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(54) **REGULATORY SEQUENCES FOR
MODULATING TRANSGENE EXPRESSION
IN PLANTS**

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(57) **ABSTRACT**

The invention relates to gene expression regulatory sequences, specifically introns that act as enhancers of gene expression, the promoter and terminator sequences endogenously associated with these introns. Presence of these intronic enhancer sequences in proximity to promoter sequences leads to enhancement of gene expression. Methods of finding such new intronic enhancer sequences and using them to generate transgenic plants are also described.

Specification includes a Sequence Listing.

UBI <intron>

CTTCAAG<gtacg.....cag>GTCGAC
(SEQ ID NO:147)

ITV (ZM-Ubi promoter + AsiSI + Acc65I (No Intron Control))

CTTGCGATCGCAGGTACCCGAC ←
(SEQ ID NO:148)

ITV + ts1 (UBI + AsiSI <ts1 intron> Acc65I)

CTTGCGATCGCAG<gtgatc.....cag>**GTACC**
(SEQ ID NO:149)

Identical

intron splicing

CTTGCGATCGCAGGTACC
(SEQ ID NO:150)

FIG. 1

UBI <intron>
CTTCAG<gtacg.....cag>GTCGAC
(SEQ ID NO:147)

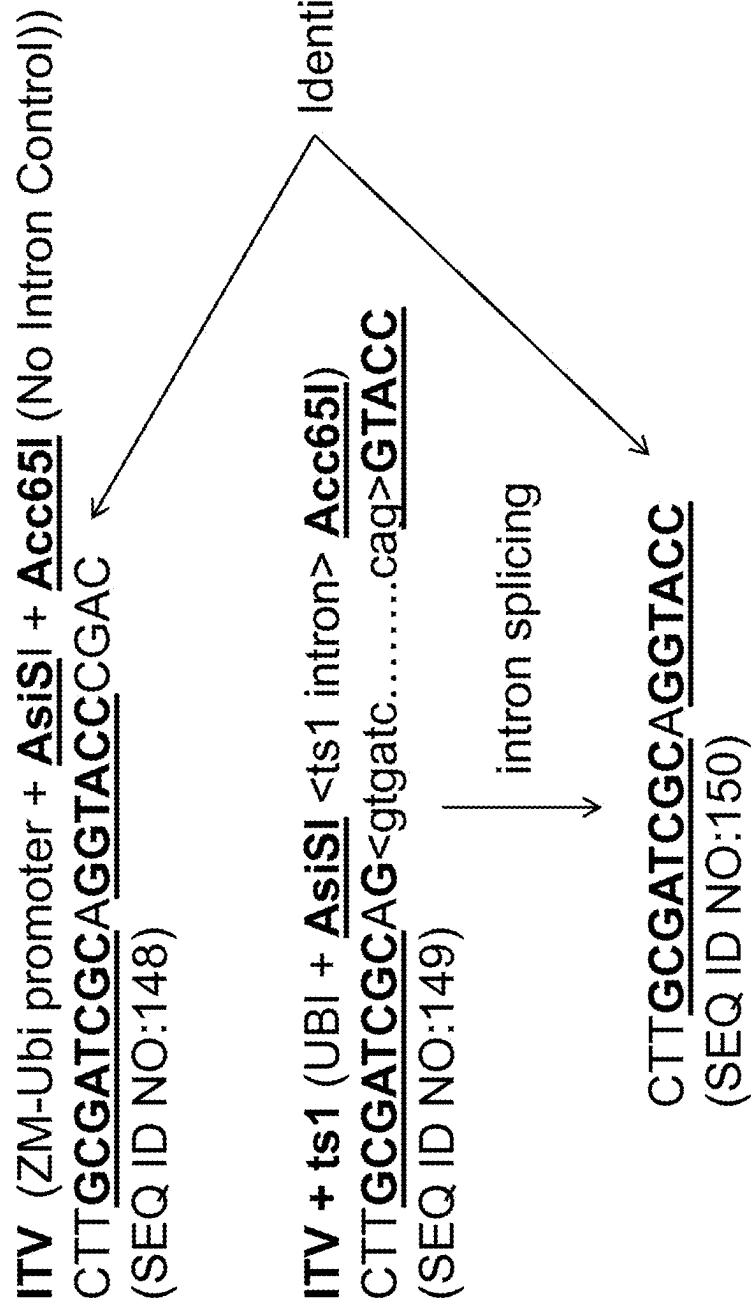


FIG. 2

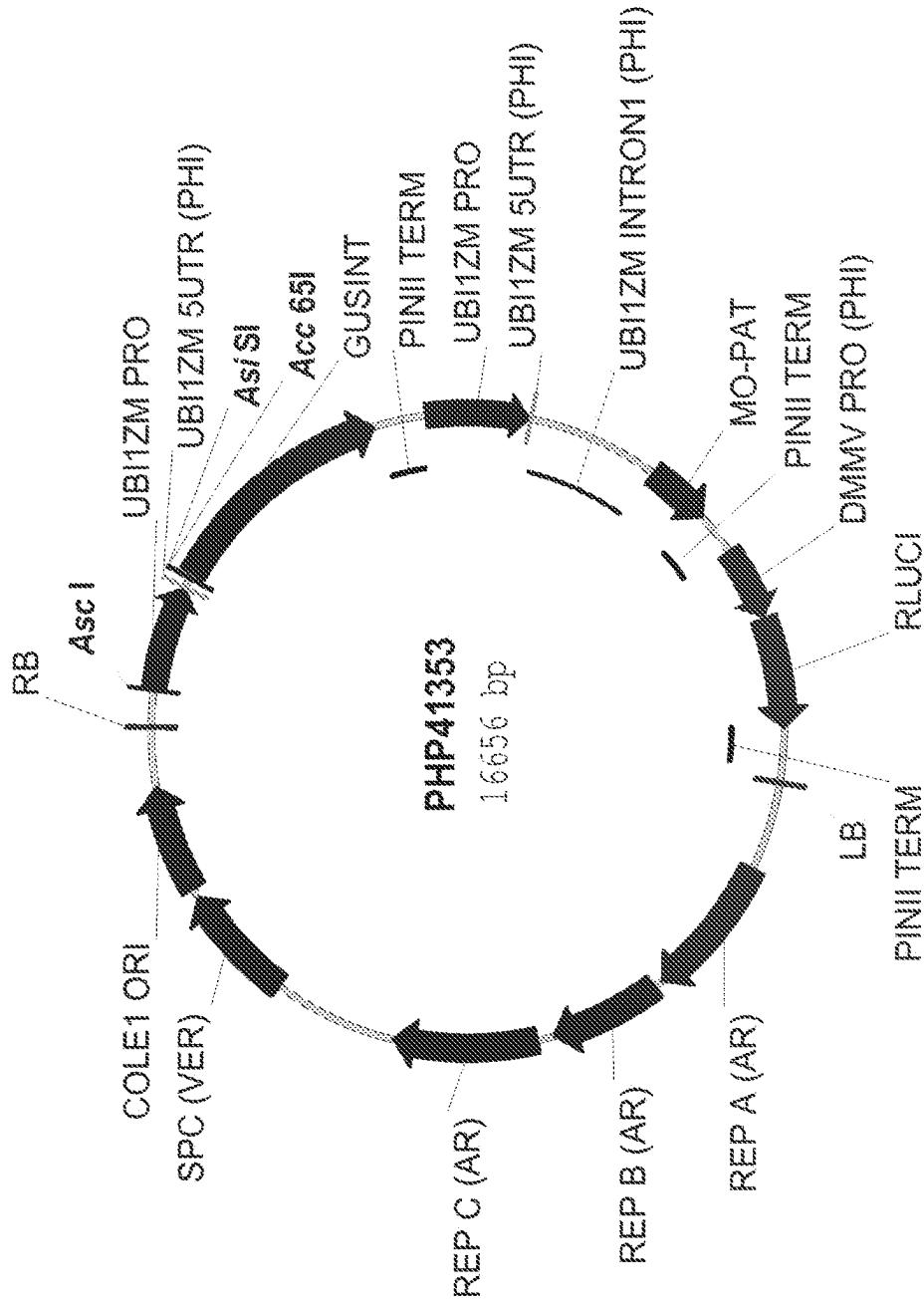


FIG. 3

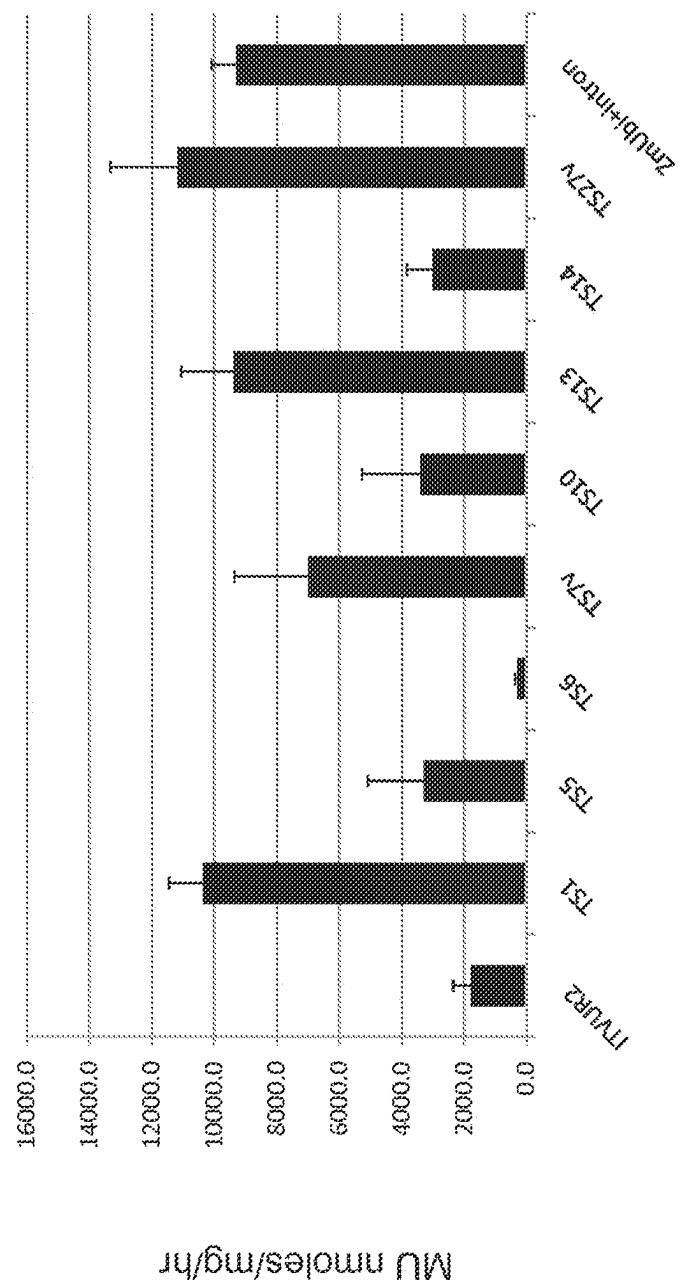


FIG. 4

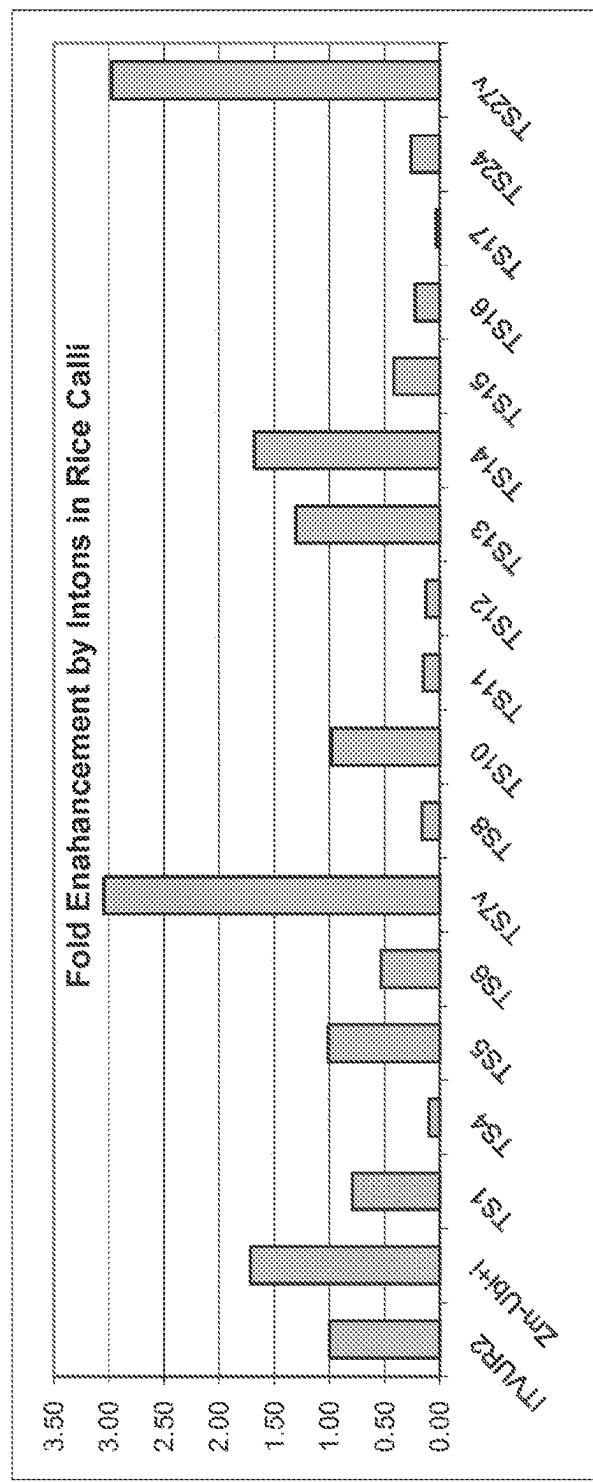
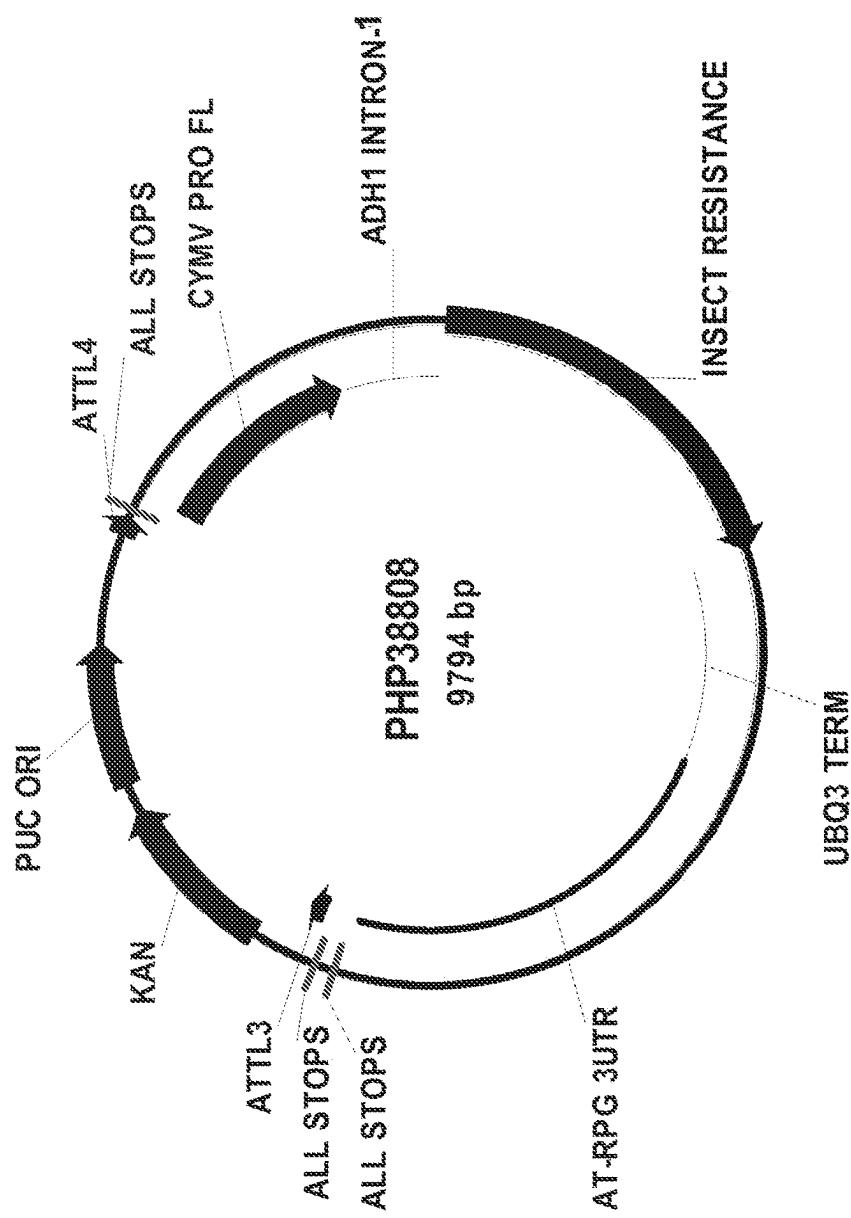


