UBIQUITIN PROMOTER PATENT STUDY

May 10, 2007

Dear Dr. Chi-Ham,

As requested, we conducted a study regarding the ubiquitin promoters as described in more detail below in "Statement of the Technology" and hereinafter referred to as the empty expression vectors. The study included a search of U.S. patents related to ubiquitin promoters, the results of which we include in the form of a CD attached hereto. The results of the search were then further searched for particular applicability to the empty expression vectors. These selected patents were then reviewed; the results of the review are included herein.

LIMITATIONS

Other patents have not been examined, and the prosecution history of the reviewed U.S. patents has not been examined. This study is limited to U.S. patents only; foreign patents have not been reviewed. No opinion is expressed concerning whether the subject invention may infringe one or more patents. No opinion is given as to the scope of the claims in the patents reviewed. This study includes the results of a reading of the claims and the specification (the "review"). No clearance is given herein and no opinion or clearance is given herein; this does not constitute a legal opinion.

The Statement of Technology includes the entirety of the information in which this study is based. Some aspects of the empty expression vectors have not been specifically provided. If no information has been provided concerning parts of the vector, step(s) in the process of using the vector or making the vector, or methods related thereto, or any other piece of information related to the empty expression vectors including intended use, it has been assumed to be unimportant to the success of the empty expression vectors. If the assumptions concerning these factors are not correct, this study and the review may be adversely affected. Also, if future substantive changes are made to the empty expression vectors those changes could also affect the review. If errors in assumption are noted or substantive changes in the empty expression vectors are made, the matter should be given further review.

The review includes a study of whether the empty expression vectors may "infringe" one or more claims of the reviewed patents. Although the language "infringe" is used, it should be understood that these reviews were done in the context of a "study" and not in the context of a legal opinion. Thus, the term "infringe", when used in the context referring to any particular claim, for purposes of this study, means that the empty expression vectors may be found to include all of the elements of the particular claim. This determination was done as part of a review, thus, only reading the claim and the specification, but not reviewing the prosecution history. Thus, no study has been completed to determine the legal scope of the claims. Thus, *all* statements made herein should be read in the context of these limitations.

1. Summary

Promoter	Patents Reviewed	Patent Infringed	Owned by a PIPRA Member
Arabidopsis thaliana UBI-3	N/A	N/A	N/A
Arabidopsis thaliana UBI-10	N/A	N/A	N/A
Sugarcane UBI-9	6,706,948 6,686,513 6,638,766	Probably Probably Probably Not	Yes (Univ. of Hawaii) Yes (Univ. of Hawaii) Yes (Univ. of Hawaii)
Rice UBI-3	6,528,701	Probably Not	Yes (Univ. of Louisiana)
Potato UBI-3	6,448,391	Probably Not	No
Maize UBI	6,977,325 6,020,190 5,614,399 5,510,474 6,054,574	Probably Not Probably Probably Probably Probably	No No No No No

2. Statement of the Technology

This letter provides a freedom-to-operate (FTO) study regarding the use of ubiquitin promoters in PIPRA's empty expression vectors (see Appendix A). The vector is a bacterial based expressional strategy that works in either sexually or asexually propagated plants. Furthermore, the vector is provided in one of two subversions: a) the transposon module; and b) the recombinase module. Each module contains a visual marker cassette, selected from one of several possible markers depending on the module, under the control of a promoter other than ubiquitin.

Each module utilizes a marker removal cassette wherein the FMV34S promoter (Univ. Cal.) expresses either a transposase or recombinase for the transposon module or recombinase module, respectively. Each removal cassette also utilizes either the nos or CaMV35S terminator.

Each module also contains a gene of interest cassette, which is driven by a promoter. While the expression vector may contain several different promoters (see Appendix A), this study is limited to the Arabidopsis thaliana UBI-3 and UBI-10, sugarcane UBI-9, rice UBI-3, potato UBI-3 and maize ubiquitin promoters.

3. Statement of the Patents Reviewed

The patents reviewed in this study are a subset of the Ubiquitin promoter patent landscape search completed in January of 2007. The patents reviewed are limited to issued United States patents that may claim an Arabidopsis thaliana UBI-3, Arabidopsis thaliana UBI-10, sugarcane UBI-9, rice UBI-3, potato UBI-3 or maize ubiquitin promoter as a composition of matter. Furthermore, this study only reviews the independent claims of the patents selected for review.[1]

Based on these criteria we selected and reviewed the following patents:

- U.S. Patent Number 6,706,948, "Sugarcane UBI9 gene promoter and methods of use thereof."
- U.S. Patent Number 6,686,513, "Sugarcane UBI9 gene promoter sequence and methods of use thereof."
- U.S. Patent Number 6,528,701, "Rice ubiquitin-derived promoter."
- U.S. Patent Number 6,448,391, "Ubiquitin-lytic peptide gene promoter."
- U.S. Patent Number 6,638,766, "Promoter of the sugarcane UBI4 gene."
- U.S. Patent Number 6,977,325, "Plant promoter sequences and methods of use for same."
- U.S. Patent Number 6,020,190, "Plant ubiquitin promoter system."
- U.S. Patent Number 5,614,399, "Plant ubiquitin promoter system."
- U.S. Patent Number 5,510,474, "Plant ubiquitin promoter system."
- U.S. Patent Number 6,054,574, "Plant ubiquitin promoter system."

4. Review

U.S. Patent Number 6,706,948, "Sugarcane UBI9 gene promoter and methods of use thereof."

One could argue that PIPRA's proposed use of the sugarcane UBI-9 promoter in the pPIPRA expression vector will probably infringe independent claims 1, 4, 5, and 6 of the '948 patent. In order to argue that claim 4 is infringed, the HindIII/XbaI fragment must be isolated from pubi9-GUS[2]. Furthermore, according to the Statement of the Technology, the sugarcane UBI-9 promoter may also be used to drive expression of a gene of interest. When these two elements are combined in the pPIPRA expression vector

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[1] Anything that infringes a dependent claim will also infringe the independent claim from which it depends. See MPEP § 608.01(n) ("A proper dependent claim shall not conceivably be infringed by anything which would not also infringe the basic claim.")

[2] Contained in Escherichia coli cells deposited as NRRLB-30116.

system, one could argue that claim 5 will probably be infringed as well. Finally, it is assumed that PIPRA researchers or PIPRA licensees will combine the sugarcane UBI-9 promoter with a gene of interest and introduce the resulting transgene into either a monocotyledonous or dicotyledonous plant cell. Argument could be made that such use of the pPIPRA expression vector will probably infringe claim 6.

It is important to note that the University of Hawaii, a PIPRA member, owns this patent. Therefore, PIPRA should examine its relationship with this patent owner to assess the risk associated with infringement of the above claims.

U.S. Patent Number 6,686,513, "Sugarcane UBI9 gene promoter sequence and methods of use thereof."

PIPRA's proposed use of the sugarcane UBI-9 promoter in the pPIPRA expression vector will probably not infringe independent claims 1 and 6 of the '513 patent. However, it is understood that PIPRA researchers or licensees will use the pPIPRA expression vector to drive expression of a gene of interest in transgenic plant cells. If the pPIPRA expression vector is used to drive expression of a gene of interest in sugarcane, tobacco, sorghum, pineapple, rice, maize, tomato, soybean, banana, or garlic cells then one could argue that claims 1 and 6 will probably be infringed.

It is important to note that the University of Hawaii, a PIPRA member, owns this patent. Therefore, PIPRA should examine its relationship with this patent owner to assess the risk associated with infringement of the above claims.

U.S. Patent Number 6,638,766, "Promoter of the sugarcane UBI4 gene."

PIPRA's proposed use of the sugarcane UBI-9 promoter in the pPIPRA expression vector will probably not infringe independent claims 1 and 4 of the '766 patent since these claims cover UBI-4 and its use to express a gene of interest.

It is important to note that, even if PIPRA chooses to use the UBI-4 promoter, the University of Hawaii, a PIPRA member, owns this patent. Therefore, PIPRA should examine its relationship with this patent owner to assess the risk associated with infringement of the above claims.

U.S. Patent Number 6,528,701, "Rice ubiquitin-derived promoter."

PIPRA's proposed use of the rice UBI-3 promoter in the pPIPRA expression vector system does not infringe any independent claim in the '701 patent. Claim 1, the broadest independent claim, is limited to a portion of SEQ ID NO 3, which is upstream of position 2785. According to the specification, SEQ ID NO 3 is the 4442 base DNA sequence of the rice UBI-2 gene. The rice UBI-3 promoter is disclosed in the '701 patent as a portion of SEQ ID NO 5 - the 1846 base DNA sequence of the rice UBI-3 fusion gene. However, no part of SEQ ID NO 5 is claimed. Therefore, the '701 patent is limited to the rice UBI-2 and the rice UBI-3 promoter does not infringe any claim.

U.S. Patent Number 6,448,391, "Ubiquitin-lytic peptide gene promoter"

PIPRA's proposed use of the potato UBI-3 promoter in the pPIPRA expression vector system does not infringe any independent claim in the '391 patent. Independent claims 1 and 2 are limited to nucleotides 1-1,788 and 1-1,1220 of SEQ ID NO 96. According to the specification, SEQ ID NO 96 codes for the potato UBI-7 promoter. The potato UBI-3 promoter is disclosed in the '391 patent as a portion of SEQ ID NO 93. However, no part of SEQ ID NO 93 is claimed. Therefore, the '391 patent is limited to the potato UBI-7 promoter and the potato UBI-3 promoter does not infringe any claim.

U.S. Patent Number 6,977,325, "Plant promoter sequences and methods of use for same"

PIPRA's proposed use of the maize UBI-1 promoter in the pPIPRA expression vector will probably not infringe independent claims 1 and 2 of the '325 patent. The claims of the '325 patent are drawn to a modified maize UBI-1 promoter wherein the two naturally occurring heat shock elements do not overlap. Thus PIPRA will not infringe these claims when using the naturally occurring maize UBI-1 promoter with overlapping heat shock elements.

U.S. Patent Number 6,020,190, "Plant ubiquitin promoter system"

One could argue that PIPRA's proposed use of the maize UBI-1 promoter in the pPIPRA expression vector will probably infringe independent claim 1 of the '190 patent, which claims the maize UBI-1 gene and its components such as intron, TATA box, and heat shock elements and a downstream plant expressible gene of interest.

U.S. Patent Number 5,614,399, "Plant ubiquitin promoter system"

PIPRA's proposed use of the maize UBI-1 promoter in the pPIPRA expression vector will probably not infringe independent claims 1 of the '399 patent unless the maize UBI-1 promoter is used under heat stress conditions in a transformed plant cell.

U.S. Patent Number 5,510,474, "Plant ubiquitin promoter system"

One could argue that PIPRA's proposed use of the maize UBI-1 promoter in the pPIPRA expression vector will probably infringe independent claim 1 of the '474 patent, which claims the maize UBI-1 gene and its components such as intron, TATA box, and heat shock elements.

U.S. Patent Number 6,054,574, "Plant ubiquitin promoter system"

One could argue that PIPRA's proposed use of the maize UBI-1 promoter in the pPIPRA expression vector will probably infringe independent claims 1, 9, and 18 of the '574 patent. Infringement turns on whether or not the use of the maize UBI-1 promoter 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.* If PIPRA's use of the maize UBI-1 promoter in the pPIPRA vector is not capable of achieving constitutive gene expression in monocots that is about one-third that obtained in by the promoter in inducible gene expression in monocots than one can argue that PIPRA does not infringe claim 1. Infringement of claim 9 requires use of maize UBI-1 in an expression vector.

5. Conclusion

Based on the foregoing review, we came to the following conclusions. PIPRA's use of the
Arabidopsis thaliana UBI-3, Arabidopsis thaliana UBI-10, Rice UBI-3, or Potato UBI-3 promoter will not infringe any patent reviewed in this study. Use of the
Sugarcane UBI-9 promoter will probably infringe claims 1, 4, 5, and 6 of the '948 patent and claims 1 and 6 of the '513 patent but not claims 1 and 4 of the '766 patent.
Finally, use of the Maize UBI-1 promoter will probably infringe claim 1 of the '190 patent, claim 1 of the '399 patent, claim 1 of the '474 patent and claims 1, 9 and 18 of the '574 patent.

APPENDIX A – DESCRIPTION OF pPIPRA EXPRESSION VECTOR SYSTEM

PIPRA
public intellectual property resource for agriculture

Biotechnology Resources
Plant Transformation System with Maximum FTO

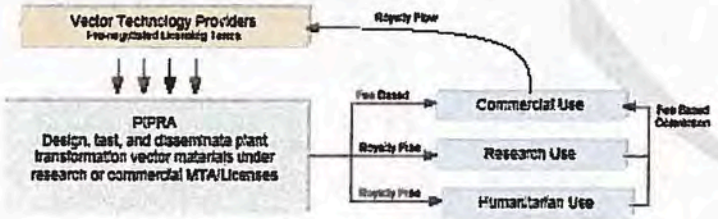
PIPRA will integrate biological, legal, and regulatory considerations to develop, test, and distribute effective plant transformation systems with maximum FTO to meet a range of research needs. The plant transformation system is designed to address major IP hurdles encountered during agricultural development of improved crops.

The plant transformation system will incorporate:

- features that conveniently and effectively meet routine and specialized research applications
- technologies that can be deployed for both commercial and humanitarian applications, with known licensing terms/access conditions
- plant-based technologies
- technologies that address regulatory considerations
- technologies that address public acceptance issues

Overview of the project

1. **IP Strategy**
   a. FTO analyses
   b. Technology pooling and license negotiation
   c. Material transfer agreement development
   d. Vector distribution strategy
2. **Plant Transformation System Development**
   a. All plant-derived DNA
   b. Marker-free strategies
   c. DNA transfer sequences from Agrobacterium (T/DNA) and plants (P/DNA)
   d. Tested by delivery with Agrobacterium and Agrobacterium alternatives
   e. Plant derived selectable markers vs. kanamycin
3. **Transformation into Model Crops**
   a. Monocots
   b. Dicots
4. **Validation of Transformation System**
   a. Transformation efficiency
   b. Efficacy of selectable marker cassettes
   c. Vectors tested at PIPRA member institutions
5. **Collaborative Pilot Projects**
   PIPRA's laboratory facilities are located in the University of California Davis, USA. Contact PIPRA to discuss collaborative opportunities to develop improved crops.



PIPRA

public intellectual property resource for agriculture

Biotechnology Resources
Plant Transformation System with Maximum FTO





Selectable Visual Markers



- Promoter: MAS, BRDC/Purdue Univ.
- Selectable Markers:
  - Peptide Deformylase, Univ. Kentucky
  - Atwbc19, Univ. of Tennessee
  - Cytosine Deaminase, In process
- Terminator: MAS, BRDC, Purdue

Visual Marker



- Promoter: FMV34S, Univ. California
- Selectable/Fluorescent Marker: Phytochrome, Univ. California
- Terminator: nos or CaMV35S, Public Domain--verify

Promoters to drive Gene of Interest



- Promoters:
  - Constitutive:
    - FMV34S, Univ. California
    - Arabidopsis Actin 2, Public Domain
    - Rice Actin 1, Cornell Univ.
    - MAS/SuperMAS, BRDC/Purdue Univ.
  - Fruit: Tomato E8 Promoter, Public Domain
  - Seed: Brassica napus Cruciferin, Public Domain
  - Environmentally/Chemically Inducible:
    - Light Regulated Rubisco Small Subunit, Public Domain
    - *Estrogen Inducible Promoter, Rockefeller Univ. --verify*
  - Promoter-less: Option to insert researcher's promoter of interest
- Multi Cloning Site: modified PUC18, Public Domain
- Terminator: Pea-E9 Rubisco Terminator, Public Domain

Marker Removal Technologies



- Promoter: FMV34S, Univ. California
- Marker Free System, Transposase-based System, Univ. California
- Terminator: nos or CaMV35S, Public Domain--verify



- Promoter: FMV34S, Univ. California
- Marker Free System, Recombinase-based System, USDA
- Terminator: nos or CaMV35S, Public Domain--verify

APPENDIX B – CLAIM CHARTS

U.S. Patent No.	6,706,948
Inventor(s):	Albert; Henrik H. (Honolulu, HI), Wei; Hairong (Honolulu, HI)
Assignee(s):	The United States of America as represented by the Secretary of Agriculture (Washington, DC); University of Hawaii (Honolulu, HI)
Filed:	March 17, 1999
Issued:	March 16, 2004
Expiration Date:	March 17, 2019
Maintenance Fee Due:	September 16, 2007 (maintenance fee due at 3.5 years)
Status:	Active
Priority Information Summary:	This application claims priority to now abandoned provisional application: 60/078,768, filed on 3/19/1998.

Claims of U.S. 6,706,948	Support in specification of U.S. 6,706,948
1. A substantially purified nucleic acid sequence comprising	"As used herein, the term "purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated. An "isolated nucleic acid sequence" is therefore a purified nucleic acid sequence. "Substantially purified" molecules are at least 60% free, preferably at least 75% free, and more preferably at least 90% free from other components with which they are naturally associated." (col. 9, lines 39-46)
a nucleotide sequence selected from the group consisting of	**A nucleotide sequence** "The invention relates to nucleic acid sequences isolated from sugarcane and to methods of using them. In particular, the invention relates to nucleotide sequences which are derived from sugarcane polyubiquitin genes and which are capable of directing constitutive expression of a nucleic acid sequence of interest that is operably linked to the sugarcane polyubiquitin nucleotide sequences." (col. 1, lines 10-17) "In a preferred embodiment, the nucleotide sequence is characterized by having promoter activity.... the promoter activity is constitutive. In another embodiment, nucleotide sequence is double-stranded. In yet another embodiment, the nucleotide sequence is single-stranded." (col. 2, lines 10-15)
SEQ ID NO:7,	**SEQ ID NO: 7** "The invention also provides a substantially purified nucleic acid sequence comprising a portion of a nucleotide sequence selected from the group consisting of SEQ ID NO:7 and the complement thereof. In a preferred embodiment, the portion is characterized by having promoter activity. In a more preferred embodiment, the promoter activity is constitutive. In an alternative preferred embodiment, the portion is double-stranded. In another alternative preferred embodiment, the portion is single-stranded. In yet another alternative preferred embodiment, the portion comprises the nucleotide sequence selected from the group consisting of the nucleotides from 1 to 242, from 245 to 787, from 788 to 1020, from 1021 to 1084, from 1085 to 1168, from 1169 to 1173, from 1174 to 1648, from 1649 to 1802, from 1 to 377, from 378 to 442, and from 443 to 1802." (col. 2, lines 27-42)

Claims of U.S. 6,706,948	Support in specification of U.S. 6,706,948
	FIG. 10 shows SEQ: 7 - the portion of the UBI4 gene which corresponds to nucleotides 1-1802 of SEQ ID NO: 5. (col. 7, 23-28) FIGS. 5 shows SEQ:5 (nucleotides 1 to 5512 of the 5551 nucleotide sequence deposited as GenBank accession number AF093504) of sugarcane polyubiquitin ubi4 gene. (col. 6, lines 53-57)
the complement of SEQ ID NO:7,	**The complement of SEQ ID NO:7** "As used herein, the terms "complementary" or "complementarity" are used in reference to nucleotide sequences related by the base-pairing rules. For example, the sequence 5'-AGT-3' is complementary to the sequence 5'-ACT-3'. Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases is not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acids is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. A "complement" of a nucleic acid sequence as used herein refers to a nucleotide sequence whose nucleic acids show total complementarity to the nucleic acids of the nucleic acid sequence." (col. 9, lines 48-64)
SEQ ID NO:10, and	**SEQ ID NO:10** "Further provided by the invention is a substantially purified nucleic acid sequence comprising a portion of a nucleotide sequence selected from the group consisting of SEQ ID NO:10 and the complement thereof. In a preferred embodiment, the portion is characterized by having promoter activity. In a more preferred embodiment, the promoter activity is constitutive. In an alternative preferred embodiment, the portion is double-stranded. In another alternative preferred embodiment, the portion is single-stranded. In yet another alternative preferred embodiment, the portion comprises the nucleotide sequence selected from the group consisting of the nucleotides 1 to 3600, from 3602 to 3612, from 3614 to 3688, from 1 to 2248, from 2249 to 2313, from 2314 to 3688, and from 1671 to 2248." (col. 2, lines 53-66) FIG. 11 shows SEQ: 10 - the portion of the UBI9 gene upstream of the translation start codon, which corresponds to nucleotides 1-3688 of SEQ: 8. (col. 7, lines 29-33) FIG. 8 shows SEQ: 8 (GenBank accession number AF093505) of the sugarcane polyubiquitin ubi9 gene. (col. 7, lines 10-13) "Similarly, it is the inventors' view that the sequence upstream of the translation start codon of the ubi9 gene (a) is as shown in FIG. 11 (SEQ ID NO:10) (i.e., total number of nucleotides being 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3'), (b) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3', assuming substitution of the "T" at position 4 with a "C"), or (c) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3689, with the ten nucleotides at the 5'-end

