a gene under the control of a promoter and said enhancer or fragment, said enhancer obtained from the regulatory region of BN115 from <em>Brassica napus</em>.</td>
</tr>
<tr>
<td>Claim 16</td>
<td>An isolated negative regulatory element capable of repressing gene expression at 22°C, obtained from the regulatory region of BN115 from <em>Brassica napus</em> and comprising nucleotides 461–623 of SEQ ID NO:1.</td>
</tr>
</tbody>
</table>
Claim 1
An isolated DNA molecule comprising a 5' regulatory region of a low temperature-responsive gene BN115 from *Brassica napus*, comprising nucleotides 156–1362 of SEQ ID NO:1.

Claim 5
An isolated DNA molecule comprising a 5' regulatory region of a low temperature-responsive gene BN115 from *Brassica napus*, comprising nucleotides 1–1271 of SEQ ID NO:1.

Claim 7
An isolated cold inducible promoter, or a functional fragment thereof capable of regulating the expression of a gene in response to a change in temperature, said cold inducible promoter comprising nucleotides 1–1271 of SEQ ID NO:1.

Claim 8
An isolated DNA molecule comprising a sequence defined by nucleotides 1–1271 of SEQ ID NO:1, a fragment thereof, or a nucleotide sequence that hybridizes to said sequence defined by nucleotides 1–1271 of SEQ ID NO:1 under stringent hybridization conditions, wherein said DNA molecule, said fragment thereof, or said nucleotide sequence exhibit low temperature activity, said stringent hybridization conditions are selected from:

i) hybridization at 4X SSC at 42°C followed by washing in 0.1X SSC at 65°C for an hour; and
ii) hybridization at 4X SSC, 50% formamide at 42°C followed by washing in 0.1X SSC at 65°C for an hour.

Claim 13
An isolated enhancer comprising a sequence defined by nucleotides 156–623 of SEQ ID NO:1, a functional fragment thereof, or a nucleotide sequence that hybridizes to said sequence defined by nucleotides 156–623 of SEQ ID NO:1 under stringent hybridization conditions, wherein said enhancer, fragment or nucleotide sequence are each capable of mediating expression of a gene, said stringent hybridization conditions are selected from:

i) hybridization at 4X SSC at 42°C followed by washing in 0.1X SSC at 65°C for an hour; and
ii) hybridization at 4X SSC, 50% formamide at 42°C followed by washing in 0.1X SSC at 65°C for an hour.

Claim 16
An isolated negative regulatory element capable of repressing gene expression at 22°C, obtained from the regulatory region of BN115 from *Brassica napus* and comprising nucleotides 461–623 of SEQ ID NO:1.

Light-regulated promoters
A. Light-inducible

Patent granted to Calgene Inc.

**US 5 750 385**

[view in patent database]

Claim 1
A method for obtaining a plant having a modified phenotype, said method comprising;

Transforming a host plant cell with a DNA construct under genomic integration conditions, wherein said construct comprises as operably linked components in the direction of transcription, a promoter region obtainable from a gene, wherein transcription of said gene is light-inducible in a plant chloroplast containing tissue, a DNA sequence of interest other than the native coding sequence of said gene, and a transcription termination region, wherein said components are functional in a plant cell,

Whereby said DNA construct becomes integrated into a genome of said plant cell; regenerating a plant from said transformed plant cell, and growing said plant under conditions whereby said DNA sequence of interest is expressed and a plant having said modified phenotype is obtained.

Claim 2
A method for altering the phenotype of chloroplast containing A DNA sequence comprising a light-inducible promoter derived from a myxobacterium, and a nucleotide sequence containing a restriction site located to enable a nucleotide sequence which is to be expressed to be placed under the control of the said promoter.

Patent granted to the University of Warwick
## Actual granted independent claims

### CA 1321563

**Claim 1**
A recombinant DNA sequence containing a light-inducible promoter derived from a myxobacterium.

**Claim 2**
A DNA sequence comprising a light-inducible promoter derived from a myxobacterium, and a nucleotide sequence containing a restriction site located to enable a nucleotide sequence which is to be expressed to be placed under the control of the said promoter.