FIG. 5



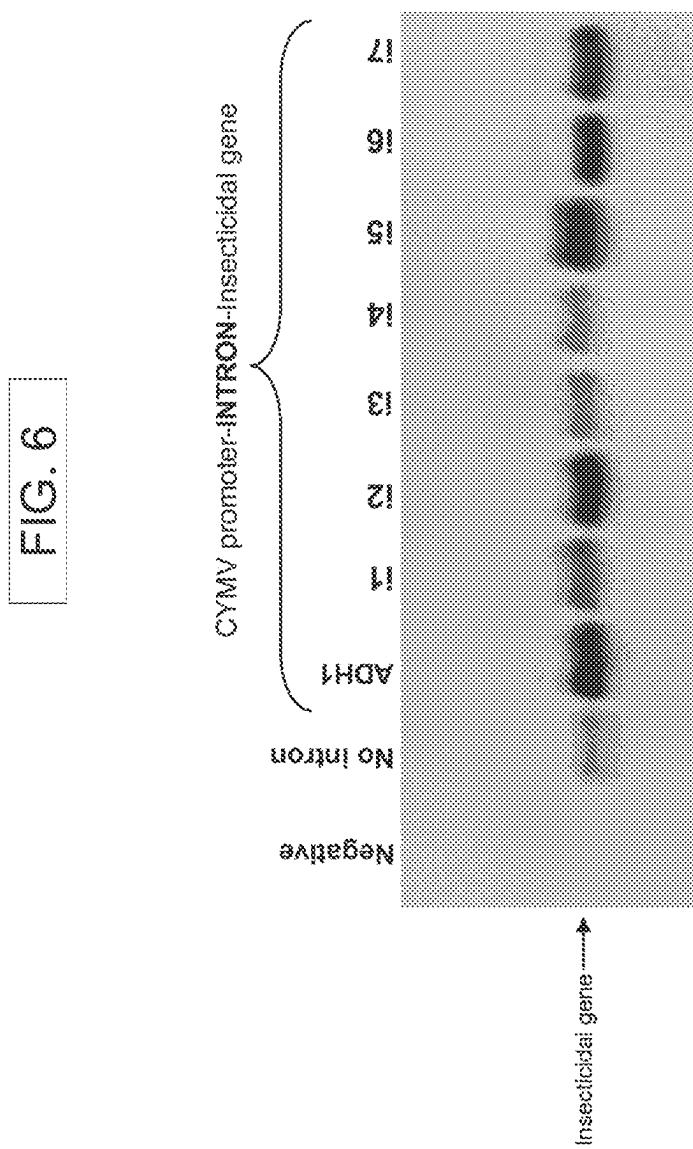


FIG. 7

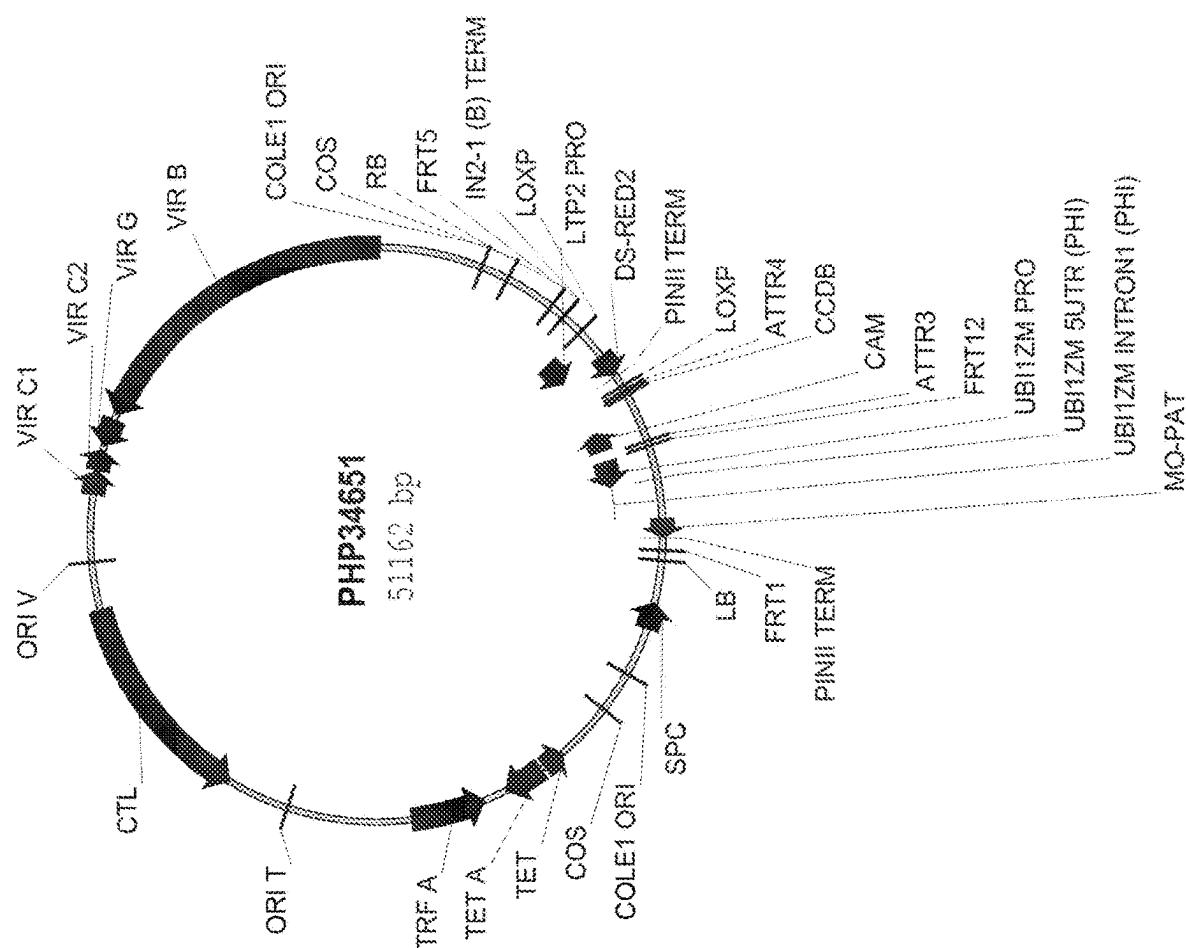


FIG. 8

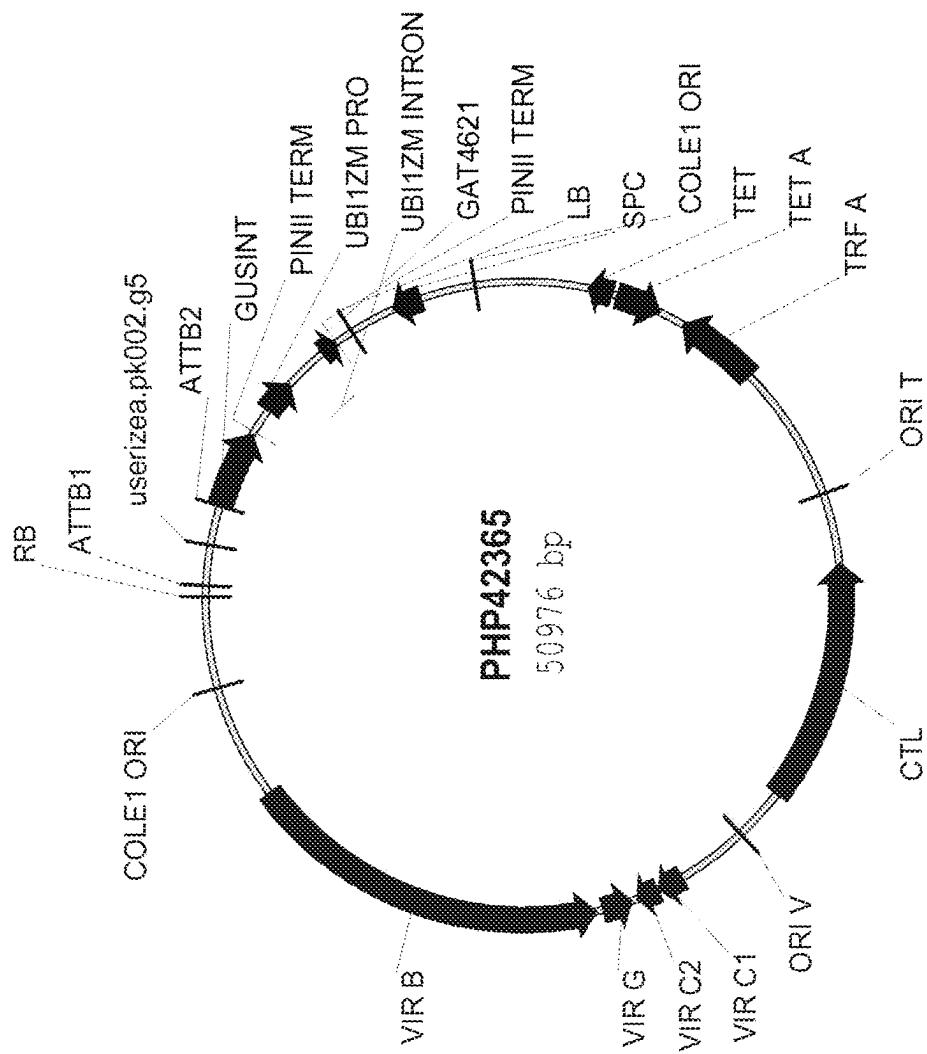


FIG. 9

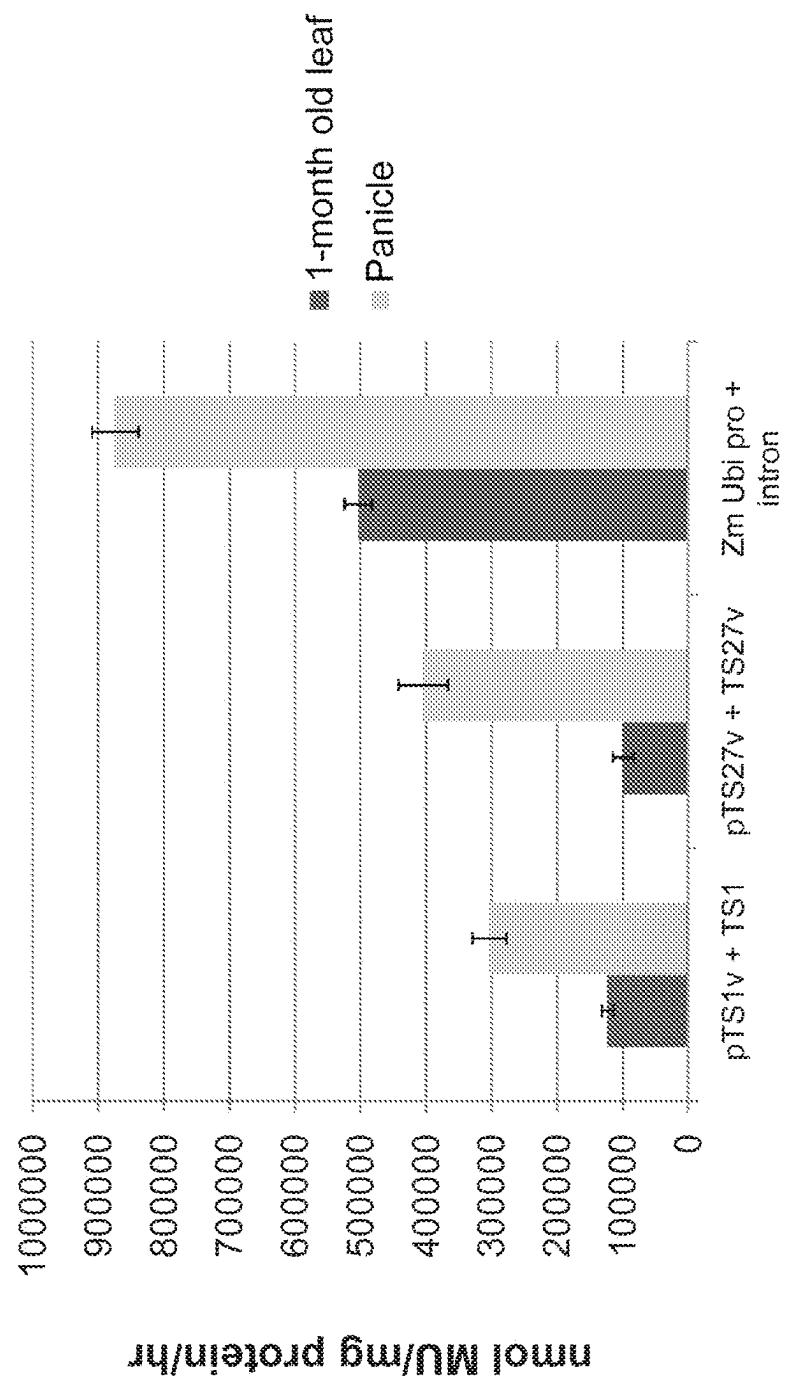


FIG. 10

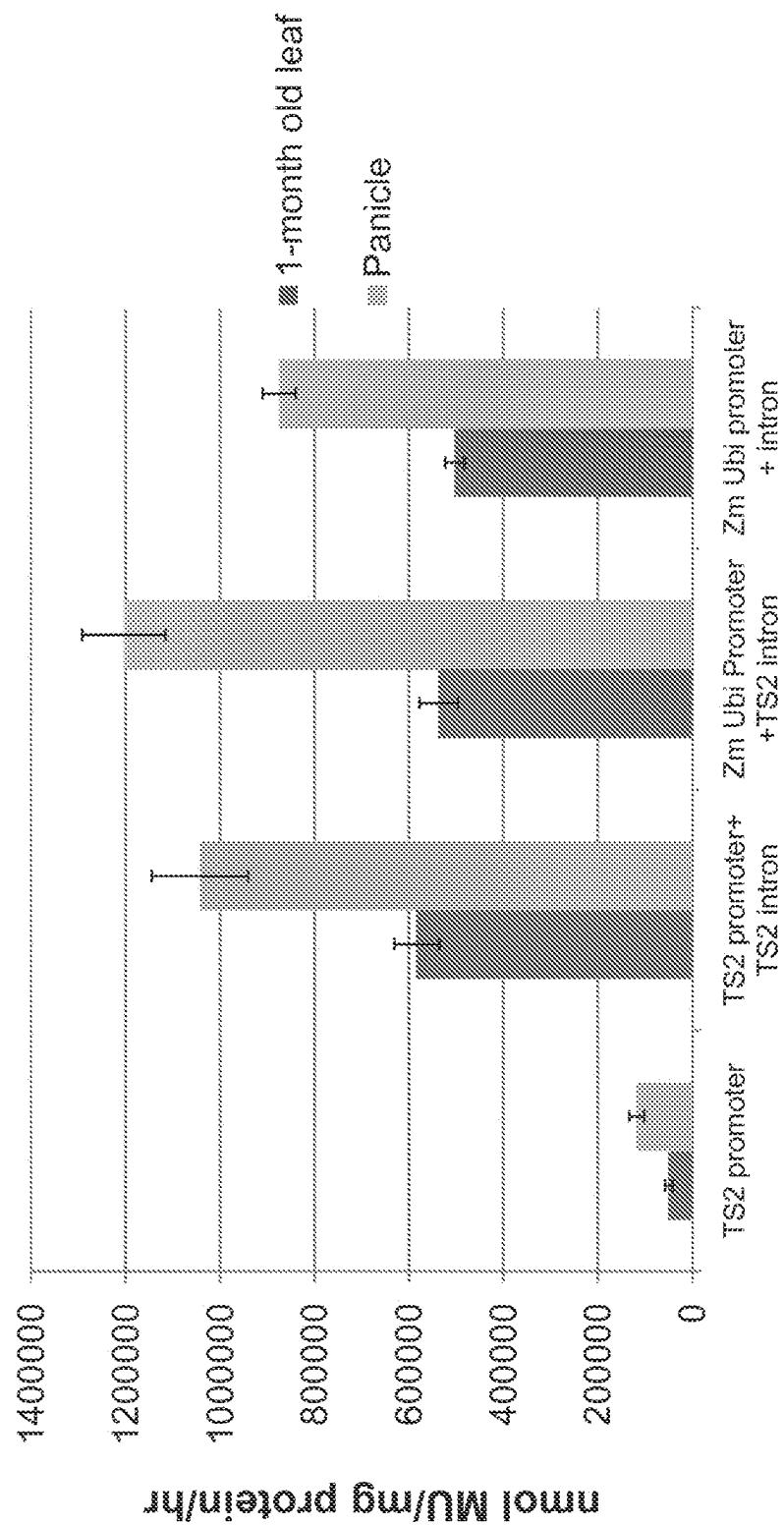


FIG. 11

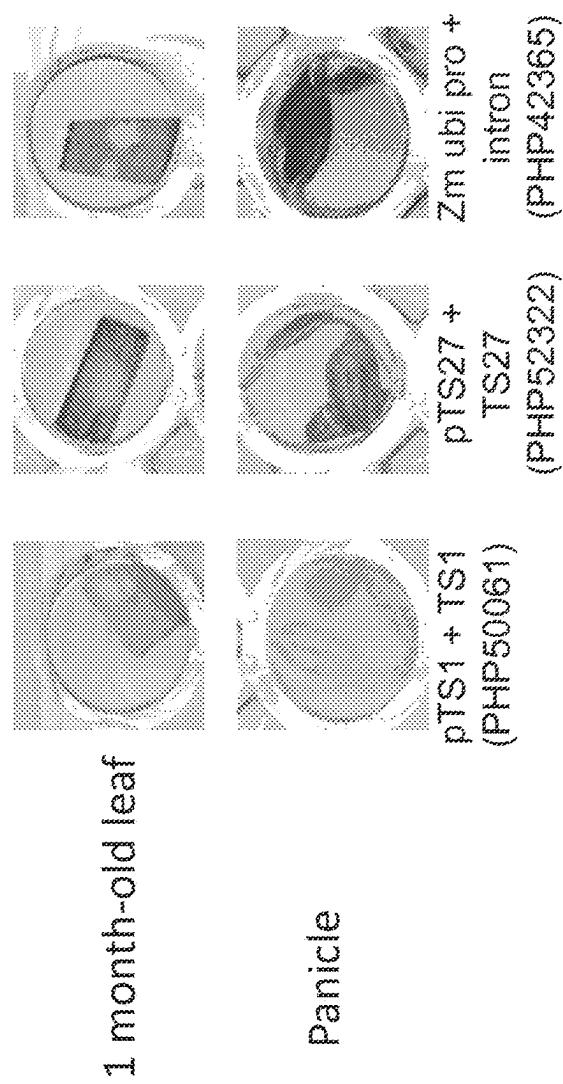


FIG. 12

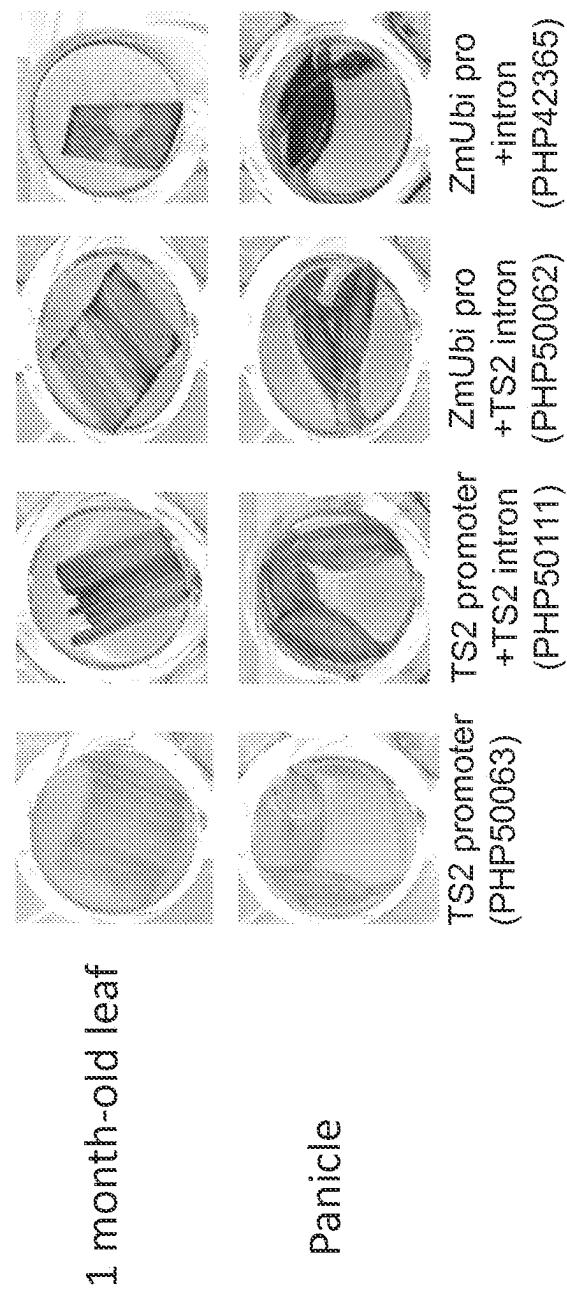
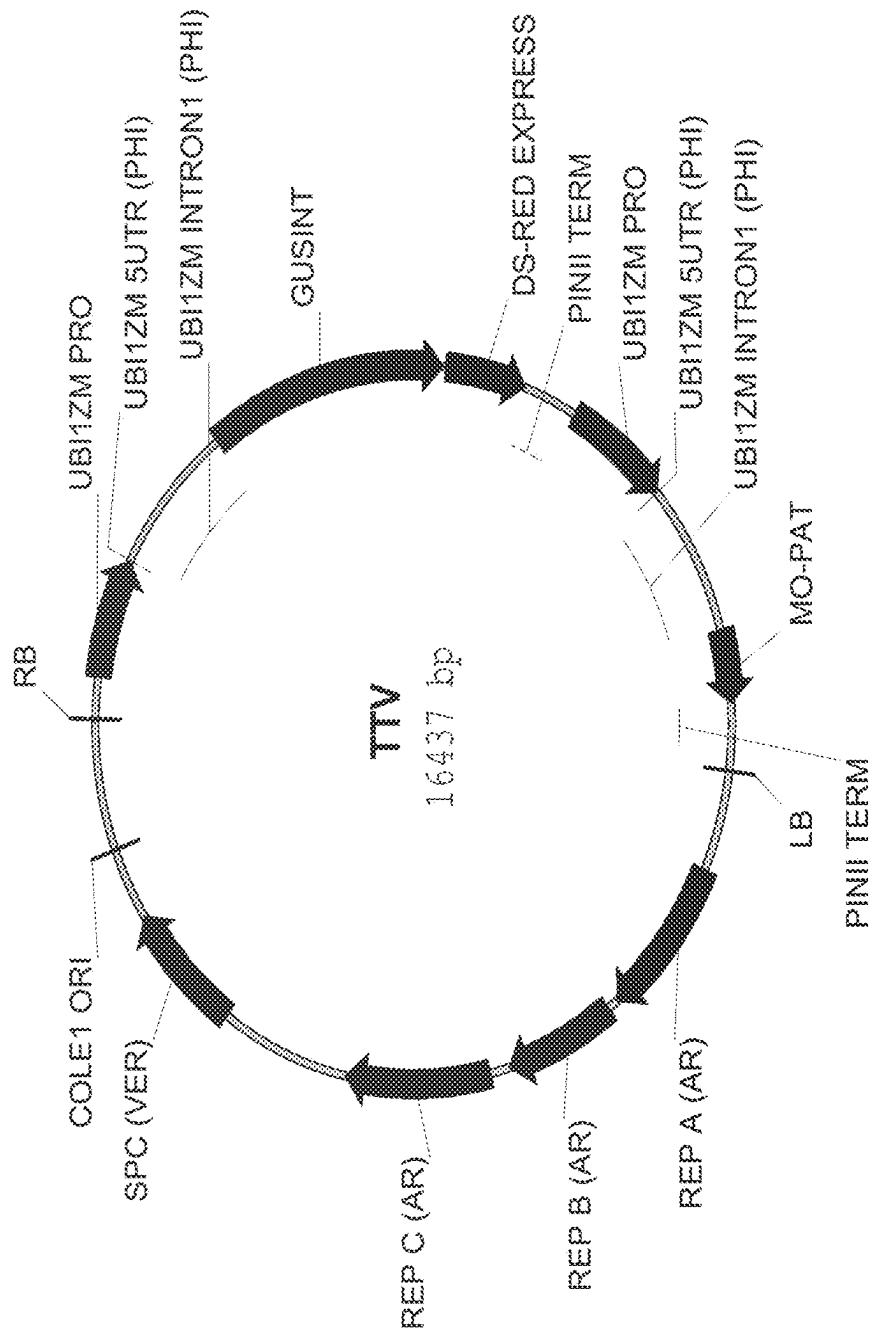


FIG. 13



REGULATORY SEQUENCES FOR MODULATING TRANSGENE EXPRESSION IN PLANTS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of Indian Provisional Application No. 1340/DELNP/2010, filed Jun. 9, 2010, and U.S. Provisional Application No. 61/372,515, filed Aug. 11, 2010, the entire contents of each is herein incorporated by reference.