Claims of U.S. 6,706,948	Support in specification of U.S. 6,706,948
	being 5'-AAGCTTTTGn-3', assuming insertion of a "C" at position 4. Accordingly, any reference herein to SEQ ID NO:10 is intended to mean each and every one of the three nucleotide sequences described in the preceding sentence." (col. 15, lines 41-56) "The nucleotide sequence upstream of the translation start codon of the ubi9 gene (i.e., nucleotides 1-3691 of SEQ ID NO:3, and nucleotides 1-3691 of SEQ ID NO:8) contained three regions: (a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of SEQ ID NO:3, and nucleotides 1-2248 of SEQ ID NOs:8 and 10), (b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of SEQ ID NO:3, and nucleotides 2249-2313 of SEQ ID NOs:8 and 10), and (c) an intron sequence (i.e., nucleotides 2314-3688 of SEQ ID NO:3, and nucleotides 2314-3688 of SEQ ID NOs:8 and 10)." (col. 15, line 62 – col. 16, line 5). "The invention also contemplates at least a portion of SEQ ID NOs:7 and 10, and homologs thereof having promoter activity. The term "promoter activity" ... refers to the ability of the nucleic acid sequence to initiate transcription of an operably linked nucleotide sequence into mRNA." (col. 17, lines 49-54)
the complement of SEQ ID NO:10.	**The complement of SEQ ID NO:10** "As used herein, the terms "complementary" or "complementarity" are used in reference to nucleotide sequences related by the base-pairing rules. For example, the sequence 5'-AGT-3' is complementary to the sequence 5'-ACT-3'. Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases is not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acids is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. A "complement" of a nucleic acid sequence as used herein refers to a nucleotide sequence whose nucleic acids show total complementarity to the nucleic acids of the nucleic acid sequence." (col. 9, lines 48-64)
4. A substantially purified nucleic acid sequence comprising	"As used herein, the term "purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated. An "isolated nucleic acid sequence" is therefore a purified nucleic acid sequence. "Substantially purified" molecules are at least 60% free, preferably at least 75% free, and more preferably at least 90% free from other components with which they are naturally associated." (col. 9, lines 39-46)
the HindIII/XbaI fragment isolated from plasmid pubi9-GUS contained in Escherichia coli cells deposited as NRRLB-30116.	**The HindIII/XbaI fragment... from... pubi9-GUS contained in Escherichia coli cells deposited as NRRLB-30116** "Also provided herein is 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, the complement of the fragment, homologs of the fragment, and homologs of the complement of the fragment. In a preferred embodiment, the nucleotide sequence is SEQ ID NO:10." (col. 3, lines 36-39)

Claims of U.S. 6,706,948	Support in specification of U.S. 6,706,948
	"An approximately 7.2 kb HindIII-EcoRI fragment, which contains SEQ ID NO:8 plus approximately 2kb additional downstream sequence, was cloned in the plasmid vector pBluescript II KS+(Stratagene) to form plasmid pubi9. This plasmid was transformed into E. coli DH5a host cells and deposited with the Agriculture Research Service culture collection (NRRL), under the terms of the Budapest Treaty on March 8, 1999 as accession number NRRLB-30113. An XbaI restriction site was added at the 3' end of the ubi9 promoter shown in SEQ ID NO: 10 by way of PCR amplification with an XbaI adapter primer. The ubi9 promoter so modified was ligated upstream of a GUS coding sequence and a NOS 3' terminator in the vector plasmid pUC19 to form pubi9-GUS. This plant expression plasmid was transformed into E. coli DH5a host cells and deposited with the NRRL under the terms of the Budapest Treaty on Mar. 15, 1999 as accession number NRRLB-30116. Subclone pubi9 (FIGS. 4, 6, 7 and 8) contained five copies of the polyubiquitin coding repeat, 244 bp of 3' UTR.... A possible poly-A addition signal was present 221 bp down stream of the TAA stop codon, and there was approximately 2 kb additional downstream sequence.... an intron was located immediately 5' of the initiation codon; this intron was 1374 bp. 5' of this intron were 65 bp (in SEQ ID NO:8) and 67 bp (in SEQ ID NO:3).... The subclone contained an additional 2247 bp of upstream sequence, including a TATA consensus sequence at-30 bp relative to the beginning of the cDNA clones.... approximately 344 bp upstream of transcription start codon, is an apparent insertion of approximately 200 bp.... This 200 bp region was delimited by 17 bp imperfect inverted repeats....The nature of this possible insertion event has not been investigated; however, it has features of miniature inverted-repeat transposable elements (MITEs) [Wessler et al., Curr Opin Genet Dev 5, 814-21 (1995)]. Without limiting the invention to any particular mechanism, this insertion is not believed to have a functional role in the promoter activity of the polyubiquitin ubi9 promoter since this insertion is inserted in the 3' UTR (not the promoter) of the glucose transporter gene, and since it is not present in the polyubiquitin ubi4 gene.... the ubi9 gene also contained two HSE-like sequences about 320 bp upstream of the transcription start codon; however, both of these HSE-like sequences lacked the invariant G residue." (col. 33, line 41 – col. 34, line 54)
5. A transgenic plant cell comprising	**A transgenic plant cell** "In a preferred embodiment, the transgenic plant cell expresses the nucleic acid sequence of interest. In a more preferred embodiment, the expression is constitutive. In an alternative embodiment, the transgenic plant cell is derived from a monocotyledonous plant. In a more preferred embodiment, the monocotyledonous plant is selected from the group consisting of sugarcane, maize, sorghum, pineapple, rice, barley, oat, wheat, rye, yam, onion, banana, coconut, date, and hop. In another alternative embodiment, the transgenic plant cell is derived from a dicotyledonous plant. In a more preferred embodiment, the dicotyledonous plant is selected from the group consisting of tobacco, tomato, soybean, and papaya." (col.4, line 62 – col. 5, line 8) "The term "plant" as used herein refers to a plurality of plant cells which are largely differentiated into a structure that is present at any stage of a plant's

Claims of U.S. 6,706,948	Support in specification of U.S. 6,706,948
	development. Such structures include, but are not limited to, a fruit, shoot, stem, leaf, flower petal, etc. The term "plant tissue" includes differentiated and undifferentiated tissues of plants including, but not limited to, roots, shoots, leaves, pollen, seeds, tumor tissue and various types of cells in culture (e.g., single cells, protoplasts, embryos, callus, protocorm-like bodies, etc.). Plant tissue may be in planta, in organ culture, tissue culture, or cell culture." (col. 13, lines 12-23)
a nucleic acid sequence comprising	
a double-stranded nucleotide sequence listed as SEQ ID NO:10,	**SEQ ID NO:10** "Further provided by the invention is a substantially purified nucleic acid sequence comprising a portion of a nucleotide sequence selected from the group consisting of SEQ ID NO:10 and the complement thereof. In a preferred embodiment, the portion is characterized by having promoter activity. In a more preferred embodiment, the promoter activity is constitutive. In an alternative preferred embodiment, the portion is double-stranded. In another alternative preferred embodiment, the portion is single-stranded. In yet another alternative preferred embodiment, the portion comprises the nucleotide sequence selected from the group consisting of the nucleotides 1 to 3600, from 3602 to 3612, from 3614 to 3688, from 1 to 2248, from 2249 to 2313, from 2314 to 3688, and from 1671 to 2248." (col. 2, lines 53-66) FIG. 11 shows SEQ: 10 - the portion of the UBI9 gene upstream of the translation start codon, which corresponds to nucleotides 1-3688 of SEQ: 8. (col. 7, lines 29-33) FIG. 8 shows SEQ: 8 (GenBank accession number AF093505) of the sugarcane polyubiquitin ubi9 gene. (col. 7, lines 10-13) "Similarly, it is the inventors' view that the sequence upstream of the translation start codon of the ubi9 gene (a) is as shown in FIG. 11 (SEQ ID NO:10) (i.e., total number of nucleotides being 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3'), (b) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3', assuming substitution of the "T" at position 4 with a "C"), or (c) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3689, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3', assuming insertion of a "C" at position 4. Accordingly, any reference herein to SEQ ID NO:10 is intended to mean each and every one of the three nucleotide sequences described in the preceding sentence." (col. 15, lines 41-56) "The nucleotide sequence upstream of the translation start codon of the ubi9 gene (i.e., nucleotides 1-3691 of SEQ ID NO:3, and nucleotides 1-3691 of SEQ ID NO:8) contained three regions: (a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of SEQ ID NO:3, and nucleotides 1-2248 of SEQ ID NOs:8 and 10), (b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of SEQ ID NO:3, and nucleotides 2249-2313 of SEQ ID NOs:8 and 10), and (c) an intron sequence (i.e., nucleotides 2314-3688 of SEQ ID NO:3, and

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	nucleotides 2314-3688 of SEQ ID NOs:8 and 10)." (col. 15, line 62 – col. 16, line 5). "The invention also contemplates at least a portion of SEQ ID NOs:7 and 10, and homologs thereof having promoter activity. The term "promoter activity" … refers to the ability of the nucleic acid sequence to initiate transcription of an operably linked nucleotide sequence into mRNA." (col. 17, lines 49-54)
wherein said nucleotide sequence is operably linked to a nucleic acid sequence of interest.	**Operably linked to a nucleic acid sequence of interest** "In yet another alternative embodiment, the nucleic acid sequence of interest is a sense sequence. In a more preferred embodiment, the sense sequence encodes a protein selected from the group consisting of .beta.-glucuronidase, luciferase, .beta.-galactosidase, 1-aminocyclopropane-1-carboxylic acid deaminase, sucrose phosphate synthase, 5-enolpyruvyl-3- phosphoshikimate synthase, acetolactate synthase, RNase, wheat germ agglutinin, sweetness protein, and Bacillus thuringiensis crystal toxin proteins. In a further alternative embodiment, the nucleic acid sequence of interest is an antisense sequence. In a more preferred embodiment, the antisense sequence is selected from the group consisting of an antisense sequence to ACC synthase, to ethylene inducible sequences, and to polyphenol oxidase." (col. 5, lines 8-21) "The term "nucleotide sequence of interest" refers to any nucleotide sequence, the manipulation of which may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (e.g., reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product, (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.)." (col. 8 line 66 – col. 9, line 9) Specific Examples: (col. 22, line 32 – col. 24, line 6) - I-arninocyclopropane-l-carboxylic acid (ACC) - sucrose phosphate synthase enzyme - 5-enolpyruvyl-3- phosphoshikimate synthase (EPSP synthase) - acetolactate synthase - RNase - wheat germ agglutinin - nucleic acid sequences, which encode the sweetness protein - Bacillus thuringiensis (B.t.) crystal toxin proteins - nucleic acid sequence which encodes an antisense RNA that hybridizes with a genomic plant DNA sequence (e.g. ethylene inducible sequences in fruit, ACC synthase gene, polyphenol oxidase)
6. A method for expressing a nucleic acid sequence of interest in a plant cell, comprising:	
a) providing: i) a plant cell;	**Providing a plant cell** "In another preferred embodiment, the plant cell is derived from a monocotyledonous plant. In yet a more preferred embodiment, the

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	monocotyledonous plant is selected from the group consisting of sugarcane, maize, sorghum, pineapple, rice, barley, oat, wheat, rye, yam, onion, banana, coconut, date, and hop. In another alterative more preferred embodiment the plant cell is derived from a dicotyledonous plant. In a yet more preferred embodiment, the dicotyledonous plant is selected from the group consisting of tobacco, tomato, soybean, and papaya." (col. 5, lines 50-60) "The term "plant" as used herein refers to a plurality of plant cells which are largely differentiated into a structure that is present at any stage of a plant's development. Such structures include, but are not limited to, a fruit, shoot, stem, leaf, flower petal, etc. The term "plant tissue" includes differentiated and undifferentiated tissues of plants including, but not limited to, roots, shoots, leaves, pollen, seeds, tumor tissue and various types of cells in culture (e.g., single cells, protoplasts, embryos, callus, protocorm-like bodies, etc.). Plant tissue may be in planta, in organ culture, tissue culture, or cell culture." (col. 13, lines 12-23)
ii) a nucleic acid sequence of interest; and	**Providing a nucleic acid sequence of interest** "In yet another alternative embodiment, the nucleic acid sequence of interest is a sense sequence. In a more preferred embodiment, the sense sequence encodes a protein selected from the group consisting of .beta.-glucuronidase, luciferase, .beta.-galactosidase, 1-aminocyclopropane-1-carboxylic acid deaminase, sucrose phosphate synthase, 5-enolpyruvyl-3- phosphoshikimate synthase, acetolactate synthase, RNase, wheat germ agglutinin, sweetness protein, and Bacillus thuringiensis crystal toxin proteins. In a further alternative embodiment, the nucleic acid sequence of interest is an antisense sequence. In a more preferred embodiment, the antisense sequence is selected from the group consisting of an antisense sequence to ACC synthase, to ethylene inducible sequences, and to polyphenol oxidase." (col. 5, lines 8-21) "The term "nucleotide sequence of interest" refers to any nucleotide sequence, the manipulation of which may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (e.g., reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product, (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.)." (col. 8 line 66 – col. 9, line 9) Specific Examples: (col. 22, line 32 – col. 24, line 6) - I-arninocyclopropane-l-carboxylic acid (ACC) - sucrose phosphate synthase enzyme - 5-enolpyruvyl-3- phosphoshikimate synthase (EPSP synthase) - acetolactate synthase - RNase - wheat germ agglutinin - nucleic acid sequences, which encode the sweetness protein - Bacillus thuringiensis (B.t.) crystal toxin proteins - nucleic acid sequence which encodes an antisense RNA that hybridizes with a genomic plant DNA sequence (e.g. ethylene inducible sequences in fruit, ACC synthase gene, polyphenol oxidase)

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iii) a nucleotide sequence selected from the group consisting of	**Providing a nucleotide sequence selected from the group consisting of**
SEQ ID NO: 10, and	**SEQ ID NO:10** "Further provided by the invention is a substantially purified nucleic acid sequence comprising a portion of a nucleotide sequence selected from the group consisting of SEQ ID NO:10 and the complement thereof. In a preferred embodiment, the portion is characterized by having promoter activity. In a more preferred embodiment, the promoter activity is constitutive. In an alternative preferred embodiment, the portion is double-stranded. In another alternative preferred embodiment, the portion is single-stranded. In yet another alternative preferred embodiment, the portion comprises the nucleotide sequence selected from the group consisting of the nucleotides 1 to 3600, from 3602 to 3612, from 3614 to 3688, from 1 to 2248, from 2249 to 2313, from 2314 to 3688, and from 1671 to 2248." (col. 2, lines 53-66) FIG. 11 shows SEQ: 10 - the portion of the UBI9 gene upstream of the translation start codon, which corresponds to nucleotides 1-3688 of SEQ: 8. (col. 7, lines 29-33) FIG. 8 shows SEQ: 8 (GenBank accession number AF093505) of the sugarcane polyubiquitin ubi9 gene. (col. 7, lines 10-13) "Similarly, it is the inventors' view that the sequence upstream of the translation start codon of the ubi9 gene (a) is as shown in FIG. 11 (SEQ ID NO:10) (i.e., total number of nucleotides being 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3'), (b) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3', assuming substitution of the "T" at position 4 with a "C"), or (c) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3689, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3', assuming insertion of a "C" at position 4. Accordingly, any reference herein to SEQ ID NO:10 is intended to mean each and every one of the three nucleotide sequences described in the preceding sentence." (col. 15, lines 41-56) "The nucleotide sequence upstream of the translation start codon of the ubi9 gene (i.e., nucleotides 1-3691 of SEQ ID NO:3, and nucleotides 1-3691 of SEQ ID NO:8) contained three regions: (a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of SEQ ID NO:3, and nucleotides 1-2248 of SEQ ID NOs:8 and 10), (b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of SEQ ID NO:3, and nucleotides 2249-2313 of SEQ ID NOs:8 and 10), and (c) an intron sequence (i.e., nucleotides 2314-3688 of SEQ ID NO:3, and nucleotides 2314-3688 of SEQ ID NOs:8 and 10)." (col. 15, line 62 – col. 16, line 5). "The invention also contemplates at least a portion of SEQ ID NOs:7 and 10, and homologs thereof having promoter activity. The term "promoter activity" … refers to the ability of the nucleic acid sequence to initiate transcription of an operably linked nucleotide sequence into mRNA." (col.

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	17, lines 49-54)
the complement of SEQ ID NO:10;	**The compliment of SEQ ID NO: 10** "As used herein, the terms "complementary" or "complementarity" are used in reference to nucleotide sequences related by the base-pairing rules. For example, the sequence 5'-AGT-3' is complementary to the sequence 5'-ACT-3'. Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases is not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acids is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. A "complement" of a nucleic acid sequence as used herein refers to a nucleotide sequence whose nucleic acids show total complementarity to the nucleic acids of the nucleic acid sequence." (col. 9, lines 48-64)
b) operably linking said nucleic acid sequence of interest to said nucleotide sequence to produce a transgene; and	**Operably linking nucleic acid sequence of interest and nucleotide sequence to produce a transgene** "The term "transgene" as used herein refers to any nucleic acid sequence which is introduced into the genome of a cell by experimental manipulations. A transgene may be an "endogenous DNA sequence," or a "heterologous DNA sequence" (i.e., "foreign DNA"). The term "endogenous DNA sequence" refers to a nucleotide sequence which is naturally found in the cell into which it is introduced so long as it does not contain some modification (e.g., a point mutation, the presence of a selectable marker gene, etc.) relative to the naturally-occurring sequence. The term "heterologous DNA sequence" refers to a nucleotide sequence which is ligated to, or is manipulated to become ligated to, a nucleic acid sequence to which it is not ligated in nature, or to which it is ligated at a different location in nature. Heterologous DNA is not endogenous to the cell into which it is introduced, but has been obtained from another cell. Heterologous DNA also includes an endogenous DNA sequence which contains some modification. Generally, although not necessarily, heterologous DNA encodes RNA and proteins that are not normally produced by the cell into which it is expressed. Examples of heterologous DNA include reporter genes, transcriptional and translational regulatory sequences, selectable marker proteins (e.g., proteins which confer drug resistance), etc." (col. 8 lines 1-24) "The terms "operably linked," "in operable combination," and "in operable order" as used herein refer to the linkage of nucleic acid sequences in a manner such that a nucleic acid molecule is capable of directing the transcription of nucleic acid sequence of interest and/or the synthesis of a polypeptide sequence of interest." (col. 17, lines 54-60)
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	**Introducing transgene into plant cell to produce transgenic plant cell** "The term "transgenic" when used in reference to a cell refers to a cell which contains a transgene, or whose genome has been altered by the introduction of a transgene. The term "transgenic" when used in reference to a tissue or to a plant refers to a tissue or plant, respectively, which comprises one or more cells that contain a transgene, or whose genome has been altered by the introduction of a transgene. Transgenic cells, tissues and