**Claim 8**
A recombinant DNA sequence comprising a light-inducible promoter derived from a myxobacterium, operatively linked to a DNA sequence coding for a polypeptide to be expressed, the coding DNA sequence not being operatively linked to the promoter in nature.

**Claim 11**
A method of producing a polypeptide which comprises:

1. positioning a gene sequence coding for the polypeptide—under the operative control of a light-inducible promoter derived from a myxobacterium to produce a recombinant DNA molecule;
2. transforming a competent bacterial host with the said recombinant DNA molecule; and
3. subjecting the host to light to induce the promoter whereby the gene is expressed.

### B. Light-repressible

**Patents filed by Suntory LTD**

**Actual granted independent claims**

### NZ 508 103

**Claim 1**
A DNA fragment containing the sequence of SEQ ID NO: 1 as a core sequence, whereby expression of a gene placed downstream of said DNA fragment is repressed in the presence of light.

**Claim 4**
A promoter containing the nucleotide sequence of SEQ ID NO: 1 as a core sequence, whereby expression of a gene placed downstream of said promoter is promoted in the dark but repressed in the presence of light.

**Note:** Actual filed independent claims of patent applications EP 1 077 257 & CA 2328139 AA are the same as the granted independent claims of the New Zealander patent.

**Patents granted to Mycogen Plant Science**

**Actual granted independent claims**

### US 5 639 952

(A view in patent database)

**Claim 1**
A method for the expression of a structural gene in a plant cell under conditions of darkness comprising the steps of:

1. transforming said plant cell with a recombinant molecule comprising: a plant, dark- and light-active maize Cab promoter/regulatory system, wherein said promoter/regulatory system is the promoter/regulatory system of Cab AB1084 or cross-hybridizes with the promoter/regulatory system of Cab AB1 084 under stringent conditions, and a heterologous plant-expressible structural gene that is under the regulatory control of said plant Cab promoter/regulatory system in said plant cell, and
2. maintaining conditions of darkness for expression of said structural gene in said transformed plant cell.
Claim 8
A method for enhancing the level of expression obtained in the dark of a plant-expressible gene in a plant cell by exposing said plant cell to conditions of illumination comprising the steps of:

a) transforming said plant cell with a recombinant molecule comprising:
   a plant, dark- and light-active maize Cab promoter/regulatory system,
   wherein said promoter/regulatory system is the promoter/regulatory system of Cab AB1084 or cross-hybridizes with the promoter/regulatory system of Cab AB1084 under stringent conditions, and
   a heterologous plant-expressible structural gene that is under the regulatory control of said plant Cab promoter/regulatory system in said plant cell, and
   b) maintaining conditions of darkness for expression of said structural gene in said transformed plant cell, and
   c) applying conditions of illumination to said transformed plant cell such that enhancement by a factor of about 3– to 6-fold in expression of said structural gene is obtained.

Claim 15
A plant grown from a plant cell,
wherein said plant cell is transformed with a recombinant molecule comprising a plant, dark- and light-active maize Cab promoter/regulatory system,
wherein said promoter/regulatory system is the promoter/regulatory system of Cab AB1084 or cross-hybridizes with the promoter/regulatory system of Cab AB1084 under stringent conditions, and
a heterologous plant-expressible structural gene that is under the regulatory control of said plant Cab promoter/regulatory system in said plant cell.

US 5 656 496
(view in patent database)

Claim 1
A recombinant DNA molecule comprising:

a) a dark- and light-active Cab promoter/regulatory system which cross-hybridizes with the Cab AB1084 promoter/regulatory system under stringent conditions, and wherein said promoter/regulatory system:
   (i) functions to direct the expression of a structural gene under its control during conditions of darkness and
   (ii) is stimulated to direct enhanced expression of said structural gene under light conditions, and
b) a heterologous plant-expressible structural gene wherein said structural gene is placed under the regulatory control of said plant promoter/regulatory system.

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