FIELD OF INVENTION

[0002] The present invention relates to the generation of transgenic plants, particularly to the use of promoter and intron sequences to regulate gene expression in plants.

BACKGROUND

[0003] Recent advances in plant genetic engineering have opened new doors to engineer plants to have improved characteristics or traits. These transgenic plants characteristically have recombinant DNA constructs in their genome that have a protein-coding region operably linked to at least one regulatory region that is the promoter. The promoter can be a strong or weak promoter, or a constitutive or tissue-specific promoter. Besides the promoter, the expression level of the gene product can be modulated by other regulatory elements such as introns. Introns are intervening, non-coding sequences that are present in most eukaryotic genes. Introns have been reported to affect the levels of gene expression. This effect is known as Intron Mediated Enhancement (IME) of gene expression (Lu et al., *Mol Genet Genomics* (2008) 279:563-572). Callis et al. (*Genes Dev.* 1987 1:1183-1200) showed that the presence of the first intron from maize alcohol dehydrogenase-1 (Adh1) gene increased the expression levels of transgenes in cultured maize cells up to 100-fold when compared to intronless constructs. Mascarenhas et al. (*Plant Mol. Biol.*, 1990, 15:913-920) showed that other introns from the maize Adh1 gene could also increase heterologous gene expression in maize protoplasts. Vasil et al. (*Plant Physiol.*, 1989, 91:1575-1579) reported that the constructs containing Shrunken-1 (Sh-1) first intron had much higher expression levels of the reporter gene in plant protoplasts, when compared to the constructs with promoter alone, or to constructs with promoter and Adh-1 first intron. Identifying novel regulatory sequences can lead to finer modulation of gene expression in transgenic plants.