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said transgenic plant cell.	plants may be produced by several methods including the introduction of a "transgene" comprising nucleic acid (usually DNA) into a target cell or integration of the transgene into a chromosome of a target cell by way of human intervention, such as by the methods described herein." (col. 7, line 55 – 67) "In one embodiment, the expression vectors are introduced into plant cells by particle mediated gene transfer." (col. 24, lines 48-49) "Alternatively, an expression vector may be inserted into the genome of plant cells by infecting the cells with a bacterium..." (col. 25, lines 6-8) "Other methods are also available for the introduction of expression vectors into plant tissue, e.g., electroinjection (Nan et al. (1995) In "Biotechnology in Agriculture and Forestry," Ed. Y.P.S. Bajaj, Springer-Verlag Berlin Heidelberg, Vol 34:145-155; Griesbach (1992) HortScience 27:620); fusion with liposomes, lysosomes, cells, minicells or other fusible lipid-surfaced bodies (Fraley et al. (1982) Proc. Natl. Acad. Sci. USA 79:1859-1863); polyethylene glycol (Krens et al. (1982) nature 296:72-74); chemicals that increase free DNA uptake; transformation using virus, and the like." (col. 26, lines 50-60)
7. A method for expressing a nucleic acid sequence of interest in a plant cell, comprising:	
a) providing: i) a plant cell;	**Providing a plant cell** "In another preferred embodiment, the plant cell is derived from a monocotyledonous plant. In yet a more preferred embodiment, the monocotyledonous plant is selected from the group consisting of sugarcane, maize, sorghum, pineapple, rice, barley, oat, wheat, rye, yam, onion, banana, coconut, date, and hop. In another alterative more preferred embodiment the plant cell is derived from a dicotyledonous plant. In a yet more preferred embodiment, the dicotyledonous plant is selected from the group consisting of tobacco, tomato, soybean, and papaya." (col. 5, lines 50-60) "The term "plant" as used herein refers to a plurality of plant cells which are largely differentiated into a structure that is present at any stage of a plant's development. Such structures include, but are not limited to, a fruit, shoot, stem, leaf, flower petal, etc. The term "plant tissue" includes differentiated and undifferentiated tissues of plants including, but not limited to, roots, shoots, leaves, pollen, seeds, tumor tissue and various types of cells in culture (e.g., single cells, protoplasts, embryos, callus, protocorm-like bodies, etc.). Plant tissue may be in planta, in organ culture, tissue culture, or cell culture." (col. 13, lines 12-23)
ii) a nucleic acid sequence of interest; and	**Providing a nucleic acid sequence of interest** "In yet another alternative embodiment, the nucleic acid sequence of interest is a sense sequence. In a more preferred embodiment, the sense sequence encodes a protein selected from the group consisting of .beta.-glucuronidase, luciferase, .beta.-galactosidase, 1-aminocyclopropane-1-carboxylic acid deaminase, sucrose phosphate synthase, 5-enolpyruvyl-3- phosphoshikimate synthase, acetolactate synthase, RNase, wheat germ agglutinin, sweetness protein, and Bacillus thuringiensis crystal toxin proteins. In a further

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	alternative embodiment, the nucleic acid sequence of interest is an antisense sequence. In a more preferred embodiment, the antisense sequence is selected from the group consisting of an antisense sequence to ACC synthase, to ethylene inducible sequences, and to polyphenol oxidase." (col. 5, lines 8-21) "The term "nucleotide sequence of interest" refers to any nucleotide sequence, the manipulation of which may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (e.g., reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product, (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.)." (col. 8 line 66 – col. 9, line 9) Specific Examples: (col. 22, line 32 – col. 24, line 6) - I-arninocyclopropane-l-carboxylic acid (ACC) - sucrose phosphate synthase enzyme - 5-enolpyruvyl-3- phosphoshikimate synthase (EPSP synthase) - acetolactate synthase - RNase - wheat germ agglutinin - nucleic acid sequences, which encode the sweetness protein - Bacillus thuringiensis (B.t.) crystal toxin proteins - nucleic acid sequence which encodes an antisense RNA that hybridizes with genomic plant DNA (e.g. ethylene inducible sequences in fruit, ACC synthase gene, polyphenol oxidase)
iii) a promoter comprising	**Providing a promoter** "The term "promoter," "promoter element," or "promoter sequence" as used herein, refers to a DNA sequence which when ligated to a nucleotide sequence of interest is capable of controlling the transcription of the nucleotide sequence of interest into mRNA." (col. 11, line 48-52)
SEQ ID NO:10;	**SEQ ID NO: 10** "Further provided by the invention is a substantially purified nucleic acid sequence comprising a portion of a nucleotide sequence selected from the group consisting of SEQ ID NO:10 and the complement thereof. In a preferred embodiment, the portion is characterized by having promoter activity. In a more preferred embodiment, the promoter activity is constitutive. In an alternative preferred embodiment, the portion is double-stranded. In another alternative preferred embodiment, the portion is single-stranded. In yet another alternative preferred embodiment, the portion comprises the nucleotide sequence selected from the group consisting of the nucleotides 1 to 3600, from 3602 to 3612, from 3614 to 3688, from 1 to 2248, from 2249 to 2313, from 2314 to 3688, and from 1671 to 2248." (col. 2, lines 53-66) FIG. 11 shows SEQ: 10 - the portion of the UBI9 gene upstream of the translation start codon, which corresponds to nucleotides 1-3688 of SEQ: 8. (col. 7, lines 29-33) FIG. 8 shows SEQ: 8 (GenBank accession number AF093505) of the sugarcane polyubiquitin ubi9 gene. (col. 7, lines 10-13)

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	"Similarly, it is the inventors' view that the sequence upstream of the translation start codon of the ubi9 gene (a) is as shown in FIG. 11 (SEQ ID NO:10) (i.e., total number of nucleotides being 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3'), (b) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3', assuming substitution of the "T" at position 4 with a "C"), or (c) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3689, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3', assuming insertion of a "C" at position 4. Accordingly, any reference herein to SEQ ID NO:10 is intended to mean each and every one of the three nucleotide sequences described in the preceding sentence." (col. 15, lines 41-56) "The nucleotide sequence upstream of the translation start codon of the ubi9 gene (i.e., nucleotides 1-3691 of SEQ ID NO:3, and nucleotides 1-3691 of SEQ ID NO:8) contained three regions: (a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of SEQ ID NO:3, and nucleotides 1-2248 of SEQ ID NOs:8 and 10), (b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of SEQ ID NO:3, and nucleotides 2249-2313 of SEQ ID NOs:8 and 10), and (c) an intron sequence (i.e., nucleotides 2314-3688 of SEQ ID NO:3, and nucleotides 2314-3688 of SEQ ID NOs:8 and 10)." (col. 15, line 62 – col. 16, line 5). "The invention also contemplates at least a portion of SEQ ID NOs:7 and 10, and homologs thereof having promoter activity. The term "promoter activity" ... refers to the ability of the nucleic acid sequence to initiate transcription of an operably linked nucleotide sequence into mRNA." (col. 17, lines 49-54)
b) operably linking said nucleic acid sequence of interest to said promoter to produce a transgene;	**Operably linking nucleic acid sequence of interest to promoter to produce transgene** "The term "transgene" as used herein refers to any nucleic acid sequence which is introduced into the genome of a cell by experimental manipulations. A transgene may be an "endogenous DNA sequence," or a "heterologous DNA sequence" (i.e., "foreign DNA"). The term "endogenous DNA sequence" refers to a nucleotide sequence which is naturally found in the cell into which it is introduced so long as it does not contain some modification (e.g., a point mutation, the presence of a selectable marker gene, etc.) relative to the naturally-occurring sequence. The term "heterologous DNA sequence" refers to a nucleotide sequence which is ligated to, or is manipulated to become ligated to, a nucleic acid sequence to which it is not ligated in nature, or to which it is ligated at a different location in nature. Heterologous DNA is not endogenous to the cell into which it is introduced, but has been obtained from another cell. Heterologous DNA also includes an endogenous DNA sequence which contains some modification. Generally, although not necessarily, heterologous DNA encodes RNA and proteins that are not normally produced by the cell into which it is expressed. Examples of heterologous DNA include reporter genes, transcriptional and translational regulatory sequences, selectable marker proteins (e.g., proteins which confer drug

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	resistance), etc." (col. 8 lines 1-24) "The terms "operably linked," "in operable combination," and "in operable order" as used herein refer to the linkage of nucleic acid sequences in a manner such that a nucleic acid molecule is capable of directing the transcription of nucleic acid sequence of interest and/or the synthesis of a polypeptide sequence of interest." (col. 17, lines 54-60)
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	**Introducing transgene into plant cell to produce transgenic plant cell** "The term "transgenic" when used in reference to a cell refers to a cell which contains a transgene, or whose genome has been altered by the introduction of a transgene. The term "transgenic" when used in reference to a tissue or to a plant refers to a tissue or plant, respectively, which comprises one or more cells that contain a transgene, or whose genome has been altered by the introduction of a transgene. Transgenic cells, tissues and plants may be produced by several methods including the introduction of a "transgene" comprising nucleic acid (usually DNA) into a target cell or integration of the transgene into a chromosome of a target cell by way of human intervention, such as by the methods described herein." (col. 7, line 55 – 67) "In one embodiment, the expression vectors are introduced into plant cells by particle mediated gene transfer." (col. 24, lines 48-49) "Alternatively, an expression vector may be inserted into the genome of plant cells by infecting the cells with a bacterium…" (col. 25, lines 6-8) "Other methods are also available for the introduction of expression vectors into plant tissue, e.g., electroinjection (Nan et al. (1995) In "Biotechnology in Agriculture and Forestry," Ed. Y.P.S. Bajaj, Springer-Verlag Berlin Heidelberg, Vol 34:145-155; Griesbach (1992) HortScience 27:620); fusion with liposomes, lysosomes, cells, minicells or other fusible lipid-surfaced bodies (Fraley et al. (1982) Proc. Natl. Acad. Sci. USA 79:1859-1863); polyethylene glycol (Krens et al. (1982) nature 296:72-74); chemicals that increase free DNA uptake; transformation using virus, and the like." (col. 26, lines 50-60)
d) identifying said transgenic plant cell.	**Identifying transgenic plant cell** "Plants, plant cells and tissues transformed with a heterologous nucleic acid sequence of interest are readily detected using methods known in the art including, but not limited to, restriction mapping of the genomic DNA, PCR-analysis, DNA-DNA hybridization, DNA-RNA hybridization, DNA sequence analysis and the like. Additionally, selection of transformed plant cells may be accomplished using a selection marker gene." (col. 26, line 62 – col. 27, line 2)

Claim Constructions: '948

Claim 1, on its face, and as interpreted by the patent specification recites:

Substantially purified nucleic acid sequences, which are derived from sugarcane polyubiquitin genes and are capable of directing expression of an operably linked nucleic acid sequence of interest. These substantially purified nucleic acid sequences may be double or single stranded and have constitutive promoter activity.

The first nucleic acid sequence is nucleotides 1-1802 of the sugarcane polyubiquitin UBI-4 gene (GenBank accession number AF093504).

The second nucleic acid sequence is the complement of nucleotides 1-1802 of the sugarcane polyubiquitin UBI-4 gene (GenBank accession number AF093504).

The third nucleotide sequence, in general, is nucleotides 1-3688 of the sugarcane polyubiquitin UBI-9 gene (GenBank accession number AF093505). However, the sequence upstream of the translation start codon of the UBI-9 gene may take the following forms: a) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3'; b) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3', assuming substitution of the "T" at position 4 with a "C"; or c) the total number of nucleotides is 3689, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3', assuming insertion of a "C" at position 4. Furthermore, the nucleotide sequence upstream of the translation start codon of the UBI-9 gene contains three regions: a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of the sugarcane polyubiquitin UBI-9 gene); b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of the sugarcane polyubiquitin UBI-9 gene), and c) an intron sequence (i.e., nucleotides 2314-3688 of the sugarcane polyubiquitin UBI-9 gene).

The fourth nucleotide sequence is the complement of nucleotides 1-3688 of the sugarcane polyubiquitin UBI-9 gene (GenBank accession number AF093505) and each of the variations (a-c) listed above.

While it is unclear as to whether this claim covers fragments or homologous sequences of the ubiquitin promoter, arguments can be made based on the specification that claim 1 might cover fragments as short as 10 contiguous base pairs or a sequence having at least 61% homology to SEQ ID No: 7. The specification states that more preferably fragments must be at least 20 nucleotide bases long.

Claim 4, on its face, and as interpreted by the patent specification recites:

Substantially purified nucleic acid sequence, which is derived from sugarcane polyubiquitin genes and is capable of directing expression of an operably linked nucleic acid sequence of interest. The substantially purified nucleic acid sequence may be double or single stranded and have constitutive promoter activity. The nucleic acid sequence is

the HindIII/XbaI fragment isolated from plasmid pubi9-GUS contained in Escherichia coli cells deposited as NRRLB-30116. The HindIII/XbaI fragment is a sugarcane polyubiquitin UBI-9 promoter with an XbaI restriction site added at the 3' end.

Claim 5, on its face, and as interpreted by the patent specification recites:

A transgenic monocotyledonous or dicotyledonous plant cell that contains a double stranded sugarcane UBI-9 promoter operably linked to any nucleotide sequence, the manipulation of which, may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art.

The sugarcane UBI-9 promoter is, in general, nucleotides 1-3688 of the sugarcane polyubiquitin UBI-9 gene (GenBank accession number AF093505). However, the sequence upstream of the translation start codon of the UBI-9 gene may take the following forms: a) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3'; b) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3', assuming substitution of the "T" at position 4 with a "C"; or c) the total number of nucleotides is 3689, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3', assuming insertion of a "C" at position 4. Furthermore, the nucleotide sequence upstream of the translation start codon of the UBI-9 gene contains three regions: a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of the sugarcane polyubiquitin UBI-9 gene); b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of the sugarcane polyubiquitin UBI-9 gene), and c) an intron sequence (i.e., nucleotides 2314-3688 of the sugarcane polyubiquitin UBI-9 gene).

Specific examples of nucleotide sequences, the manipulation of which may be deemed desirable by one of ordinary skill in the art include: I-arninocyclopropane-l-carboxylic acid (ACC); sucrose phosphate synthase enzyme; 5-enolpyruvyl-3- phosphoshikimate synthase (EPSP synthase); acetolactate synthase; RNase; wheat germ agglutinin; nucleic acid sequences, which encode the sweetness protein; Bacillus thuringiensis (B.t.) crystal toxin proteins; and nucleic acid sequence which encodes an antisense RNA that hybridizes with a genomic plant DNA sequence (e.g. ethylene inducible sequences in fruit, ACC synthase gene, polyphenol oxidase).

Claim 6, on its face, and as interpreted by the patent specification recites:

A method for expressing any nucleotide sequence, the manipulation of which, may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art, in a transgenic monocotyledonous or dicotyledonous plant cell. The first step is providing the following elements: i) monocotyledonous or dicotyledonous plant cells; ii) any nucleotide sequence, the manipulation of which, may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art (e.g. I-arninocyclopropane-l-carboxylic acid (ACC)); and iii) a sugarcane UBI-9 promoter or its complementary sequence.

The sugarcane UBI-9 promoter is, in general, nucleotides 1-3688 of the sugarcane polyubiquitin UBI-9 gene (GenBank accession number AF093505). However, the sequence upstream of the translation start codon of the UBI-9 gene may take the following forms: a) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3'; b) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3', assuming substitution of the "T" at position 4 with a "C"; or c) the total number of nucleotides is 3689, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3', assuming insertion of a "C" at position 4. Furthermore, the nucleotide sequence upstream of the translation start codon of the UBI-9 gene contains three regions: a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of the sugarcane polyubiquitin UBI-9 gene); b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of the sugarcane polyubiquitin UBI-9 gene), and c) an intron sequence (i.e., nucleotides 2314-3688 of the sugarcane polyubiquitin UBI-9 gene).

The second step is to link the desired nucleotide sequence and the sugarcane UBI-9 promoter (or its complement) in a manner such that a nucleic acid molecule is capable of directing the transcription of the desired nucleic acid sequence and/or the synthesis of a desired polypeptide sequence.

The third step is to introduce the transgene created in the second step into the monocotyledonous or dicotyledonous plant cells by particle mediated gene transfer, infecting the cells with a bacterium, electroinjection, fusion with liposomes, lysosomes, cells, minicells or other fusible lipid-surfaced bodies, polyethylene glycol, chemicals that increase free DNA uptake; transformation using virus, or other known methods. This third step must produce a transgenic plant cell that expresses the desired nucleotide sequence.

Claim 7, on its face, and as interpreted by the patent specification recites:

A method that includes all of the steps recited in claim 6, but also includes the step of identifying the transgenic plant cell produced in step three of claim 6. The transgenic plant cell may be identified by restriction mapping of the genomic DNA, PCR-analysis, DNA-DNA hybridization, DNA-RNA hybridization, DNA sequence analysis, selection marker genes, and other known methods.

U.S. Patent No.	6,686,513
Inventor(s):	Albert; Henrik H. (Honolulu, HI), Wei; Hairong (Honolulu, HI)
Assignee(s):	The United States of America as represented by the Secretary of Agriculture (Washington, DC); University of Hawaii (Honolulu, HI)
Filed:	October 20, 2000
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Maintenance Fee Due:	August 3, 2007 (maintenance fee due at 3.5 years)
Status:	Active
Priority Information Summary:	This is a continuation-in-part of application Ser. No. 09/270,976, filed Mar. 17, 1999, which claims priority to provisional application Serial No. 60/078,767, filed Mar. 19, 1998, now abandoned.

Claims of U.S. 6,686,513	Support in specification of U.S. 6,686,513
1. A transgenic plant cell comprising	**Transgenic plant cell** "The term "transgenic" when used in reference to a cell refers to a cell which contains a transgene, or whose genome has been altered by the introduction of a transgene. The term "transgenic" when used in reference to a tissue or to a plant refers to a tissue or plant, respectively, which comprises one or more cells that contain a transgene, or whose genome has been altered by the introduction of a transgene. Transgenic cells, tissues and plants may be produced by several methods including the introduction of a "transgene" comprising nucleic acid (usually DNA) into a target cell or integration of the transgene into a chromosome of a target cell by way of human intervention, such as by the methods described herein." (col. 8, lines 50-62) "The term "plant" as used herein refers to a plurality of plant cells which are largely differentiated into a structure that is present at any stage of a plant's development. Such structures include, but are not limited to, a fruit, shoot, stem, leaf, flower petal, etc. The term "plant tissue" includes differentiated and undifferentiated tissues of plants including, but not limited to, roots, shoots, leaves, pollen, seeds, tumor tissue and various types of cells in culture (e.g., single cells, protoplasts, embryos, callus, protocorm-like bodies, etc.). Plant tissue may be in planta, in organ culture, tissue culture, or cell culture." (col. 14, line 9-19)
a promoter comprising	**Promoter** "The term "promoter"... as used herein, refers to a DNA sequence which when ligated to a nucleotide sequence of interest is capable of controlling the transcription of the nucleotide sequence of interest into mRNA." (col. 12, lines 45-49)
SEQ ID NO:10,	**SEQ ID NO: 10** FIG. 11 shows the nucleotide sequence (SEQ ID NO:10) of the portion of the ubi9 gene upstream of the translation start codon which was ligated to the gene encoding .beta.-glucuronidase (GUS) in plasmids pubi9-GUS and pCAM-ubi9-GUS. SEQ ID NO:10 corresponds to nucleotides 1-3688 of SEQ ID NO:8.

Claims of U.S. 6,686,513	Support in specification of U.S. 6,686,513
	FIGS. 8 shows the nucleotide sequence (SEQ ID NO:8) (GenBank accession number AF093505) of the ubi9 gene. "It is noted that while the 5' end of the ubi9 gene was obtained by cleavage with HindIII which recognizes the sequence 5'-AAGCTT-3', repeated sequencing of the 5'-end of the ubi9 gene showed instead the sequence 5'-AAGTTT-3' (FIGS. 8 and 11). Thus, it is the inventors' view that the sequence of the full-length ubi9 gene (a) is as shown in FIG. 8A (SEQ ID NO:8) (i.e., total number of nucleotides being 5174, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3') (SEQ ID NO:13), (b) is as shown in FIG. 8A (SEQ ID NO:8) with the exception that it has a total number of nucleotides of 5174, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3' (SEQ ID NO: 14), assuming substitution of the "T" at position 4 with a "C"), or (c) is as shown in FIG. 8A (SEQ ID NO:8) with the exception that it has a total number of nucleotides of 5175, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3' (SEQ ID NO: 15), assuming insertion of a "C" at position 4. Accordingly, any reference herein to SEQ ID NO:8 is intended to mean each and every one of the three nucleotide sequences described in the preceding sentence. Similarly, it is the inventors' view that the sequence upstream of the translation start codon of the ubi9 gene (a) is as shown in FIG. 11 (SEQ ID NO:10) (i.e., total number of nucleotides being 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3') (SEQ ID NO:16), (b) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3' (SEQ ID NO:14), assuming substitution of the "T" at position 4 with a "C"), or (c) is as shown in FIG. 11 (SEQ ID NO:10) with the exception that it has a total number of nucleotides of 3689, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3' (SEQ ID NO:15), assuming insertion of a "C" at position 4. Accordingly, any reference herein to SEQ ID NO:10 is intended to mean each and every one of the three nucleotide sequences described in the preceding sentence. Fragments of the ubi9 gene sequence which were identical in the initially determined and the subsequently determined nucleic acid sequences were as follows (the nucleotide number refers to the nucleotide number in SEQ ID NO:8): Fragment A: 1-3600; fragment B: 3602-3612; and fragment C: 3614-3691. The nucleotide sequence upstream of the translation start codon of the ubi9 gene (i.e., nucleotides 1- 3691 of SEQ ID NO:3, and nucleotides 1-3691 of SEQ ID NO:8) contained three regions: (a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of SEQ ID NO:3, and nucleotides 1-2248 of SEQ ID NOs:8 and 10), (b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of SEQ ID NO:3, and nucleotides 2249-2313 of SEQ ID NOs:8 and 10), and (c) an intron sequence (i.e., nucleotides 2314-3688 of SEQ ID NO:3, and nucleotides 2314-3688 of SEQ ID NOs:8 and 10)." (col. 16, line 14 – col. 17, line 39)
wherein said promoter is operably linked to a nucleic acid sequence of interest, and	**Promoter operably linked to nucleic acid sequence of interest** "The term "nucleotide sequence of interest" refers to any nucleotide sequence, the manipulation of which may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (e.g., reporter genes, selection marker genes, oncogenes,

Claims of U.S. 6,686,513	Support in specification of U.S. 6,686,513
	drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product, (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.)." (col. 9, line 61 – col. 10, line 4) "The terms "operably linked," "in operable combination," and "in operable order" as used herein refer to the linkage of nucleic acid sequences in a manner such that a nucleic acid molecule is capable of directing the transcription of nucleic acid sequence of interest and/or the synthesis of a polypeptide sequence of interest." (col. 19, lines 26-32)
said plant cell is selected from sugar cane, tobacco, sorghum, pineapple, rice, maize, tomato, soybean, banana, and garlic.	**Plant cell is sugar cane, tobacco, sorghum, pineapple, rice, maize, tomato, soybean, banana, or garlic**
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;	**Providing plant cell** "The term "plant" as used herein refers to a plurality of plant cells which are largely differentiated into a structure that is present at any stage of a plant's development. Such structures include, but are not limited to, a fruit, shoot, stem, leaf, flower petal, etc. The term "plant tissue" includes differentiated and undifferentiated tissues of plants including, but not limited to, roots, shoots, leaves, pollen, seeds, tumor tissue and various types of cells in culture (e.g., single cells, protoplasts, embryos, callus, protocorm-like bodies, etc.). Plant tissue may be in planta, in organ culture, tissue culture, or cell culture." (col. 14, line 9-19)
ii) a nucleic acid sequence of interest; and	**Providing a nucleic acid sequence of interest** "The term "nucleotide sequence of interest" refers to any nucleotide sequence, the manipulation of which may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (e.g., reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product, (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.)." (col. 9, line 61 – col. 10, line 4) Specific examples: (col. 24, line 9 – col. 25, line 50) - 1-aminocyclopropane-1-carboxylic acid (ACC) - sucrose phosphate synthase enzyme - 5-enolpyruvyl-3-phosphoshikimate synthase (EPSP) - RNase - wheat germ agglutinin - sweetness protein - Bacillus thuringiensis (B.t.) crystal toxin proteins

Claims of U.S. 6,686,513	Support in specification of U.S. 6,686,513
	- antisense RNA that hybridizes with a genomic plant DNA sequence (e.g. ethylene inducible sequences in fruit, ACC synthase gene, polyphenol oxidase)
iii) a promoter comprising	**Providing a promoter** "The term "promoter"... as used herein, refers to a DNA sequence which when ligated to a nucleotide sequence of interest is capable of controlling the transcription of the nucleotide sequence of interest into mRNA." (col. 12, lines 45-49) "the promoter sequence is SEQ ID NO:10 which is derived from the sugarcane ubi9 gene."
SEQ ID NO: 10;	**SEQ ID NO: 10** FIG. 11 shows the nucleotide sequence (SEQ ID NO:10) of the portion of the ubi9 gene upstream of the translation start codon which was ligated to the gene encoding .beta.-glucuronidase (GUS) in plasmids pubi9-GUS and pCAM-ubi9-GUS. SEQ ID NO:10 corresponds to nucleotides 1-3688 of SEQ ID NO:8. FIGS. 8 shows the nucleotide sequence (SEQ ID NO:8) (GenBank accession number AF093505) of the ubi9 gene.
b) operably linking said nucleic acid sequence of interest to said promoter to produce a transgene; and	**Operably linking nucleic acid sequence of interest to promoter to produce a transgene** "The term "transgene" as used herein refers to any nucleic acid sequence which is introduced into the genome of a cell by experimental manipulations. A transgene may be an "endogenous DNA sequence," or a "heterologous DNA sequence" (i.e., "foreign DNA"). The term "endogenous DNA sequence" refers to a nucleotide sequence which is naturally found in the cell into which it is introduced so long as it does not contain some modification (e.g., a point mutation, the presence of a selectable marker gene, etc.) relative to the naturally-occurring sequence. The term "heterologous DNA sequence" refers to a nucleotide sequence which is ligated to, or is manipulated to become ligated to, a nucleic acid sequence to which it is not ligated in nature, or to which it is ligated at a different location in nature. Heterologous DNA is not endogenous to the cell into which it is introduced, but has been obtained from another cell. Heterologous DNA also includes an endogenous DNA sequence which contains some modification. Generally, although not necessarily, heterologous DNA encodes RNA and proteins that are not normally produced by the cell into which it is expressed. Examples of heterologous DNA include reporter genes, transcriptional and translational regulatory sequences, selectable marker proteins (e.g., proteins which confer drug resistance), etc." (col. 8 line 63 – col. 9, line 19) "The terms "operably linked," "in operable combination," and "in operable order" as used herein refer to the linkage of nucleic acid sequences in a manner such that a nucleic acid molecule is capable of directing the transcription of nucleic acid sequence of interest and/or the synthesis of a polypeptide sequence of interest." (col. 19, lines 26-32)
c) introducing said transgene into said plant cell to produce a transgenic plant cell under	**Introducing transgene into plant cell to produce a transgenic plant cell** "The term "transgenic" when used in reference to a cell refers to a cell which contains a transgene, or whose genome has been altered by the introduction of a transgene. The term "transgenic" when used in reference to a tissue or to

Claims of U.S. 6,686,513	Support in specification of U.S. 6,686,513
conditions such that said nucleic acid sequence of interest is expressed in said transgenic plant cell.	a plant refers to a tissue or plant, respectively, which comprises one or more cells that contain a transgene, or whose genome has been altered by the introduction of a transgene. Transgenic cells, tissues and plants may be produced by several methods including the introduction of a "transgene" comprising nucleic acid (usually DNA) into a target cell or integration of the transgene into a chromosome of a target cell by way of human intervention, such as by the methods described herein." (col. 8, lines 50-62) "In one embodiment, the expression vectors are introduced into plant cells by particle mediated gene transfer." (col. 26, line 25) "Alternatively, an expression vector may be inserted into the genome of plant cells by infecting the cells with a bacterium, including but not limited to an Agrobacterium strain previously transformed with the nucleic acid sequence of interest." (col. 26, lines 50-54) "Other methods are also available for the introduction of expression vectors into plant tissue, e.g., electroinjection (Nan et al. (1995) In "Biotechnology in Agriculture and Forestry," Ed. Y. P. S. Bajaj, Springer-Verlag Berlin Heidelberg, Vol 34:145-155; Griesbach (1992) HortScience 27:620); fusion with liposomes, lysosomes, cells, minicells or other fusible lipid-surfaced bodies (Fraley et al. (1982) Proc. Natl. Acad. Sci. USA 79:1859-1863); polyethylene glycol (Krens et al. (1982) nature 296:72-74); chemicals that increase free DNA uptake; transformation using virus, and the like." (col. 28, lines 28-28)

Claim Constructions: '513

Claim 1, on its face, and as interpreted by the patent specification recites:

A sugarcane, tobacco, sorghum, pineapple, rice, maize, tomato, soybean, banana, or garlic cell that contains a transgene or whose genome has been altered by the introduction of a transgene. The transgene is any nucleotide sequence, the manipulation of which may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art, that is linked to the sugarcane UBI-9 promoter (or its complement) in a manner such that the promoter is capable of directing the transcription of the desired nucleic acid sequence and/or the synthesis of a desired polypeptide sequence.

Nucleotide sequences that may be deemed desirable include, but are not limited to, coding sequences of structural genes (e.g., reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product, (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.). The following are specific examples of desirable nucleotide sequences: I-arninocyclopropane-l-carboxylic acid (ACC); sucrose phosphate synthase enzyme; 5-enolpyruvyl-3-phosphoshikimate synthase (EPSP synthase); acetolactate synthase; RNase; wheat germ agglutinin; nucleic acid sequences, which encode the sweetness protein, Bacillus thuringiensis (B.t.) crystal toxin proteins; and nucleic acid sequence which encodes an antisense RNA that hybridizes with a genomic plant DNA sequence (e.g. ethylene inducible sequences in fruit, ACC synthase gene, polyphenol oxidase).

The sugarcane UBI-9 promoter is, in general, nucleotides 1-3688 of the sugarcane polyubiquitin UBI-9 gene (GenBank accession number AF093505). However, the sequence upstream of the translation start codon of the UBI-9 gene may take the following forms: a) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3'; b) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3', assuming substitution of the "T" at position 4 with a "C"; or c) the total number of nucleotides is 3689, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3', assuming insertion of a "C" at position 4. Furthermore, the nucleotide sequence upstream of the translation start codon of the UBI-9 gene contains three regions: a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of the sugarcane polyubiquitin UBI-9 gene); b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of the sugarcane polyubiquitin UBI-9 gene), and c) an intron sequence (i.e., nucleotides 2314-3688 of the sugarcane polyubiquitin UBI-9 gene).

While it is unclear as to whether this claim covers fragments or homologous sequences of the ubiquitin promoter, arguments can be made based on the specification that claim 1 might cover fragments as short as 10 contiguous base pairs or a sequence having at least 61% homology to SEQ ID No: 7. The specification states that more preferably fragments must be at least 20 nucleotide bases long.

Claim 6, on its face, and as interpreted by the patent specification recites:

A method for expressing any nucleotide sequence, the manipulation of which, may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art, in a sugarcane, tobacco, sorghum, pineapple, rice, maize, tomato, soybean, banana, or garlic cell.

The first step is providing the following elements: i) sugarcane, tobacco, sorghum, pineapple, rice, maize, tomato, soybean, banana, or garlic cells; ii) any nucleotide sequence, the manipulation of which, may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art; and iii) a sugarcane UBI-9 promoter.

Nucleotide sequences that may be deemed desirable include, but are not limited to, coding sequences of structural genes (e.g., reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product, (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.). The following are specific examples of desirable nucleotide sequences: I-arninocyclopropane-l-carboxylic acid (ACC); sucrose phosphate synthase enzyme; 5-enolpyruvyl-3-phosphoshikimate synthase (EPSP synthase); acetolactate synthase; RNase; wheat germ agglutinin; nucleic acid sequences, which encode the sweetness protein; Bacillus thuringiensis (B.t.) crystal toxin proteins; and nucleic acid sequence which encodes an antisense RNA that hybridizes with a genomic plant DNA sequence (e.g. ethylene inducible sequences in fruit, ACC synthase gene, polyphenol oxidase).

The sugarcane UBI-9 promoter is, in general, nucleotides 1-3688 of the sugarcane polyubiquitin UBI-9 gene (GenBank accession number AF093505). However, the sequence upstream of the translation start codon of the UBI-9 gene may take the following forms: a) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGTTTTGnT-3'; b) the total number of nucleotides is 3688, with the ten nucleotides at the 5'-end being 5'-AAGCTTTGnT-3', assuming substitution of the "T" at position 4 with a "C"; or c) the total number of nucleotides is 3689, with the ten nucleotides at the 5'-end being 5'-AAGCTTTTGn-3', assuming insertion of a "C" at position 4. Furthermore, the nucleotide sequence upstream of the translation start codon of the UBI-9 gene contains three regions: a) an upstream of the 5' UTR sequence (i.e., nucleotides 1-2248 of the sugarcane polyubiquitin UBI-9 gene); b) a 5' UTR sequence (i.e., nucleotides 2249-2313 of the sugarcane polyubiquitin UBI-9 gene), and c) an intron sequence (i.e., nucleotides 2314-3688 of the sugarcane polyubiquitin UBI-9 gene).

The second step is to link the desired nucleic acid sequences and the sugarcane UBI-9 promoter in a manner such that the promoter is capable of directing the transcription of the desired nucleic acid sequence and/or the synthesis of a desired polypeptide sequence.

The third step is to introduce the transgene created in the second step into the sugarcane, tobacco, sorghum, pineapple, rice, maize, tomato, soybean, banana, or garlic cells by

particle mediated gene transfer, infecting the cells with a bacterium, electroinjection, fusion with liposomes, lysosomes, cells, minicells or other fusible lipid-surfaced bodies, polyethylene glycol, chemicals that increase free DNA uptake, transformation using virus, or other known methods. This third step must produce a transgenic plant cell that expresses the desired nucleotide sequence.

U.S. Patent No.	6,528,701
Inventor(s):	Wang; Jianlin (Baton Rouge, LA), Oard; James H. (Baton Rouge, LA)
Assignee(s):	Board of Supervisors of Louisiana State University and Agricultural and Mechanical College (Baton Rouge, LA)
Filed:	February 29, 2000
Issued:	March 4, 2003
Expiration Date:	February 29, 2020
Maintenance Fee Due:	September 4, 2010 (maintenance fee due at 7.5 years)
Status:	Active
Priority Information Summary:	The benefit of the Mar. 2, 1999 filing date of provisional application 60/198,241 (which was a conversion of non-provisional application 09/260,687), now abandoned, is claimed under 35 U.S.C. § 119(e).

Claims of U.S. 6,528,701	Support in specification of U.S. 6,528,701
1. An isolated nucleotide sequence comprising	
a rice ubiquitin promoter capable of controlling constitutive expression of	**Rice ubiquitin promoter capable of controlling constitutive expression** "This invention pertains to four novel ubiquitin promoters derived from ubiquitin genes isolated from rice (Oryza sativa L.), promoters that efficiently drive constitutive gene expression in transgenic plants" (col. 1, lines 7-10) "'Expression' is the transcription or translation of a structural gene." (col. 5, lines 11-12) "A 'promoter' is that portion of the DNA upstream from the coding region that contains the binding site for RNA polymerase II to initiate transcription of the DNA." (col. 5, lines 19-21) "It is expected that the rice ubiquitin promoters can be combined with any structural gene for efficient constitutive expression in transgenic plants...." (col. 6, lines 15-20)
a nucleic acid encoding a polypeptide,	**Nucleic acid encoding a polypeptide** "The two rice polyubiquitin promoters isolated from RUBQ1 and RUBQ2 have been used to drive GUS gene expression in rice." (col. 5, lines 46-49) "The rice ubiquitin promoter can be used in combination with any structural gene to make a chimeric gene..." (col. 5, lines 64-65) "The rice ubiquitin promoters can be used to regulate a variety of structural genes.... It is expected that the rice ubiquitin promoters can be combined with any structural gene for efficient constitutive expression in transgenic plants...." (col. 6, lines 6-20) "The promoters can thus be used to express a variety of structural genes in different plants, including genes for herbicide resistance, resistance to plant pathogens, and tolerance to drought or other adverse environmental conditions." (col. 6, lines 27-32)

Claims of U.S. 6,528,701	Support in specification of U.S. 6,528,701
wherein said nucleotide sequence comprises at least a portion of SEQ ID NO:3 which is upstream of position 2785 and	**At least a portion of SEQ ID NO: 3 upstream of position 2785** "The present invention relates to promoters from rice ubiquitin genes and the use of those promoters to drive gene expression in transformed plants. Four promoters from rice ubiquitin genes have been sequenced and cloned. Two polyubiquitin genes, RUBQ1 (SEQ ID NO 1) and RUBQ2 (SEQ ID NO 3), and two ubiquitin-fusion genes, RUBQ3 (SEQ ID NO 5) and RUBQ4 (SEQ ID NO 8), from rice have been characterized. Both polyubiquitin genes, RUBQ1 and RUBQ2, contain six ubiquitin monomers in the coding region with an intron immediately upstream of the coding region. (FIGS. 1 and 2) The two ubiquitin-fusion genes, RUBQ3 (SEQ ID NO 5) and RUBQ4 (SEQ ID NO 9), contain a single ubiquitin monomer with one putative intron within the ubiquitin monomer-coding region. (FIGS. 3 and 4). The polyubiquitin gene, RUBQ2, is expressed at high levels in all rice tissues tested." (col. 5, lines 31-45) "The 4442 base DNA sequence of the other polyubiquitin gene, RUBQ2, is shown in FIG. 2 (SEQ ID NO 3). The ubiquitin-coding region is found from positions 2786 to 4159. A 926 base intron is located immediately upstream of the coding region, extending from positions 1824 to 2785. A 2.8 kb RUBQ2-promoter can be isolated as a PstI and XhoI restriction fragment from RUBQ2. This promoter contains the 962 base intron followed by a 48 base ubiquitin-coding region. Again, a fusion protein is produced when a structural gene is inserted downstream of this RUBQ2-promoter with the 48 base ubiquitin-coding region. A TATA box is found at position 1712, approximately 30 bases upstream of the putative cap site. Within the promoter region, a GC box is found at position 1454 (259 bases upstream of the TATA box). One interesting feature of this promoter is an enhancer_core consensus sequence beginning at position 1019 (694 bases upstream of the TATA box). This enhancer core consensus sequence 5'GGTGTGGAAA(or TTT)G-3' (SEQ ID NO 18) has been found in both animal and plant genes and is believed to function as a global enhancement element for gene expression (Khoury et al., Enhancer Elements. Cell, vol. 33, pp. 313-314 (1983)). Additionally, a single heat shock consensus sequence is found beginning at position 1536 (176 bases upstream of the TATA box)." (col. 8, lines 13-37)
wherein said portion retains promoter activity	**Portion of SEQ ID NO: 3 retains promoter activity** "It should be also noted that a shortened promoter that will still retain promoter activity can be obtained using restriction sites within the promoter. In particular, the 48 base coding region could be removed from both promoters of RUBQ1 and RUBQ2 by exonuclease digestion." (col. 9, lines 59-64)

Claim Constructions: '701

Claim 1, on its face, and as interpreted by the patent specification recites:

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.

A nucleic acid sequence encoding the rice polyubiquitin UBI-2 promoter, wherein the nucleic acid sequence retains promoter activity.

The rice UBI-2 gene is a 4442 base DNA sequence. The ubiquitin-coding region is found from positions 2786 to 4159. A 926 base intron is located immediately upstream of the coding region, extending from positions 1824 to 2785. A 2.8 kb rice UBI-2 promoter can be isolated as a PstI and XhoI restriction fragment from the rice UBI-2 gene. This promoter contains the 962 base intron followed by a 48 base ubiquitin-coding region. A TATA box is found at position 1712, approximately 30 bases upstream of the putative cap site. Within the promoter region, a GC box is found at position 1454 (259 bases upstream of the TATA box). Another feature of this promoter is an enhancer core consensus sequence, 5'GGTGTGGAAA(or TTT)G-3', beginning at position 1019 (694 bases upstream of the TATA box). Additionally, a single heat shock consensus sequence is found beginning at position 1536.

U.S. Patent No.	6,448,391
Inventor(s):	Garbarino, Joan (Berkeley, CA); Belknap, William (Albany, CA)
Assignee(s):	The United States of America as represented by the Secretary of Agriculture (Washington, DC); Demegen, Inc. (Pittsburgh, PA) [*Note: This assignee is not listed on the current Patent Assignment Abstract of Title*]
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Status:	Active
Priority Information Summary:	This application is a continuation of application Ser. No. 08/801,028, filed Feb. 19, 1997, now U.S. Pat. No. 6,018,102, which is a continuation of application Ser. No. 08/279,472, filed Jul. 22, 1994 now abandoned.

Claims of U.S. 6,448,391	Support in specification of U.S. 6,448,391
1. A promoter comprising	**Promoter** "The term "promoter" as used herein refers to an untranslated (i.e. one that does not result in a peptide or protein product) sequence upstream of the polypeptide coding region of a nucleotide sequence that controls transcription of a gene." (col. 6, lines 3-7)
the sequence of nucleotides 1-1,788 of SEQ ID NO. 96.	**Nucleotides 1-1,788 of SEQ ID NO: 96** FIG. 2 is a map of a recombinant nucleic acid expression vector pUCUbi7-LP98 containing a 1220 bp polyubiquitin promoter region and 568 bp intron linked to a 228 bp coding region for a ubiquitin polypeptide with a six bp BamHI site at the 3' end (SEQ ID NO. 96) that is fused at its 3' end to a gene coding for a lytic peptide (D5D*, SEQ ID NO. 98) "Preferably, the ubiquitin promoter is a potato plant ubiquitin promoter and most preferably it is the potato Ubi3 or Ubi7 promoter." (col. 6, lines 32-34) "In embodiments wherein the isolated nucleocide sequence codes for the potato Ubi7 promoter linked to a gene coding for a ubiquitin polypeptide it has a nucleotide sequence according to SEQ ID NO. 96. The Ubi7 nucleotide sequence according to SEQ ID No. 96 includes an intron that is part of the ubiquitin transcription unit. The intron is not required for gene expression from the Ubi7 promoter, thus the Ubi7 promoter region without the intron can be considered as a specific functional equivalent of the Ubi7 promoter. The Ubi7 promoter alone, with or without the intron, has utility as a wound inducible promoter in eukaryotes." (col. 6, lines 39-49)
2. A promoter comprising	**Promoter** "The term "promoter" as used herein refers to an untranslated (i.e. one that does not result in a peptide or protein product) sequence upstream of the polypeptide coding region of a nucleotide sequence that controls transcription of a gene." (col. 6, lines 3-7)

Claims of U.S. 6,448,391	Support in specification of U.S. 6,448,391
the sequence of nucleotides 1-1220 of SEQ ID NO. 96.	**Nucleotides 1-1220 of SEQ ID NO: 96** FIG. 2 is a map of a recombinant nucleic acid expression vector pUCUbi7-LP98 containing a 1220 bp polyubiquitin promoter region and 568 bp intron linked to a 228 bp coding region for a ubiquitin polypeptide with a six bp BamHI site at the 3' end (SEQ ID NO. 96) that is fused at its 3' end to a gene coding for a lytic peptide (D5D*, SEQ ID NO. 98) "Preferably, the ubiquitin promoter is a potato plant ubiquitin promoter and most preferably it is the potato Ubi3 or Ubi7 promoter." (col. 6, lines 32-34) "In embodiments wherein the isolated nucleocide sequence codes for the potato Ubi7 promoter linked to a gene coding for a ubiquitin polypeptide it has a nucleotide sequence according to SEQ ID NO. 96. The Ubi7 nucleotide sequence according to SEQ ID No. 96 includes an intron that is part of the ubiquitin transcription unit. The intron is not required for gene expression from the Ubi7 promoter, thus the Ubi7 promoter region without the intron can be considered as a specific functional equivalent of the Ubi7 promoter. The Ubi7 promoter alone, with or without the intron, has utility as a wound inducible promoter in eukaryotes." (col. 6, lines 39-49)

Claim Constructions: '391

Claim 1, on its face, and as interpreted by the patent specification recites:

A pototao UBI-7 promoter containing a 1220 bp polyubiquitin promoter region and 568 bp intron.

Claim 2, on its face, and as interpreted by the patent specification recites:

A pototao UBI-7 promoter containing a 1220 bp polyubiquitin promoter region.

U.S. Patent No.	6,638,766
Inventor(s):	Albert; Henrik H., Wei; Hairong
Assignee(s):	The United States of America as represented by the Secretary of Agriculture; University of Hawaii
Filed:	May 24, 2001
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Expiration Date:	August 4, 2021
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Status:	Active
Priority Information Summary:	This is a continuation of co-pending application Ser. No. 09/270,976 (pending) filed on Mar. 17, 1999, which claims priority to provisional application Ser. No. 60/078,768, filed on Mar. 19, 1998.

Claims of U.S, 6,638,766	Support in specification of U.S, 6,638,766
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.	**Substantially purified:** "As used herein, the term "purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated. An "isolated nucleic acid sequence" is therefore a purified nucleic acid sequence. "Substantially purified" molecules are at least 60% free, preferably at least 75% free, and more preferably at least 90% free from other components with which they are naturally associated." 9:35-42. "FIG. 10 shows the nucleotide sequence (SEQ ID NO:7) of the portion of the ubi4 gene which was ligated to the gene encoding .beta.-glucuronidase (GUS) in plasmids pubi4-GUS and 4PI-GUS. SEQ ID NO:7 corresponds to nucleotides 1-1802 of SEQ ID NO:5." 7:19-23. "The invention provides the nucleic acid sequence of two members of the sugarcane polyubiquitin family, the ubi4 gene and the ubi9 gene. Referring to the ubi4 gene, the initially determined nucleic acid sequence (SEQ ID NO:1) of the ubi4 gene including the translation start codon (ATG) and the sequence upstream of the translation start codon is shown in FIG. 3A, and the nucleic acid sequence (SEQ ID NO:2) of the ubi4 gene including the translation stop codon and sequences downstream of the translation stop codon is shown in FIG. 3B. The subsequently determined nucleic acid sequence (SEQ ID NO:5) of the entire ubi4 gene is shown in FIG. 5A with the subsequently determined nucleic acid sequence (SEQ ID NO:7) located upstream of the translation start codon of the ubi4 gene being shown in FIG. 10. The nucleotide sequence of FIG. 5A represents nucleotides 1 to 5512 of the 5551 nucleotide sequence deposited as GenBank accession number AF093504." 14:41-57. **Complement:** "As used herein, the terms "complementary" or "complementarity" are used in reference to nucleotide sequences related by the base-pairing rules. For example, the sequence 5'-AGT-3' is complementary to the sequence 5'-ACT-3'. Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases is not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acids

Claims of U.S. 6,638,766	Support in specification of U.S. 6,638,766
	is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands." 9:43-55. "A "complement" of a nucleic acid sequence as used herein refers to a nucleotide sequence whose nucleic acids show total complementarity to the nucleic acids of the nucleic acid sequence." 9:57-60. "The term "homology" when used in relation to nucleic acids refers to a degree of complementarity. There may be partial homology (i.e., partial identity) or complete homology (i.e., complete identity). A partially complementary sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid and is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe (i.e., an oligonucleotide which is capable of hybridizing to another oligonucleotide of interest) will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence to a target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target which lacks even a partial degree of complementarity (e.g., less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target." 9:60-10:19. **Seq ID: No. 7** "FIG. 10 shows the nucleotide sequence (SEQ ID NO:7) of the portion of the ubi4 gene which was ligated to the gene encoding β-glucuronidase (GUS) in plasmids pubi4-GUS and 4PI-GUS. SEQ ID NO:7 corresponds to nucleotides 1-1802 of SEQ ID NO:5." 7:20-24. "FIG. 5 shows the nucleotide sequence (SEQ ID NO:5) (nucleotides 1 to 5512 of the 5551 nucleotide sequence deposited as GenBank accession number AF093504) (A) and translated amino acid sequence (SEQ ID NO:6) (B) of sugarcane polyubiquitin ubi4 gene." 6:51-55. "The invention provides the nucleic acid sequence of two members of the sugarcane polyubiquitin family, the ubi4 gene and the ubi9 gene. Referring to the ubi4 gene, the initially determined nucleic acid sequence (SEQ ID NO:1) of the ubi4 gene including the translation start codon (ATG) and the sequence upstream of the translation start codon is shown in FIG. 3A, and the nucleic acid sequence (SEQ ID NO:2) of the ubi4 gene including the translation stop codon and sequences downstram of the translation stop codon is shown in FIG. 3B. The subsequently determined nucleic acid sequence (SEQ ID NO:5) of the entire ubi4 gene is shown in FIG. 5A with the subsequently determined nucleic acid sequence (SEQ ID NO:7) located upstream of the translation start codon of the ubi4 gene being shown in FIG. 10. The nucleotide sequence of FIG. 5A represents nucleotides 1 to 5512 of the 5551 nucleotide sequence deposited as GenBank accession number AF093504." 14:41-57.

Claims of U.S. 6,638,766	Support in specification of U.S. 6,638,766
	"The present invention is not limited to SEQ ID NO:7 but specifically contemplates portions thereof. As used herein the term "portion" when made in reference to a nucleic acid sequence refers to a fragment of that sequence. The fragment may range in size from ten (10) contiguous nucleotide residues to the entire nucleic acid sequence minus one nucleic acid residue. Thus, a nucleic acid sequence comprising "at least a portion of" a nucleotide sequence comprises from ten (10) contiguous nucleotide residues of the nucleotide sequence to the entire nucleotide sequence." 16:30-39. "In a preferred embodiment, portions contemplated to be within the scope of the invention include, but are not limited to, portions larger than 20 nucleotide bases, more preferably larger than 100 nucleotide bases, the sequence upstream of the 5' UTR sequence (i.e., nucleotide sequence from position 1 to 377 of SEQ ID NO:7), the 5' UTR sequence (i.e., nucleotides sequence from position 378 to 442 of SEQ ID NO:7), and the intron sequence (i.e., nucleotide sequence from position 443 to 1802 of SEQ ID NO:7). In an alternative preferred embodiment, portions within the scope of the invention include portions larger than 20 nucleotide bases, more preferably larger than 100 nucleotide bases, those sequences which are upstream of the translation start codon and which are identical in the initially determined and subsequently determined sequence of the ubi4 gene, and are exemplified by the nucleotide sequence from position 1 to 242, from position 245 to 787, from position 788 to 1020, from position 1021 to 1084, from position 1085 to 1168, from position 1169 to 1173, from position 1174 to 1648, and from position 1649 to 1802 of SEQ ID NO:7. In yet another alternative preferred embodiment, the portion contains the 377 bp sequence which is upstream of the 5'UTR and which is highly homologous (>90% identity) in both the ubi4 and ubi9 gene sequences, i.e., the nucleotide sequence from position 1 to 377 of SEQ ID NO:7." 16:40-64. "The invention provided herein is not limited to SEQ ID NO:7 and 10, homologs thereof, and portions thereof, having promoter activity, but includes sequences having no promoter activity (i.e., non-functional homologs and non-functional portions of homologs). This may be desirable, for example, where a portion of SEQ ID NOs:7 and 10 is used as a probe to detect the presence of SEQ ID NOs:7 and 10, respectively, or of portions thereof in a sample." 19:29-36. "The sequences of the present invention are not limited to SEQ ID NOs:7 and 10 and portions thereof, but also include homologs of SEQ ID NOs:7 and 10, as well as portions of these homologs. A nucleotide sequence which is a "homolog" of SEQ ID NOs:7 and 10 is defined herein as a nucleotide sequence which exhibits greater than 61% identity (but not 100% identity) to the sequence of SEQ ID NOs:7 and 10, respectively." 17:26-33.
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.	**Transgenic plant cell:** "In an alternative embodiment, the transgenic plant cell is derived from a monocotyledonous plant. In a more preferred embodiment, the monocotyledonous plant is selected from the group consisting of sugarcane, maize, sorghum, pineapple, rice, barley, oat, wheat, rye, yam, onion, banana, coconut, date, and hop. In another alternative embodiment, the transgenic plant cell is derived from a dicotyledonous plant. In a more preferred embodiment, the dicotyledonous plant is selected from the group consisting of tobacco, tomato, soybean, and papaya."3:1-10.

Claims of U.S. 6,638,766	Support in specification of U.S. 6,638,766
	Seq ID: No. 7 "FIG. 10 shows the nucleotide sequence (SEQ ID NO:7) of the portion of the ubi4 gene which was ligated to the gene encoding β-glucuronidase (GUS) in plasmids pubi4-GUS and 4PI-GUS. SEQ ID NO:7 corresponds to nucleotides 1-1802 of SEQ ID NO:5." 7:20-24. "FIG. 5 shows the nucleotide sequence (SEQ ID NO:5) (nucleotides 1 to 5512 of the 5551 nucleotide sequence deposited as GenBank accession number AF093504) (A) and translated amino acid sequence (SEQ ID NO:6) (B) of sugarcane polyubiquitin ubi4 gene." 6:51-55. "The invention provides the nucleic acid sequence of two members of the sugarcane polyubiquitin family, the ubi4 gene and the ubi9 gene. Referring to the ubi4 gene, the initially determined nucleic acid sequence (SEQ ID NO:1) of the ubi4 gene including the translation start codon (ATG) and the sequence upstream of the translation start codon is shown in FIG. 3A, and the nucleic acid sequence (SEQ ID NO:2) of the ubi4 gene including the translation stop codon and sequences downstram of the translation stop codon is shown in FIG. 3B. The subsequently determined nucleic acid sequence (SEQ ID NO:5) of the entire ubi4 gene is shown in FIG. 5A with the subsequently determined nucleic acid sequence (SEQ ID NO:7) located upstream of the translation start codon of the ubi4 gene being shown in FIG. 10. The nucleotide sequence of FIG. 5A represents nucleotides 1 to 5512 of the 5551 nucleotide sequence deposited as GenBank accession number AF093504." 14:41-57. "The present invention is not limited to SEQ ID NO:7 but specifically contemplates portions thereof. As used herein the term "portion" when made in reference to a nucleic acid sequence refers to a fragment of that sequence. The fragment may range in size from ten (10) contiguous nucleotide residues to the entire nucleic acid sequence minus one nucleic acid residue. Thus, a nucleic acid sequence comprising "at least a portion of" a nucleotide sequence comprises from ten (10) contiguous nucleotide residues of the nucleotide sequence to the entire nucleotide sequence." 16:30-39. "In a preferred embodiment, portions contemplated to be within the scope of the invention include, but are not limited to, portions larger than 20 nucleotide bases, more preferably larger than 100 nucleotide bases, the sequence upstream of the 5' UTR sequence (i.e., nucleotide sequence from position 1 to 377 of SEQ ID NO:7), the 5' UTR sequence (i.e., nucleotides sequence from position 378 to 442 of SEQ ID NO:7), and the intron sequence (i.e., nucleotide sequence from position 443 to 1802 of SEQ ID NO:7). In an alternative preferred embodiment, portions within the scope of the invention include portions larger than 20 nucleotide bases, more preferably larger than 100 nucleotide bases, those sequences which are upstream of the translation start codon and which are identical in the initially determined and subsequently determined sequence of the ubi4 gene, and are exemplified by the nucleotide sequence from position 1 to 242, from position 245 to 787, from position 788 to 1020, from position 1021 to 1084, from position 1085 to 1168, from position 1169 to 1173, from position 1174 to 1648, and from position 1649 to 1802 of SEQ ID NO:7. In yet another alternative preferred embodiment, the portion contains the 377 bp sequence which is upstream of the 5'UTR and which is highly homologous (>90% identity) in both the ubi4 and ubi9 gene sequences, i.e., the nucleotide sequence from position 1 to 377 of SEQ ID NO:7." 16:40-64.

Claims of U.S. 6,638,766	Support in specification of U.S. 6,638,766
	"The invention provided herein is not limited to SEQ ID NO:7 and 10, homologs thereof, and portions thereof, having promoter activity, but includes sequences having no promoter activity (i.e., non-functional homologs and non-functional portions of homologs). This may be desirable, for example, where a portion of SEQ ID NOs:7 and 10 is used as a probe to detect the presence of SEQ ID NOs:7 and 10, respectively, or of portions thereof in a sample." 19:29-36. "The sequences of the present invention are not limited to SEQ ID NOs:7 and 10 and portions thereof, but also include homologs of SEQ ID NOs:7 and 10, as well as portions of these homologs. A nucleotide sequence which is a "homolog" of SEQ ID NOs:7 and 10 is defined herein as a nucleotide sequence which exhibits greater than 61% identity (but not 100% identity) to the sequence of SEQ ID NOs:7 and 10, respectively." 17:26-33. **Operably linked:** Additionally provided by the invention is a method for expressing a nucleic acid sequence of interest in a plant cell, comprising: a) providing: i) a plant cell; ii) a nucleic acid sequence of interest; and iii) a nucleotide sequence selected from the group consisting of SEQ ID NO:7, the complement of SEQ ID NO:7, homologs of SEQ ID NO:7, homologs of the complement of SEQ ID NO:7; SEQ ID NO:10, the complement of SEQ ID NO:10, homologs of SEQ ID NO:10, and homologs of the complement of SEQ ID NO:10; b) operably linking the nucleic acid sequence of interest to the nucleotide sequence to produce a transgene; and c) introducing the transgene into the plant cell to produce a transgenic plant cell under conditions such that the nucleic acid sequence of interest is expressed in the transgenic plant cell. 5:30-44. The terms "operably linked," "in operable combination," and "in operable order" as used herein refer to the linkage of nucleic acid sequences in a manner such that a nucleic acid molecule is capable of directing the transcription of nucleic acid sequence of interest and/or the synthesis of a polypeptide sequence of interest. 17:53-59. **Sequence of Interest:** Promoter activity may be determined using methods known in the art. For example, a candidate nucleotide sequence whose promoter activity is to be determined is ligated in-frame to a nucleic acid sequence of interest (e.g., a reporter gene sequence, a selectable marker gene sequence) to generate a reporter vector, introducing the reporter vector into plant tissue using methods described herein, and detecting the expression of the reporter gene (e.g., detecting the presence of encoded mRNA or encoded protein, or the activity of a protein encoded by the reporter gene). 17:60-18:3. The term "nucleotide sequence of interest" refers to any nucleotide sequence, the manipulation of which may be deemed desirable for any reason (e.g., confer improved qualities), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (e.g., reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, etc.), and non-coding regulatory sequences which do not encode an mRNA or protein product, (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.). 8:62-9:5.

Claim Constructions: '766

Claim 1, on its face, and as interpreted by the patent specification recites:

A nucleic acid encoding the sugarcane polyubiquitin ubi4 gene, identified under the label SEQ ID No. 7, or its complementary sequence, wherein the polyubiquitin is at least 60% free from components from which it is naturally associated.

While it is unclear as to whether this claim covers fragments or homologous sequences of the ubiquitin promoter, arguments can be made based on the specification that claim 1 might cover fragments as short as 10 contiguous base pairs or a sequence having at least 61% homology to SEQ ID No: 7. The specification states that more preferably fragments must be at least 20 nucleotide bases long.

Claim 4, on its face, and as interpreted by the patent specification recites:

A transgenic plant cell having a DNA sequence encoding the sugarcane polyubiquitin ubi4 gene, identified under the label SEQ ID No. 7 and a nucleic acid sequence of interest, wherein the sugarcane polyubiquitin ubi4 gene is capable of directing the transcription of the nucleic acid sequence of interest and/or the synthesis of the nucleic acid sequence of interest.

The nucleotide sequence of interest includes, but is not limited to, a coding sequence of a structural gene (e.g., reporter gene, selection marker gene, oncogene, drug resistance gene, growth factor, etc.), and a non-coding regulatory sequence which do not encode an mRNA or protein product, (e.g., promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, etc.).

U.S. Patent No.	6,977,325
Inventor(s):	Jilka; Joseph M., Hood; Elizabeth E., Howard; John A.
Assignee(s):	
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Status:	Active
Priority Information Summary:	This application is a continuation of U.S. application Ser. No. 09/590,558, filed Jun. 9, 2000, now abandoned.

Claims of U.S. 6,977,325	Support in specification of U.S. 6,977,325
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.	**Engineered Ubiquitin promoter:** "The invention discloses novel promoter sequences capable of expressing genes in plant cells. The promoters include engineered versions of the maize ubiquitin promoter to increase expression levels beyond those observed with the native ubiquitin promoter and alter the tissue preference. Expression constructs, vectors, transgenic plants and methods are also disclosed." Abstract. "According to the invention, several engineered versions of a maize ubiquitin promoter are described which provide for expression levels that are higher than that achieved with native ubiquitin promoters and which spatially provide for altered expression levels in the embryo and endosperm of seed of regenerated plants." 3:29-34. "According to the invention, ubiquitin promoters are provided which differ from prior ubiquitin promoters primarily in the area of the heat shock region which comprises overlapping heat shock elements, to remove one of the elements, to remove the overlap of the sequences, or to delete both elements entirely. In a preferred embodiment binding domains for transcription factors may be inserted in this area. The interaction between the overlapping heat shock elements and the intron region with the rest of the 5' sequence in the ubiquitin promoter is unknown and was previously thought to be critical for full promoter function. See Quail, supra. Applicants have found that the promoter not only still functions adequately, despite prior teachings to the contrary but quite surprisingly have discovered that engineering in this region increases expression over the previous ubiquitin promoter system and alters the expression ratio of the protein from embryo to endosperm." 3:41-62. "As used herein, the term "maize ubiquitin promoter", or "ubiquitin promoter", or "ubiquitin-1 promoter" or "Ubi-1 promoter" shall include a 5' promoter region from a gene encoding ubiquitin, or protein with the functional characteristics of ubiquitin, and shall include the 5' region of the maize ubiquitin gene described in Quail, bases -899-1092 including sequences which are capable of hybridizing under conditions of high stringency thereto. As used herein the term "engineered ubiquitin promoter" or "Ubi-1 promoter variant" shall include a ubiquitin promoter which has a heat shock region that is engineered from its native state and which is capable of directing expression in a plant cell.

Claims of U.S. 6,977,325	Support in specification of U.S. 6,977,325
	As used herein the term "heat shock region" shall include an area of a ubiquitin promoter sequence which comprises two overlapping heat shock elements and includes bases -214 to -189 of the sequence disclosed in Quail." 14:14-30. **Plant Cell:** "By "host cell" is meant a cell which contains a vector and supports the replication and/or expression of the vector. Host cells may be prokaryotic cells such as E. coli, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells. Preferably, host cells are monocotyledonous or dicotyledonous plant cells. A particularly preferred monocotyledonous host cell is a maize host cell." 6:26-32. "As used herein, the term "plant" can include reference to whole plants, plant parts or organs (e.g., leaves, stems, roots, etc.), plant cells, seeds and progeny of same. Plant cell, as used herein, further includes, without limitation, cells obtained from or found in: seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. Plant cells can also be understood to include engineered cells, such as protoplasts, obtained from the aforementioned tissues. The class of plants which can be used in the methods of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants. Particularly preferred plants include maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, and millet." 7:45-60. **Heat Shock Region:** "As used herein the term "heat shock region" shall include an area of a ubiquitin promoter sequence which comprises two overlapping heat shock elements and includes bases -214 to -189 of the sequence disclosed in Quail." 14:27-30. "According to the invention novel promoters have been designed which include ubiquitin promoter variants with engineering primarily of the heat shock region at -214-190 of the ubiquitin promoter. Typically this region is comprised of two overlapping heat shock elements having the following sequence: CTGGACCCC TCTCGA GAGTTCCGCT (SEQ ID NO:1) The 5' heat shock consensus sequence is underlined. The 3' heat shock consensus sequence is [double underlined]. As can be seen, the overlap is a CTCGA 5-mer. According to the invention, novel promoters are designed which do not include two overlapping heat shock elements. Variants included, deletion of both heat shock elements, deletion of the 3' element, deletion of the 5' element, and removal of the overlap so that the two elements are adjacent." 16:28-45. "In yet another embodiment a transcription binding factor can be added in the engineered heat shock element region, to add in transcription of the sequences following the promoter." 17:15-18.

Claims of U.S. 6,977,325	Support in specification of U.S. 6,977,325
	Seq ID No: 4: *maize ubi-1 Heat shock element* **TABLE 1** Engineering of Ubi-1 promoter HSE DNA construct — DNA sequence[1] — HSE engineering — Transgenic lines PGN7062 — CTGGACCCCTCTCGAGAGTTCCGCT (SEQ ID NO:1) — wild type — GSB PGN7547 — ---------- — HSEs deleted — GSC PGN7565 — CTGGACCCCTCTCGA---------- (SEQ ID NO:2) — 3'HSE deleted — GSD PGN7583 — ----------CTCGAGAGTTCCGCT (SEQ ID NO:3) — 5'HSE deleted — GSE PGN7600 — CTGGACCCCTCTCGACTCGAGAGTTC CGCT (SEQ ID NO:4) — HSEs adjacent — GSF
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.	**heterologous structural gene or open reading frame:** "As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially engineered from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived, or, if from the same species, one or both are substantially engineered from their original form. A heterologous protein may originate from a foreign species or, if from the same species, is substantially engineered from its original form by deliberate human intervention." 4:14-25. "As used herein, the term "structural gene" includes any nucleotide sequence the expression of which is desired in a plant cell. A structural gene can include an entire sequence encoding a protein, an open reading frame or any portion thereof or also antisense. Examples of structural genes are included hereinafter are intended for illustration and not limitation." 10:52-58. "Structural Gene Likewise, by means of the present invention, heterologous nucleotide sequences can be expressed in transformed plants. More particularly, plants can be genetically engineered to express various phenotypes of agronomic interest. Exemplary genes include but are not limited to: plant disease resistance genes, (Martin et al., Science 262: 1432 (1993) (tomato Pto gene for resistance to Pseudomonas syringae pv. tomato encodes a protein kinase)); a Bacillus thuringiensis protein, (Geiser et al., Gene 48: 109 (1986); a lectin, (Van Damme et al., Plant Molec. Biol. 24: 25 (1994)); a vitamin-binding protein, (such as avidin. see PCT application US93/06487); an enzyme inhibitor, (Abe et al., J. Biol. Chem. 262: 16793 (1987)); an insect-specific hormone or pheromone, (see, for example, Hammock et al., Nature 344: 458 (1990)); an insect-specific peptide or neuropeptide, (Regan, J. Biol. Chem. 269: 9 (1994)); an insect-specific venom, (Pang et al., Gene 116: 165 (1992); an enzyme responsible for an hyperaccumulation of a monterpene; an

Claims of U.S. 6,977,325	Support in specification of U.S. 6,977,325
	enzyme involved in the engineering, including the post-translational engineering, of a biologically active molecule; for example, a glycolytic enzyme, a proteolytic enzyme; (See PCT application WO 93/02197); a molecule that stimulates signal transduction, (for example, Botella et al., Plant Molec. Biol. 24: 757 (1994)); a hydrophobic moment peptide, (PCT application WO 95/16776); a membrane permease, (Jaynes et al., Plant Sci. 89: 43 (1993)); a viral-invasive protein or a complex toxin derived therefrom, (Beachy et al., Ann. Rev. Phytopathol.28: 451 (1990)); (Taylor et al., Abstract #497, SEVENTH IN'L SYMPOSIUM ON MOLECULAR PLANT-MICROBE INTERACTIONS (Edinburgh, Scotland, 1994)); a virus-specific antibody, (Tavladoraki et al., Nature 366: 469 (1993)); a developmental-arrestive protein produced in nature by a pathogen or a parasite, (Lamb et al., Bio/Technology 10: 1436 (1992)); a developmental-arrestive protein produced in nature by a plant, (Logemann et al., Bio/Technology 10: 305 (1992)); a herbicide that inhibits the growing point or meristem, such as an imidazalinone or a sulfonylurea, (Lee et al.,EMBO J. 7: 1241 (1988)); Glyphosate (resistance imparted by mutant 5-enolpyruvl-3-phosphikimate synthase (EPSP) and aroA genes, respectively) (U.S. Pat. No. 4,940,835); a herbicide that inhibits photosynthesis, such as a triazine (psbA and gs+ genes) and a benzonitrile (nitrilase gene). (Przibilla et al., Plant Cell 3: 169 (1991)); Engineered fatty acid metabolism, for example, by transforming a plant with an antisense gene of stearoyl-ACP desaturase to increase stearic acid content of the plant. See Knultzon et al., Proc. Natl. Acad. Sci. USA 89: 2624 (1992); decreased phytate content, (Van Hartingsveldt et al., Gene 127: 87 (1993)); engineered carbohydrate composition, for example, by transforming plants with a gene coding for an enzyme that alters the branching pattern of starch. (See Shiroza et al., J. Bacteriol. 170: 810 (1988)); genes that controls cell proliferation and growth of the embryo and/or endosperm such as cell cycle regulators (Bogre L et al., "Regulation of cell division and the cytoskeleton by mitogen-activated protein kinases in higher plants." Results Probl Cell Differ 27:95-117 (2000). 18:22-19:36. **expression construct:** As used herein, a "expression cassette" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements which permit transcription of a particular nucleic acid in a host cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid to be transcribed, and a promoter. 11:18-27. **Engineered Ubiquitin promoter:** "The invention discloses novel promoter sequences capable of expressing genes in plant cells. The promoters include engineered versions of the maize ubiquitin promoter to increase expression levels beyond those observed with the native ubiquitin promoter and alter the tissue preference. Expression constructs, vectors, transgenic plants and methods are also disclosed." Abstract. "According to the invention, several engineered versions of a maize ubiquitin promoter are described which provide for expression levels that are higher than that achieved with native ubiquitin promoters and which spatially provide for altered expression levels in the embryo and endosperm of seed of regenerated plants." 3:29-34.

Claims of U.S. 6,977,325	Support in specification of U.S. 6,977,325
	"According to the invention, ubiquitin promoters are provided which differ from prior ubiquitin promoters primarily in the area of the heat shock region which comprises overlapping heat shock elements, to remove one of the elements, to remove the overlap of the sequences, or to delete both elements entirely. In a preferred embodiment binding domains for transcription factors may be inserted in this area. The interaction between the overlapping heat shock elements and the intron region with the rest of the 5' sequence in the ubiquitin promoter is unknown and was previously thought to be critical for full promoter function. See Quail, supra. Applicants have found that the promoter not only still functions adequately, despite prior teachings to the contrary but quite surprisingly have discovered that engineering in this region increases expression over the previous ubiquitin promoter system and alters the expression ratio of the protein from embryo to endosperm." 3:41-62. "As used herein, the term "maize ubiquitin promoter", or "ubiquitin promoter", or "ubiquitin-1 promoter" or "Ubi-1 promoter" shall include a 5' promoter region from a gene encoding ubiquitin, or protein with the functional characteristics of ubiquitin, and shall include the 5' region of the maize ubiquitin gene described in Quail, bases -899-1092 including sequences which are capable of hybridizing under conditions of high stringency thereto. As used herein the term "engineered ubiquitin promoter" or "Ubi-1 promoter variant" shall include a ubiquitin promoter which has a heat shock region that is engineered from its native state and which is capable of directing expression in a plant cell. As used herein the term "heat shock region" shall include an area of a ubiquitin promoter sequence which comprises two overlapping heat shock elements and includes bases -214 to -189 of the sequence disclosed in Quail." 14:14-30. **operably linked:** "As used herein "operably linked" includes reference to a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame." 8:37-44. **Plant Cell:** "By "host cell" is meant a cell which contains a vector and supports the replication and/or expression of the vector. Host cells may be prokaryotic cells such as E. coli, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells. Preferably, host cells are monocotyledonous or dicotyledonous plant cells. A particularly preferred monocotyledonous host cell is a maize host cell." 6:26-32. "As used herein, the term "plant" can include reference to whole plants, plant parts or organs (e.g., leaves, stems, roots, etc.), plant cells, seeds and progeny of same. Plant cell, as used herein, further includes, without limitation, cells obtained from or found in: seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. Plant cells can also be understood to include engineered cells, such as protoplasts, obtained from the aforementioned tissues. The class of plants which can be used in the methods of the invention

Claims of U.S. 6,977,325	Support in specification of U.S. 6,977,325
	is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants. Particularly preferred plants include maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, and millet." 7:45-60. **Heat Shock Region:** "As used herein the term "heat shock region" shall include an area of a ubiquitin promoter sequence which comprises two overlapping heat shock elements and includes bases -214 to -189 of the sequence disclosed in Quail." 14:27-30. "According to the invention novel promoters have been designed which include ubiquitin promoter variants with engineering primarily of the heat shock region at -214-190 of the ubiquitin promoter. Typically this region is comprised of two overlapping heat shock elements having the following sequence: CTGGACCCC TCTCGA GAGTTCCGCT (SEQ ID NO:1) The 5' heat shock consensus sequence is underlined. The 3' heat shock consensus sequence is [double underlined]. As can be seen, the overlap is a CTCGA 5-mer. According to the invention, novel promoters are designed which do not include two overlapping heat shock elements. Variants included, deletion of both heat shock elements, deletion of the 3' element, deletion of the 5' element, and removal of the overlap so that the two elements are adjacent." 16:28-45. "In yet another embodiment a transcription binding factor can be added in the engineered heat shock element region, to add in transcription of the sequences following the promoter." 17:15-18. **Seq ID No: 4:** *maize ubi-1 Heat shock element* **TABLE 1** Engineering of Ubi-1 promoter HSE DNA construct — DNA sequence[1] — HSE engineering — Transgenic lines PGN7062 — CTGGACCCCTCTCGAGAGTTCCGCT (SEQ ID NO:1) — wild type — GSB PGN7547 — ---------- — HSEs deleted — GSC PGN7565 — CTGGACCCCTCTCGA---------- (SEQ ID NO:2) — 3'HSE deleted — GSD PGN7583 — ----------CTCGAGAGTTCCGCT (SEQ ID NO:3) — 5'HSE deleted — GSE PGN7600 — CTGGACCCCTCTCGACTCGAGAGTTCCGCT (SEQ ID NO:4) — HSEs adjacent — GSF

Claim Constructions: '325

Claim 1, on its face, and as interpreted by the patent specification recites:

A modified maize ubiquitin promoter wherein the two heat shock elements that usually overlap are modified to be adjacent to each other. The sequence of the modified heat shock elements is as follows: CTGGACCCCTCTCGACTCGAGAGTTCCGCT (first heat shock element in plain text, the second heat shock element underlined).

The modified maize ubiquitin promoter with two adjacent heat shock elements is capable of directing expression of a nucleotide sequence in plant cells, tissues or whole plants whether monocotyledonous or dicotyledonous.

Claim 2, on its face, and as interpreted by the patent specification recites:

A method of expressing a gene from a source other than the host, or if from the host, substantially modified from its native form, or expressing a fragment or antisense portion of a gene in a plant cell, which may be monocotyledonous or dicotyledonous.

The method includes the step of introducing an expression construct into the plant cell. The expression construct having a modified maize ubiquitin promoter and a nucleotide sequence, which expression is desired, wherein the nucleotide sequence is functionally linked to the promoter so that the promoter sequence initiates and mediates transcription of the nucleotide sequence.

The modified maize ubiquitin is modified from its native form in such a way that the two heat shock elements that usually overlap are modified to be adjacent to each other. The sequence of the modified heat shock elements is as follows: CTGGACCCCTCTCGACTCGAGAGTTCCGCT (first heat shock element in plain text, the second heat shock element underlined).

A narrower possible interpretation of claim 2 is possible wherein the promoter sequence and nucleotide sequence are contiguous.

U.S. Patent No.	6,020,190
Inventor(s):	Quail; Peter H., Christensen; Alan H., Hershey; Howard P., Sharrock; Robert A., Sullivan; Thomas D.
Assignee(s):	Mycogen Plant Science, Inc.
Filed:	November 18, 1996
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Maintenance Fee Due:	Information Unavailable
Status:	Active
Priority Information Summary:	This is a division of application Ser. No. 08/462,092, filed Jun. 5, 1995 now U.S. Pat. No.5,614,399, which is a division of application Ser. No. 08/296,268, filed Aug. 25, 1994 (now U.S. Pat. No. 5,510,474), which is a continuation of application Ser. No. 08/191,134, filed Feb. 3, 1994 (now abandoned), which is a continuation of application Ser. No. 08/076,363, filed Jun. 11, 1993 (now abandoned), which is a continuation of application Ser. No. 07/670,496, filed Mar. 15, 1991 (now abandoned), which is a continuation of application Ser. No. 07/194,824, filed May 17, 1988 (now abandoned).

Claims of U.S. 6,020,190	**Support in specification of U.S. 6,020,190**
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	**Ubiquitin** "The vector herein described employs a maize ubiquitin promoter to control expression of..." 10:54-55. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 11:10-21. "FIG. 2 documents the DNA sequence and the deduced amino acid sequence of ubiquitin gene 1." 7:17-18. **Regulatory system:** "Other sequences conferring regulatory influences on transcription can be found within the promoter region and extending as far as 1000 bp or more 5' from the cap site." 8:8-11.

Claims of U.S. 6,020,190	Support in specification of U.S. 6,020,190
	"In this invention, the heat shock regulatory elements function to enhance transiently the level of downstream gene expression in response to sudden temperature elevation." 8:17-20. "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." 8: 21:24. "Plant Ubiquitin Regulatory System refers to the approximately 2 kb nucleotide sequence 5' to the translation start site of the maize ubiquitin gene and comprises sequences that direct initiation of transcription, regulation of transcription, control of expression level, induction of stress genes and enhancement of expression in response to stress. The regulatory system, comprising both promoter and regulatory functions, is the DNA sequence providing regulatory control or modulation of gene expression." 9:6-14. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 11:10-21. **Heat shock element:** Heat shock elements refer to DNA sequences that regulate gene expression in response to the stress of sudden temperature elevations. The response is seen as an immediate albeit transitory enhancement in level of expression of a downstream gene. The original work on heat shock genes was done with Drosophila but many other species including plants (T. Barnett et al. (1980) Dev. Genet. 1:331-340) exhibited analogous responses to stress. The essential primary component of the heat shock element was described in Drosophila to have the consensus sequence 5'-CTGGAATNTTCTAGE-3' (where N=A, T, C, or G) (Support for this definition of N is found in the Messing et al. reference) and to be located in the region between residues -66 through -47 bp upstream to the transcriptional start site (H. Pelham et al. (1982) supra). A chemically synthesized oligonucleotide copy of this consensus sequence can replace the natural sequence in conferring heat shock inducibility. In other systems, multiple heat shock elements were identified within the promoter region. For example, Rochester et al. (1986) supra recognized two heat shock elements in the maize hsp 70 gene. 10:13-33. **Intron:** "Introns or intervening sequences refer in this work to those regions of DNA sequence that are transcribed along with the coding sequences (exons) but are then removed in the formation of the mature mRNA. Introns may occur anywhere within a transcribed sequence--between coding sequences of the same or different genes, within the coding sequence of a gene, interrupting and splitting its amino acid sequences, and within the promoter region (5' to the translation start site). Introns in the primary transcript are excised and

Claims of U.S. 6,020,190	Support in specification of U.S. 6,020,190
	the coding sequences are simultaneously and precisely ligated to form the mature mRNA. The junctions of introns and exons form the splice sites. The base sequence of an intron begins with GU and ends with AG. The same splicing signal is found in many higher eukaryotes." 10:38-51.
(b) a plant-expressible structural gene wherein said structural gene is placed under the regulatory control of said plant ubiquitin regulatory system.	**Structural gene:** "A structural gene placed under the regulatory control of the plant ubiquitin regulatory system means that a structural gene is positioned such that the regulated expression of the gene is controlled by the sequences comprising the ubiquitin regulatory system." 9:14-19. Regulatory control: "Regulatory Control refers to the modulation of gene expression induced by DNA sequence elements located primarily, but not exclusively, upstream of (5' to) the (1981)- (1981)- transcription start site." 8:12-15.

Claim Constructions: '190

Claim 1, on its face, and as interpreted by the patent specification recites:

A DNA sequence no larger than 2kb encoding a Maize ubiquitin promoter 1 regulatory system, wherein the regulatory system includes the Maize ubiquitin promoter, including from the 5' end, 899 base pairs from the transcription start site, two overlapping heat shock elements at positions -214 to -204 and a TATA box at positions -30 to -10. To the 3' side of the transcription start site, an exon extends from position 1 to 83, and an intron is immediately adjacent at positions 84 to 1093 which comprises the 3' end of the 2kb Maize ubiquitin promoter regulatory system.

A plant-expressible structural gene is positioned, usually 3' to the promoter region, such that the regulated expression of the gene is controlled by the Maize ubiquitin promoter 1 regulatory system.

U.S. Patent No.	5,614,399
Inventor(s):	Quail; Peter H., Christensen; Alan H., Hershey; Howard P., Sharrock; Robert A., Sullivan; Thomas D.
Assignee(s):	Mycogen Plant Science, Inc.
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Status:	Active
Priority Information Summary:	This is a division of application Ser. No. 08/296,268, filed 25 August 1994 (now U.S. Pat. No. 5,510,474), which is a continuation of application Ser. No. 08/191,134, filed 3 February 1994 (now abandoned), which is a continuation of application Ser. No. 08/076,363, filed 11 June 1993 (now abandoned), which is a continuation of application Ser. No. 07/670,496, filed 15 March 1991 (now abandoned), which is a continuation of application Ser. No. 07/194,824, filed 17 May 1988 (now abandoned).

Claims of U.S. 5,614,399	**Support in specification of U.S. 5,614,399**
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:	**Selective heat shock induced enhancement:** "Ubiquitin also plays a role in the cellular response to stresses, such as heat shock and increase in metal (arsenite) concentration (D. Finley et al. (1985) supra). Most living cells respond to stress (for example, exposure to temperatures a few degrees above normal physiological temperatures or to elevated concentrations of heavy metals, ethanol, oxidants and amino acid analogs) by activating a small set of genes to selectively synthesize stress proteins, also called heat shock proteins. In most organisms these stress proteins were found to have subunit molecular weights of 89, 70 and 24 kDa (U. Bond and M. Schlesinger (1985) supra). Ubiquitin, with a molecular weight of approximately 8.5 kDa, also responds to stress, since in different species (yeast, mouse, gerbil and chicken embryo fibroblasts) the levels of ubiquitin mRNA and ubiquitin protein increase as a result of different stress conditions." 1:55-2:3. **Structural Gene:** "Structural gene is that portion of a gene comprising a DNA segment encoding a protein, polypeptide or a portion thereof, and excluding the 5' sequence which drives the initiation of transcription. The structural gene may be one which is normally found in the cell or one which is not normally found in the cellular location wherein it is introduced, in which case it is termed a heterologous gene. A heterologous gene may be derived in whole or in part from any source known to the art, including a bacterial genome or episome, eukaryotic, nuclear or plasmid DNA, cDNA, viral DNA or chemically synthesized DNA. A structural gene may contain one or more modifications in either the coding or the untranslated regions which could affect the biological activity or the chemical structure of the expression product, the rate of expression or the manner of expression control. Such modifications include, but are not limited to, mutations, insertions, deletions and substitutions of one or more nucleotides. The structural gene may

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	constitute an uninterrupted coding sequence or it may include one or more introns, bound by the appropriate splice junctions. The structural gene may be a composite of segments derived from a plurality of sources, naturally occurring or synthetic. The structural gene may also encode a fusion protein. It is contemplated that the introduction into plant tissue of recombinant DNA molecules containing the promoter/structural gene/polyadenylation signal complex will include constructions wherein the structural gene and its promoter are each derived from different plant species." 8:36-63. "A structural gene placed under the regulatory control of the plant ubiquitin regulatory system means that a structural gene is positioned such that the regulated expression of the gene is controlled by the sequences comprising the ubiquitin regulatory system." 9:5-9. **Regulatory control:** "Regulatory Control refers to the modulation of gene expression induced by DNA sequence elements located primarily, but not exclusively, upstream of (5' to) the (1981)- (1981)- transcription start site." 8:3-11. **Plant Cell:** "Plant tissue includes differentiated and undifferentiated tissues of plants, including, but not limited to roots, shoots, leaves, pollen, seeds, tumor tissue and various forms of cells in culture, such as single cells, protoplasts, embryos and callus tissue. The plant tissue may be in planta or in organ, tissue or cell culture." 9:24-29.
(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	**Ubiquitin** "The vector herein described employs a maize ubiquitin promoter to control expression of..." 10:40-41. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 10:63-11:7. "FIG. 2 documents the DNA sequence and the deduced amino acid sequence of ubiquitin gene 1." 7:6-7. **Regulatory system:** "Other sequences conferring regulatory influences on transcription can be found within the promoter region and extending as far as 1000 bp or more 5' from the cap site." 7:66-8:2. "In this invention, the heat shock regulatory elements function to enhance transiently the level of downstream gene expression in response to sudden temperature elevation." 8:8-11. "Placing a structural gene under the regulatory control of a promoter or a regulatory element means positioning the structural gene such that the

Claims of U.S. 5,614,399	Support in specification of U.S. 5,614,399
	expression of the gene is controlled by these sequences." 8:12-15. "Plant Ubiquitin Regulatory System refers to the approximately 2 kb nucleotide sequence 5' to the translation start site of the maize ubiquitin gene and comprises sequences that direct initiation of transcription, regulation of transcription, control of expression level, induction of stress genes and enhancement of expression in response to stress. The regulatory system, comprising both promoter and regulatory functions, is the DNA sequence providing regulatory control or modulation of gene expression." 8:64-9:5. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 10:63-11:7. **Heat shock element:** Heat shock elements refer to DNA sequences that regulate gene expression in response to the stress of sudden temperature elevations. The response is seen as an immediate albeit transitory enhancement in level of expression of a downstream gene. The original work on heat shock genes was done with Drosophila but many other species including plants (T. Barnett et al. (1980) Dev. Genet. 1:331-340) exhibited analogous responses to stress. The essential primary component of the heat shock element was described in Drosophila to have the consensus sequence 5'-CTGGAATNTTCTAGE-3' (where N=A, T, C, or G) (Support for this definition of N is found in the Messing et al. reference) and to be located in the region between residues -66 through -47 bp upstream to the transcriptional start site (H. Pelham et al. (1982) supra). A chemically synthesized oligonucleotide copy of this consensus sequence can replace the natural sequence in conferring heat shock inducibility. In other systems, multiple heat shock elements were identified within the promoter region. For example, Rochester et al. (1986) supra recognized two heat shock elements in the maize hsp 70 gene. 10:1-19. **Intron:** "Introns or intervening sequences refer in this work to those regions of DNA sequence that are transcribed along with the coding sequences (exons) but are then removed in the formation of the mature mRNA. Introns may occur anywhere within a transcribed sequence--between coding sequences of the same or different genes, within the coding sequence of a gene, interrupting and splitting its amino acid sequences, and within the promoter region (5' to the translation start site). Introns in the primary transcript are excised and the coding sequences are simultaneously and precisely ligated to form the mature mRNA. The junctions of introns and exons form the splice sites. The base sequence of an intron begins with GU and ends with AG. The same splicing signal is found in many higher eukaryotes." 10:24-37.
(b) selectively applying stress conditions of high temperature to said	**stress conditions:** "Heat shock elements refer to DNA sequences that regulate gene expression in response to the stress of sudden temperature elevations. The response is

Claims of U.S. 5,614,399	Support in specification of U.S. 5,614,399
transformed plant cell thereby inducing enhancement in expression of said DNA sequence of interest.	seen as an immediate albeit transitory enhancement in level of expression of a downstream gene. The original work on heat shock genes was done with Drosophila but many other species including plants (T. Barnett et al. (1980) Dev. Genet. 1: 331-340) exhibited analogous responses to stress. The essential primary component of the heat shock element was described in Drosophila to have the consensus sequence 5'-CTGGAAT-TTCTAGA-3' and to be located in the region between residues -66 through -47 bp upstream to the transcriptional start site (H. Pelham and M. Bienz (1982) supra). A chemically synthesized oligonucleotide copy of this consensus sequence can replace the natural sequence in conferring heat shock inducibility. In other systems, multiple heat shock elements were identified within the promoter region. For example, Rochester et al. (1986) supra recognized two heat shock elements in the maize hsp 70 gene." 10:1-19. **high temperature:** "In higher plants, the stress response was demonstrated by increased protein synthesis in response to heat shock in soybean, pea, millet, corn, sunflower, cotton and wheat (T. Barnett et al. (1980) Dev. Genet. 1: 331-340; J. Key et al. (1981) Proc. Nat. Acad. Sci. USA 78: 3526-3530). The major differences in heat shock response seen among plant species are: (a) the amount of total protein synthesized in response to stress, (b) the size distribution of the different proteins synthesized, (c) the optimum temperature of induction of heat shock proteins and (d) the lethal (breakpoint) temperature." 3:48-58. "The heat shock response is believed to provide thermal protection or thermotolerance to otherwise nonpermissive temperatures (M. Schlesinger et al. (1982) in Heat Shock from Bacteria to Man, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., p. 329). A permissive heat shock temperature is a temperature which is high enough to induce the heat shock response but not high enough to be lethal. Thermotolerance in plant seedlings can be attained by different treatment regimes: (a) a 1 to 2 hour exposure to continuous heat shock at 40.degree. C. followed by a 45.degree. C. incubation, (b) a 30 min heat shock at 40.degree. C. followed by 2 to 3 hours at 28.degree. C. prior to the shift to 45.degree. C., (c) a 10 min heat shock at 45.degree. C. followed by about 2 hours at 28.degree. C. prior to the shift to 45.degree. C. and (d) treatment of seedlings with 50 .mu.M arsenite at 28.degree. C. for 3 hours or more prior to the shift to 45.degree. C. During the pretreatment prior to incubation at the potentially lethal temperature, heat shock proteins are synthesized and accumulated. Also, heat shock mRNA and protein syntheses occur at 45.degree. C., if the plant seedling is preconditioned as described above. When the temperature is shifted back to physiological levels (e.g., 28.degree. C.), normal transcription and translation are resumed and after 3 to 4 hours at normal temperature, there is no longer detectable synthesis of heat shock proteins (J. Key et al. (1981) Proc. Natl. Acad. Sci. USA 78: 3526-3530; M. Schlesinger et al. (1982) Trends Biochem. Sci. 1: 222-225). The heat shock proteins that were synthesized during the 40.degree. C. heat shock treatment are very stable and are not immediately degraded." 5:46-6:6. "Example 3: Heat Shock Response A. Heat Shock Treatment To heat shock, 4 to 5 day old etiolated seedlings were transferred to an incubator at 42.degree. C. and harvested 1, 3 and 8 h after transfer. Total RNA (7 .mu.g) was isolated, denatured and electrophoresed through a 1.5% agarose 3% formaldehyde gel. The RNA was transferred to Gene Screen and

Claims of U.S. 5,614,399	Support in specification of U.S. 5,614,399
	probed with single stranded RNA transcribed from linearized pCA210 using SP6 RNA polymerase. (The recombinant plasmid, pCA210, was constructed by subcloning the 975 bp insert of p6T7.2b1 into pSP64 (Promega) so that SP6 RNA polymerase synthesized a RNA probe specific for hybridization with ubiquitin mRNA.) After autoradiography, the bands were cut out and the amount of radioactivity bound to the filter was determined by liquid scintillation. From analysis of the Northern blots, levels of three ubiquitin transcripts were determined. One hour after transfer to 42.degree. C., the level of the 2.1 kb transcript increased 2.5 to 3 fold. An approximately 2 fold increase was observed for the 1.6 kb transcript, however, no increase was seen for the 0.8 kb transcript. By three hours after transfer of the seedlings to elevated temperature, the levels of the two largest ubiquitin transcripts had returned to the level observed in unshocked tissue and remained at those levels for at least another five hours. The transitory nature of ubiquitin during the heat shock response in maize may indicate that ubiquitin has a specialized role in heat shock and that only brief periods of increased levels of ubiquitin are required." 18:6-35.

Claim Constructions: '399

Claim 1, on its face, and as interpreted by the patent specification recites:

A method of enhancing constitutive expression of a structural gene by exposing a plant cell to heat induced stress, which causes an increase in transcription of genes under the control of the ubiquitin promoter.

The structural gene can be any DNA segment encoding a protein, polypeptide or portion thereof, and may be homologous or heterologous and may contain one or more introns.

The method includes the first step of transforming the plant cell with a DNA construct having an approximately 2kb Maize ubiquitin promoter 1 regulatory system, wherein the regulatory system includes the Maize ubiquitin promoter, including from the 5' end, 899 base pairs from the transcription start site, two overlapping heat shock elements at positions -214 to -204 and a TATA box at positions -30 to -10. To the 3' side of the transcription start site, an exon extends from position 1 to 83, and an intron is immediately adjacent at positions 84 to 1093 which comprises the 3' end of the 2kb Maize ubiquitin promoter regulatory system. A DNA sequence of interest is positioned, usually 3' to the promoter region, such that the regulated expression of the gene is controlled by the Maize ubiquitin promoter 1 regulatory system.

The method includes a second step of selectively applying temperatures elevated a few degrees above normal physiological temperatures to induce stress conditions to the transformed plant cell.

U.S. Patent No.	5,510,474
Inventor(s):	Quail; Peter H., Christensen; Alan H., Hershey; Howard P., Sharrock; Robert A., Sullivan; Thomas D.
Assignee(s):	Mycogen Plant Science, Inc.
Filed:	August 25, 1994
Issued:	April 23, 1996
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Status:	Active
Priority Information Summary:	This is a continuation of application Ser. No. 08/191,134, filed Feb. 3, 1994 (now abandoned), which is a continuation of application Ser. No. 08/076,363, filed Jun. 11, 1993 (now abandoned), which is a continuation of application Ser. No. 07/670,496, filed Mar. 15, 1991 (now abandoned), which is a continuation of application Ser. No. 07/194,824, filed May 17, 1988 (now abandoned).

Claims of U.S. 5,510,474	**Support in specification of U.S. 5,510,474**
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:	**Effecting Expression of coding sequences:** "A further object of this invention is to provide a recombinant DNA molecule comprising a plant expressible promoter and a plant expressible structural gene, wherein the structural gene is placed under the regulatory control of all transcription initiating and activating elements of the promoter. In particular, the plant ubiquitin promoter can be combined with a variety of DNA sequences, typically structural genes, to provide DNA constructions for regulated transcription and translation of said DNA sequences and which will allow for regulated control of expression when stressed with elevated temperatures." 6:51-61. "Expression refers to the transcription and/or translation of a structural gene." 7:40-41. "A. Heat Shock Treatment To heat shock, 4 to 5 day old etiolated seedlings were transferred to an incubator at 42.degree. C. and harvested 1, 3 and 8 h after transfer. Total RNA (7 .mu.g) was isolated, denatured and electrophoresed through a 1.5% agarose 3% formaldehyde gel. The RNA was transferred to Gene Screen and probed with single stranded RNA transcribed from linearized pCA210 using SP6 RNA polymerase. (The recombinant plasmid, pCA210, was constructed by subcloning the 975 bp insert of p6T7.2b1 into pSP64 (Pronega) so that SP6 RNA polymerase synthesized a RNA probe specific for hybridization with ubiquitin mRNA.) After autoradiography, the bands were cut out and the amount of radioactivity bound to the filter was determined by liquid scintillation. From analysis of the Northern blots, levels of three ubiquitin transcripts were determined. One hour after transfer to 42.degree. C., the level of the 2.1 kb transcript increased 2.5 to 3 fold. An approximately 2 fold increase was observed for the 1.6 kb transcript, however, no increase was seen for the 0.8 kb transcript. By three hours after transfer of the seedlings to elevated temperature, the

Claims of U.S. 5,510,474	Support in specification of U.S. 5,510,474
	levels of the two largest ubiquitin transcripts had returned to the level observed in unshocked tissue and remained at those levels for at least another five hours. The transitory nature of ubiquitin during the heat shock response in maize may indicate that ubiquitin has a specialized role in heat shock and that only brief periods of increased levels of ubiquitin are required." 18:15-45.
(a) two heat shock elements, which overlap;	**Heat shock element:** Heat shock elements refer to DNA sequences that regulate gene expression in response to the stress of sudden temperature elevations. The response is seen as an immediate albeit transitory enhancement in level of expression of a downstream gene. The original work on heat shock genes was done with Drosophila but many other species including plants (T. Barnett et al. (1980) Dev. Genet. 1:331-340) exhibited analogous responses to stress. The essential primary component of the heat shock element was described in Drosophila to have the consensus sequence 5'-CTGGAATNTTCTAGE-3' (where N=A, T, C, or G) (Support for this definition of N is found in the Messing et al. reference) and to be located in the region between residues -66 through -47 bp upstream to the transcriptional start site (H. Pelham et al. (1982) supra). A chemically synthesized oligonucleotide copy of this consensus sequence can replace the natural sequence in conferring heat shock inducibility. In other systems, multiple heat shock elements were identified within the promoter region. For example, Rochester et al. (1986) supra recognized two heat shock elements in the maize hsp 70 gene. 9:64-10:16.
(b) a promoter comprising a transcription start site;	**Ubiquitin** "The vector herein described employs a maize ubiquitin promoter to control expression of..." 10:38-39. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 10:61-11:6. "FIG. 2-1 through 2-7 documents the DNA sequence and the deduced amino acid sequence of ubiquitin gene 1." 7:10-11.
(c) an intron of about 1 kb in length; and	**Intron:** "Introns or intervening sequences refer in this work to those regions of DNA sequence that are transcribed along with the coding sequences (exons) but are then removed in the formation of the mature mRNA. Introns may occur anywhere within a transcribed sequence--between coding sequences of the same or different genes, within the coding sequence of a gene, interrupting and splitting its amino acid sequences, and within the promoter region (5' to the translation start site). Introns in the primary transcript are excised and the coding sequences are simultaneously and precisely ligated to form the mature mRNA. The junctions of introns and exons form the splice sites. The base sequence of an intron begins with GU and ends with AG. The same splicing signal is found in many higher eukaryotes." 10:21-35.

Claims of U.S. 5,510,474	Support in specification of U.S. 5,510,474
(d) a translation start site;	"The isolation and characterization of a promoter system which is active in plants to control and regulate the expression of a downstream gene is described in the present work. This DNA sequence is found as a naturally occurring region upstream of the ubiquitin structural gene isolated from a maize genomic library. The transcription start site or cap site as determined by S1 nuclease mapping is designated as base 1 and the sequences embodied within about 899 bases 5' of the transcription start site plus about 1093 bases 3' of the cap site but 5' of the translation start site constitute the ubiquitin promoter. Located within this approximately 2 kb promoter region are a TATA box (-30), two overlapping heat shock consensus elements (-204 and -214), an 83 nucleotide leader sequence immediately adjacent to the transcription start site and an intron extending from base 84 to base 1093." 6:35-50. "Leader sequence refers to a DNA sequence comprising about 100 nucleotides located between the transcription start site and the translation start site. Embodied within the leader sequence is a region that specifies the ribosome binding site." 10:17-20. "Introns or intervening sequences refer in this work to those regions of DNA sequence that are transcribed along with the coding sequences (exons) but are then removed in the formation of the mature mRNA. Introns may occur anywhere within a transcribed sequence--between coding sequences of the same or different genes, within the coding sequence of a gene, interrupting and splitting its amino acid sequences, and within the promoter region (5' to the translation start site)." 10:21-35.
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.	

Claim Constructions: '474

Claim 1, on its face, and as interpreted by the patent specification recites:

An isolated DNA fragment for regulated control of expression of coding sequences located 3' to the DNA fragment. The DNA fragment is a maize ubiquitin 1 promoter including from the 5' end, 899 base pairs from the transcription start site, two overlapping heat shock elements at positions -214 to -204 and a TATA box at positions -30 to -10. To the 3' side of the transcription start site, an exon extends from position 1 to 83, and an intron is immediately adjacent at positions 84 to 1093 which comprises the 3' end and the translation start site of the 2kb Maize ubiquitin 1 promoter regulatory system. A DNA sequence of interest is positioned, 3' to the promoter region, such that the regulated expression of the gene is controlled by the Maize ubiquitin 1 promoter regulatory system.

U.S. Patent No.	6,054,574
Inventor(s):	Quail; Peter H., Christensen; Alan H., Hershey; Howard P., Sharrock; Robert A., Sullivan; Thomas D.
Assignee(s):	Mycogen Plant Science, Inc.
Filed:	June 9, 1998
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Status:	Active
Priority Information Summary:	This is a divisional application of application Ser. No. 08/746,822, filed Nov. 18, 1996; which is a divisional application of Ser. No. 08/462,092, filed Jun. 5, 1995 (now U.S. Pat. No. 5,614,399, issued Mar. 25, 1997; which was a divisional application of Ser. No. 08/296,268, filed Aug. 25, 1994 (now U.S. Pat. No. 5,510,474, issued Apr. 23, 1996); which was a continuation application of Ser. No. 08/191,134, filed Feb. 3, 1994 (now abandoned); which was a continuation application of Ser. No. 08/076,363, filed Jun. 11, 1993 (now abandoned); which was a continuation application of Ser. No. 07/670,496; filed Mar. 15, 1991 (now abandoned); and which was a continuation application of Ser. No. 07/194,824, filed May 17, 1988 (now abandoned).

Claims of U.S. 6,054,574	Support in specification of U.S. 6,054,574
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	**Ubiquitin:** "The vector herein described employs a maize ubiquitin promoter to control expression of..." 10:54-55. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 11:10-21. "FIG. 2 documents the DNA sequence and the deduced amino acid sequence of ubiquitin gene 1." 7:17-18. **Regulatory system:** "Other sequences conferring regulatory influences on transcription can be found within the promoter region and extending as far as 1000 bp or more 5' from the cap site." 8:8-11. "In this invention, the heat shock regulatory elements function to enhance

Claims of U.S. 6,054,574	Support in specification of U.S. 6,054,574
obtained in said inducible gene expression in monocots.	transiently the level of downstream gene expression in response to sudden temperature elevation." 8:17-20. "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." 8: 21:24. "Plant Ubiquitin Regulatory System refers to the approximately 2 kb nucleotide sequence 5' to the translation start site of the maize ubiquitin gene and comprises sequences that direct initiation of transcription, regulation of transcription, control of expression level, induction of stress genes and enhancement of expression in response to stress. The regulatory system, comprising both promoter and regulatory functions, is the DNA sequence providing regulatory control or modulation of gene expression." 9:6-14. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 11:10-21. **Heat shock element:** Heat shock elements refer to DNA sequences that regulate gene expression in response to the stress of sudden temperature elevations. The response is seen as an immediate albeit transitory enhancement in level of expression of a downstream gene. The original work on heat shock genes was done with Drosophila but many other species including plants (T. Barnett et al. (1980) Dev. Genet. 1:331-340) exhibited analogous responses to stress. The essential primary component of the heat shock element was described in Drosophila to have the consensus sequence 5'-CTGGAATNTTCTAGE-3' (where N=A, T, C, or G) (Support for this definition of N is found in the Messing et al. reference) and to be located in the region between residues -66 through -47 bp upstream to the transcriptional start site (H. Pelham et al. (1982) supra). A chemically synthesized oligonucleotide copy of this consensus sequence can replace the natural sequence in conferring heat shock inducibility. In other systems, multiple heat shock elements were identified within the promoter region. For example, Rochester et al. (1986) supra recognized two heat shock elements in the maize hsp 70 gene. 10:13-33. **Intron:** "Introns or intervening sequences refer in this work to those regions of DNA sequence that are transcribed along with the coding sequences (exons) but are then removed in the formation of the mature mRNA. Introns may occur anywhere within a transcribed sequence--between coding sequences of the same or different genes, within the coding sequence of a gene, interrupting and splitting its amino acid sequences, and within the promoter region (5' to the translation start site). Introns in the primary transcript are excised and the coding sequences are simultaneously and precisely ligated to form the mature mRNA. The junctions of introns and exons form the splice sites. The base sequence of an intron begins with GU and ends with AG. The same splicing

Claims of U.S. 6,054,574	Support in specification of U.S. 6,054,574
	signal is found in many higher eukaryotes." 10:38-51. **Promoter:** "The promoter comprises the DNA sequences from the 5' nontranscribed regions of plant ubiquitin genes that initiate and regulate the transcription of genes placed under its control. In its preferred embodiment, the promoter sequence is derived from the upstream region of the ubiquitin gene from maize." 6:35-41. "Promoter refers to the nucleotide sequences at the 5' end of a structural gene which direct the initiation of transcription. Promoter sequences are necessary, but not always sufficient, to drive the expression of a downstream gene. In general, eukaryotic promoters include a characteristic DNA sequence homologous to the consensus 5'-TATAAT-3' (TATA) box about 10-30 bp 5' to the transcription start (cap) site, which, by convention, is numbered +1. Bases 3' to the cap site are given positive numbers, whereas bases 5' to the cap site receive negative numbers, reflecting their distance from the cap site. Another promoter component, the CAAT box, is often found about 30 to 70 bp 5' to the TATA box and has homology to the canonical form 5'-CCAAT-3' (R. Breathnach and P. Chambon (1981) Ann. Rev. Biochem. 50:349-383). In plants the CAAT box is sometimes replaced by a sequence known as the AGGA box, a region having adenine residues symmetrically flanking the triplet G(orT)NG (J. Messing et al. (1983), in Genetic Engineering of Plants, T. Kosuge, C. Meredith and A. Hollaender (eds.), Plenum Press, pp. 211-227). Other sequences conferring regulatory influences on transcription can be found within the promoter region and extending as far as 1000 bp or more 5' from the cap site." 7:55-8:10 **Transcription start site:** "The isolation and characterization of a promoter system which is active in plants to control and regulate the expression of a downstream gene is described in the present work. This DNA sequence is found as a naturally occurring region upstream of the ubiquitin structural gene isolated from a maize genomic library. The transcription start site or cap site as determined by S1 nuclease mapping is designated as base 1 and the sequences embodied within about 899 bases 5' of the transcription start site plus about 1093 bases 3' of the cap site but 5' of the translation start site constitute the ubiquitin promoter. Located within this approximately 2 kb promoter region are a TATA box (-30), two overlapping heat shock consensus elements (-204 and -214), an 83 nucleotide leader sequence immediately adjacent to the transcription start site and an intron extending from base 84 to base 1093." 6:42-56. "The DNA sequence of the maize ubiquitin-1 gene, lambda 7.2b1, is shown in FIG. 2. The sequence is composed of 899 bases upstream of the transcription start site, 1992 bases of 5' untranslated and intron sequences, and 1999 bases encoding seven ubiquitin protein repeats preceding 249 bases of 3' sequence. A "TATA" box is located at -30 and two overlapping heat shock elements are located at -214 and -204. The DNA sequence of the coding and 3' regions of the ubiquitin-1 gene from maize, lambda 7.2b1, is also presented in FIG. 3. The derived amino acid sequence of maize ubiquitin is shown at the top and the nucleotide sequence of the seven ubiquitin repeats is aligned underneath. A schematic of the organization of the seven complete ubiquitin units in the genomic DNA is shown in FIG. 1C." 16:14-29.

Claims of U.S. 6,054,574	Support in specification of U.S. 6,054,574
	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: "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. Thus, in the construction of heterologous promoter/structural gene combinations, the promoter is preferably positioned upstream to the gene and at a distance from the transcription start site that approximates the distance between the promoter and the gene it controls in its natural setting. As is known in the art, some variation in this distance can be tolerated without loss of promoter function. Similarly, the preferred positioning of a regulatory element with respect to a heterologous gene placed under its control reflects its natural position relative to the structural gene it naturally regulates. Again, as is known in the art, some variation in this distance can be accommodated." 8:20-36.
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	**Ubiquitin:** "The vector herein described employs a maize ubiquitin promoter to control expression of..." 10:54-55. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 11:10-21. "FIG. 2 documents the DNA sequence and the deduced amino acid sequence of ubiquitin gene 1." 7:17-18. **Regulatory system:** "Other sequences conferring regulatory influences on transcription can be found within the promoter region and extending as far as 1000 bp or more 5' from the cap site." 8:8-11. "In this invention, the heat shock regulatory elements function to enhance transiently the level of downstream gene expression in response to sudden temperature elevation." 8:17-20. "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." 8: 21:24. "Plant Ubiquitin Regulatory System refers to the approximately 2 kb nucleotide sequence 5' to the translation start site of the maize ubiquitin gene and comprises sequences that direct initiation of transcription, regulation of

Claims of U.S. 6,054,574	Support in specification of U.S. 6,054,574
	transcription, control of expression level, induction of stress genes and enhancement of expression in response to stress. The regulatory system, comprising both promoter and regulatory functions, is the DNA sequence providing regulatory control or modulation of gene expression." 9:6-14. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 11:10-21. **Heat shock element:** Heat shock elements refer to DNA sequences that regulate gene expression in response to the stress of sudden temperature elevations. The response is seen as an immediate albeit transitory enhancement in level of expression of a downstream gene. The original work on heat shock genes was done with Drosophila but many other species including plants (T. Barnett et al. (1980) Dev. Genet. 1:331-340) exhibited analogous responses to stress. The essential primary component of the heat shock element was described in Drosophila to have the consensus sequence 5'-CTGGAATNTTCTAGE-3' (where N=A, T, C, or G) (Support for this definition of N is found in the Messing et al. reference) and to be located in the region between residues -66 through -47 bp upstream to the transcriptional start site (H. Pelham et al. (1982) supra). A chemically synthesized oligonucleotide copy of this consensus sequence can replace the natural sequence in conferring heat shock inducibility. In other systems, multiple heat shock elements were identified within the promoter region. For example, Rochester et al. (1986) supra recognized two heat shock elements in the maize hsp 70 gene. 10:13-33. **Intron:** "Introns or intervening sequences refer in this work to those regions of DNA sequence that are transcribed along with the coding sequences (exons) but are then removed in the formation of the mature mRNA. Introns may occur anywhere within a transcribed sequence--between coding sequences of the same or different genes, within the coding sequence of a gene, interrupting and splitting its amino acid sequences, and within the promoter region (5' to the translation start site). Introns in the primary transcript are excised and the coding sequences are simultaneously and precisely ligated to form the mature mRNA. The junctions of introns and exons form the splice sites. The base sequence of an intron begins with GU and ends with AG. The same splicing signal is found in many higher eukaryotes." 10:38-51. **Promoter:** "The promoter comprises the DNA sequences from the 5' nontranscribed regions of plant ubiquitin genes that initiate and regulate the transcription of genes placed under its control. In its preferred embodiment, the promoter sequence is derived from the upstream region of the ubiquitin gene from maize." 6:35-41. "Promoter refers to the nucleotide sequences at the 5' end of a structural gene

Claims of U.S. 6,054,574	Support in specification of U.S. 6,054,574
	which direct the initiation of transcription. Promoter sequences are necessary, but not always sufficient, to drive the expression of a downstream gene. In general, eukaryotic promoters include a characteristic DNA sequence homologous to the consensus 5'-TATAAT-3' (TATA) box about 10-30 bp 5' to the transcription start (cap) site, which, by convention, is numbered +1. Bases 3' to the cap site are given positive numbers, whereas bases 5' to the cap site receive negative numbers, reflecting their distance from the cap site. Another promoter component, the CAAT box, is often found about 30 to 70 bp 5' to the TATA box and has homology to the canonical form 5'-CCAAT-3' (R. Breathnach and P. Chambon (1981) Ann. Rev. Biochem. 50:349-383). In plants the CAAT box is sometimes replaced by a sequence known as the AGGA box, a region having adenine residues symmetrically flanking the triplet G(orT)NG (J. Messing et al. (1983), in Genetic Engineering of Plants, T. Kosuge, C. Meredith and A. Hollaender (eds.), Plenum Press, pp. 211-227). Other sequences conferring regulatory influences on transcription can be found within the promoter region and extending as far as 1000 bp or more 5' from the cap site." 7:55-8:10 **Transcription start site:** "The isolation and characterization of a promoter system which is active in plants to control and regulate the expression of a downstream gene is described in the present work. This DNA sequence is found as a naturally occurring region upstream of the ubiquitin structural gene isolated from a maize genomic library. The transcription start site or cap site as determined by S1 nuclease mapping is designated as base 1 and the sequences embodied within about 899 bases 5' of the transcription start site plus about 1093 bases 3' of the cap site but 5' of the translation start site constitute the ubiquitin promoter. Located within this approximately 2 kb promoter region are a TATA box (-30), two overlapping heat shock consensus elements (-204 and -214), an 83 nucleotide leader sequence immediately adjacent to the transcription start site and an intron extending from base 84 to base 1093." 6:42-56. "The DNA sequence of the maize ubiquitin-1 gene, lambda 7.2b1, is shown in FIG. 2. The sequence is composed of 899 bases upstream of the transcription start site, 1992 bases of 5' untranslated and intron sequences, and 1999 bases encoding seven ubiquitin protein repeats preceding 249 bases of 3' sequence. A "TATA" box is located at -30 and two overlapping heat shock elements are located at -214 and -204. The DNA sequence of the coding and 3' regions of the ubiquitin-1 gene from maize, lambda 7.2b1, is also presented in FIG. 3. The derived amino acid sequence of maize ubiquitin is shown at the top and the nucleotide sequence of the seven ubiquitin repeats is aligned underneath. A schematic of the organization of the seven complete ubiquitin units in the genomic DNA is shown in FIG. 1C." 16:14-29. **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:** "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. Thus, in the construction of heterologous promoter/structural gene combinations, the promoter is preferably positioned upstream to the gene and at a distance from

Claims of U.S. 6,054,574	Support in specification of U.S. 6,054,574
	the transcription start site that approximates the distance between the promoter and the gene it controls in its natural setting. As is known in the art, some variation in this distance can be tolerated without loss of promoter function. Similarly, the preferred positioning of a regulatory element with respect to a heterologous gene placed under its control reflects its natural position relative to the structural gene it naturally regulates. Again, as is known in the art, some variation in this distance can be accommodated." 8:20-36.
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.	**polyadenylation signal:** "FIG. 3 demonstrates that all seven of the ubiquitin coding repeats encode an identical amino acid sequence. The nucleotide sequence of the seven repeats is shown aligned under the derived amino acid sequence. An additional 77th amino acid, glutamine, is present in the 7th repeat preceding the stop codon. A polyadenylation signal, AATAAT, is present in the 3' untranslated region, 113 bp from the stop codon." "Polyadenylation signal refers to any nucleic acid sequence capable of effecting mRNA processing, usually characterized by the addition of polyadenylic acid tracts to the 3'-ends of the mRNA precursors. The polyadenylation signal DNA segment may itself be a composite of segments derived from several sources, naturally occurring or synthetic, and may be from a genomic DNA or an RNA-derived cDNA. Polyadenylation signals are commonly recognized by the presence of homology to the canonical form 5'-AATAA-3', although variation of distance, partial "readthrough", and multiple tandem canonical sequences are not uncommon (J. Messing et al. supra). It should be recognized that a canonical "polyadenylation signal" may in fact cause transcriptional termination and not polyadenylation per se (C. Montell et al. (1983) Nature 305:600-605)." **Structural gene:** "A structural gene placed under the regulatory control of the plant ubiquitin regulatory system means that a structural gene is positioned such that the regulated expression of the gene is controlled by the sequences comprising the ubiquitin regulatory system." 9:14-19. **Regulatory control:** "Regulatory Control refers to the modulation of gene expression induced by DNA sequence elements located primarily, but not exclusively, upstream of (5' to) the (1981)- (1981)- transcription start site." 8:12-15. **Heterologous:** "The structural gene may be one which is normally found in the cell or one which is not normally found in the cellular location wherein it is introduced, in which case it is termed a heterologous gene. A heterologous gene may be derived in whole or in part from any source known to the art, including a bacterial genome or episome, eukaryotic, nuclear or plasmid DNA, cDNA, viral DNA or chemically synthesized DNA."
18. A DNA fragment, useful in effecting expression in both monocots and dicots of coding sequences placed 3' to said fragment, wherein	**Ubiquitin:** "The vector herein described employs a maize ubiquitin promoter to control expression of..." 10:54-55. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described

Claims of U.S. 6,054,574	Support in specification of U.S. 6,054,574
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:	in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 11:10-21. "FIG. 2 documents the DNA sequence and the deduced amino acid sequence of ubiquitin gene 1." 7:17-18. **Regulatory system:** "Other sequences conferring regulatory influences on transcription can be found within the promoter region and extending as far as 1000 bp or more 5' from the cap site." 8:8-11. "In this invention, the heat shock regulatory elements function to enhance transiently the level of downstream gene expression in response to sudden temperature elevation." 8:17-20. "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." 8: 21:24. "Plant Ubiquitin Regulatory System refers to the approximately 2 kb nucleotide sequence 5' to the translation start site of the maize ubiquitin gene and comprises sequences that direct initiation of transcription, regulation of transcription, control of expression level, induction of stress genes and enhancement of expression in response to stress. The regulatory system, comprising both promoter and regulatory functions, is the DNA sequence providing regulatory control or modulation of gene expression." 9:6-14. "This invention comprises the first report of an isolated and characterized plant ubiquitin promoter. The maize ubiquitin promoter system as described in the present work includes the RNA polymerase recognition and binding sites, the transcriptional initiation sequence, regulatory sequences responsible for inducible transcription and an untranslatable intervening sequence (intron) between the transcriptional start site and the translational initiation site. Two overlapping heat shock consensus promoter sequences are situated 5' (-214 and -204) of the transcriptional start site. An exon of 83 nucleotides is located immediately adjacent to the cap site and is followed by a large (approximately 1 kb) intron." 11:10-21. **said DNA is isolated or incorporated into a larger piece of DNA:** "The present invention relates to the development of a recombinant vector useful for the expression of DNA coding segments in plant cells. The vector herein described employs a maize ubiquitin promoter to control expression of an inserted DNA coding segment. The transcriptional regulatory sequences may be combined with an extrachromosomal replication system for a predetermined host. Other DNA sequences having restriction sites for gene insertion may be added to provide a vector for the regulated transcription and translation of the inserted genes in said host. The vector may also include a prokaryotic replication system allowing amplification in a prokaryotic host, markers for selection and other DNA regions. This would allow large

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	quantities of the vector to be grown in well characterized bacterial systems prior to transforming a plant or mammalian host." 10:47-61.
(a) one or more heat shock elements, which elements may or may not be overlapping;	**Heat shock element:** Heat shock elements refer to DNA sequences that regulate gene expression in response to the stress of sudden temperature elevations. The response is seen as an immediate albeit transitory enhancement in level of expression of a downstream gene. The original work on heat shock genes was done with Drosophila but many other species including plants (T. Barnett et al. (1980) Dev. Genet. 1:331-340) exhibited analogous responses to stress. The essential primary component of the heat shock element was described in Drosophila to have the consensus sequence 5'-CTGGAATNTTCTAGE-3' (where N=A, T, C, or G) (Support for this definition of N is found in the Messing et al. reference) and to be located in the region between residues -66 through -47 bp upstream to the transcriptional start site (H. Pelham et al. (1982) supra). A chemically synthesized oligonucleotide copy of this consensus sequence can replace the natural sequence in conferring heat shock inducibility. In other systems, multiple heat shock elements were identified within the promoter region. For example, Rochester et al. (1986) supra recognized two heat shock elements in the maize hsp 70 gene. 10:13-33.
(b) a promoter comprising a transcription start site; and	**Promoter:** "The promoter comprises the DNA sequences from the 5' nontranscribed regions of plant ubiquitin genes that initiate and regulate the transcription of genes placed under its control. In its preferred embodiment, the promoter sequence is derived from the upstream region of the ubiquitin gene from maize." 6:35-41. "Promoter refers to the nucleotide sequences at the 5' end of a structural gene which direct the initiation of transcription. Promoter sequences are necessary, but not always sufficient, to drive the expression of a downstream gene. In general, eukaryotic promoters include a characteristic DNA sequence homologous to the consensus 5'-TATAAT-3' (TATA) box about 10-30 bp 5' to the transcription start (cap) site, which, by convention, is numbered +1. Bases 3' to the cap site are given positive numbers, whereas bases 5' to the cap site receive negative numbers, reflecting their distance from the cap site. Another promoter component, the CAAT box, is often found about 30 to 70 bp 5' to the TATA box and has homology to the canonical form 5'-CCAAT-3' (R. Breathnach and P. Chambon (1981) Ann. Rev. Biochem. 50:349-383). In plants the CAAT box is sometimes replaced by a sequence known as the AGGA box, a region having adenine residues symmetrically flanking the triplet G(orT)NG (J. Messing et al. (1983), in Genetic Engineering of Plants, T. Kosuge, C. Meredith and A. Hollaender (eds.), Plenum Press, pp. 211-227). Other sequences conferring regulatory influences on transcription can be found within the promoter region and extending as far as 1000 bp or more 5' from the cap site." 7:55-8:10
(c) an intron of about 1 kb in length;	**Intron:** "Introns or intervening sequences refer in this work to those regions of DNA sequence that are transcribed along with the coding sequences (exons) but are then removed in the formation of the mature mRNA. Introns may occur anywhere within a transcribed sequence--between coding sequences of the same or different genes, within the coding sequence of a gene, interrupting and splitting its amino acid sequences, and within the promoter region (5' to the translation start site). Introns in the primary transcript are excised and the

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	coding sequences are simultaneously and precisely ligated to form the mature mRNA. The junctions of introns and exons form the splice sites. The base sequence of an intron begins with GU and ends with AG. The same splicing signal is found in many higher eukaryotes." 10:38-51.
and wherein said DNA fragment comprising said elements (a)-(c) is capable of regulating gene expression in both dicots and monocots.	

Claim Constructions: '574

Claim 1, on its face, and as interpreted by the patent specification recites:

A DNA sequence approximately 2kb in length encoding a Maize ubiquitin 1 promoter regulatory system, wherein the regulatory system includes the Maize ubiquitin 1 promoter, including from the 5' end, 899 base pairs from the transcription start site, two overlapping heat shock elements at positions -214 to -204 and a TATA box at positions -30 to -10. To the 3' side of the transcription start site, an exon extends from position 1 to 83, and an intron is immediately adjacent at positions 84 to 1093 which comprises the 3' end of the 2kb Maize ubiquitin promoter regulatory system.

The Maize ubiquitin 1 promoter 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, on its face, and as interpreted by the patent specification recites:

A recombinant DNA construct comprising a DNA sequence approximately 2kb in length encoding a Maize ubiquitin 1 promoter regulatory system, wherein the regulatory system includes the Maize ubiquitin 1 promoter, including from the 5' end, 899 base pairs from the transcription start site, two overlapping heat shock elements at positions -214 to -204 and a TATA box at positions -30 to -10. To the 3' side of the transcription start site, an exon extends from position 1 to 83, and an intron is immediately adjacent at positions 84 to 1093 which comprises the 3' end of the 2kb Maize ubiquitin promoter regulatory system.

The Maize ubiquitin 1 promoter regulatory system is capable of regulating constitutive and inducible gene expression of a structural gene located 3' to the maize ubiquitin 1 regulatory system, and having a and polyadenylation signal located 3' to the structural gene, 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 18, on its face, and as interpreted by the patent specification recites:

An DNA fragment for regulated control of expression of coding sequences located 3' to the DNA fragment. The DNA fragment is a maize ubiquitin 1 promoter including from the 5' end, 899 base pairs from the transcription start site, two overlapping heat shock elements at positions -214 to -204 and a TATA box at positions -30 to -10. To the 3' side of the transcription start site, an exon extends from position 1 to 83, and an intron is immediately adjacent at positions 84 to 1093 which comprises the 3' end of the 2kb Maize ubiquitin 1 promoter regulatory system and the translation start site.

The DNA fragment is placed into a larger piece of DNA to regulate expression of coding sequences in moncots and dicots. The larger piece of DNA can be any piece of DNA besides DNA that places an ubiquitin gene 3' to the DNA fragment such as an expression vector for expressing a gene in a host.