[0004] Plant genetic engineering has advanced to introducing multiple traits into commercially important plants, also known as gene stacking. This is accomplished by multigene transformation, where multiple genes are transferred to create a transgenic plant that might express a complex phenotype, or multiple phenotypes. But it is important to modulate or control the expression of each transgene optimally. The regulatory elements such as the promoter and the terminator sequences need to be diverse, to avoid introducing into the same transgenic plant repetitive sequences, which has been correlated with undesirable negative effects on transgene expression and stability (Peremarti et al (2010) *Plant Mol Biol* 73:363-378; Mette et al (1999) *EMBO J* 18:241-248; Mette et al (2000) *EMBO J* 19:5194-5201; Mourrain et al (2007) *Planta* 225:365-379, U.S. Pat. Nos.

7,632,982, 7,491,813, 7,674,950, PCT Application No. PCT/US2009/046968). Therefore it is important to discover and characterize novel regulatory elements that can be used to express heterologous nucleic acids in important crop species. Diverse regulatory regions can be used to control the expression of each transgene optimally.

SUMMARY

[0005] The present invention relates to regulatory sequences for modulating gene expression in plants. Recombinant DNA constructs comprising regulatory sequences are provided. Recombinant DNA constructs comprising intron sequences acting as enhancers of gene expression and endogenous promoter and terminator sequences corresponding to these intron sequences are provided.

[0006] Another embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the intron comprises a nucleotide sequence that has at least 95% sequence identity to SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138. In another embodiment, the intron comprises the nucleotide sequence of SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138.

[0007] One embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the promoter comprises a nucleotide sequence that has at least 95% sequence identity to SEQ ID NO: 105-117, 119, 136 or 139. In another embodiment, the promoter comprises the nucleotide sequence of SEQ ID NO: 105-117, 119, 136 or 139.

[0008] One embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the terminator comprises a nucleotide sequence that has at least 95% sequence identity to SEQ ID NOS: 140, 141, 142 or 143. In another embodiment, the terminator comprises the nucleotide sequence of SEQ ID NO: 140, 141, 142 or 143.

[0009] One embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the intron comprises a nucleotide sequence that has at least 95% identity to SEQ ID NOS: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138; and the promoter comprises a nucleotide sequence that has at least 95% identity to SEQ ID NOS: 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 119, 136 or 139.

[0010] One embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the intron comprises a nucleotide sequence that has at least 95% identity to SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138; the promoter sequence has at least 95% identity to SEQ ID NO: 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 119, 136 or 139; and the terminator has at least 95% sequence identity to SEQ ID NO: 140, 141, 142 or 143.

[0011] In one embodiment of the current invention, the intron is operably linked to the promoter, and is present downstream of the promoter, in the recombinant DNA constructs described herein. One embodiment of the present invention includes a recombinant DNA construct comprising an intron described in the present invention, operably

linked to a promoter and a heterologous polynucleotide, wherein the intron can act as enhancer of expression of the heterologous polynucleotide.

[0012] Another embodiment of the invention encompasses a recombinant DNA construct comprising an intron wherein the intron sequence comprises at least one copy of the 8-bp sequence motif of SEQ ID NO: 99; or contains at least one copy of the 8-bp sequence motif of SEQ ID NO: 99 and at least one copy of the 5-bp sequence motif of SEQ ID NO: 100, wherein the intron is capable of enhancing expression of a heterologous polynucleotide in a transgenic plant. The intron sequence can also comprise more than one copy of SEQ ID NO: 99, or can comprise one or more than one copy of SEQ ID NO: 99 and more than one copy of SEQ ID NO: 100.

[0013] Another embodiment of this invention is a method to identify novel introns that are useful for enhancing expression of a heterologous polynucleotide in a plant cell, the method comprising the steps of scanning a plurality of introns from plants for presence of SEQ ID NO: 99, selecting a sequence that contains at least one copy of SEQ ID NO: 99, measuring the efficacy of the identified intron to enhance expression of a heterologous polynucleotide in a plant.

[0014] Another embodiment of the invention is a method for identifying novel intronic sequences for enhancing transgene expression in monocotyledenous plants by identifying sequences orthologous to SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138; and measuring the enhancing effect of the identified intron on the expression of an operably linked heterologous polynucleotide.

[0015] Another embodiment of the current invention includes the promoter and the terminator sequences that are endogenously linked to the introns identified using the methods described in the current invention.

[0016] Another embodiment of the current invention is a method for modulating expression of a heterologous polynucleotide in a monocotyledenous plant comprising the steps of: (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a promoter and a heterologous polynucleotide wherein each is operably linked to an intron, wherein the intron comprises either (i) a nucleotide sequence that is orthologous to SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138; or (ii) a nucleotide sequence that contains least one copy of a sequence motif identical to SEQ ID NO: 99; and (b) regenerating a transgenic plant from a regenerable monocotyledenous plant cell after step (a) wherein the transgenic plant comprises the recombinant DNA construct; and (c) obtaining a progeny plant derived from the transgenic plant of step (b), wherein said progeny plant comprises the recombinant DNA construct and exhibits enhanced expression of the heterologous polynucleotide when compared to a plant comprising a corresponding recombinant DNA construct without the intron sequence.

[0017] In another embodiment, this invention concerns a vector, cell, plant, or seed comprising a recombinant DNA construct comprising the regulatory sequences described in the present invention.

[0018] The invention encompasses regenerated, mature and fertile transgenic plants comprising the recombinant DNA constructs described above, transgenic seeds produced therefrom, T1 and subsequent generations. The transgenic

plant cells, tissues, plants, and seeds may comprise at least one recombinant DNA construct of interest.

[0019] In one embodiment, the plant comprising the regulatory sequences described in the present invention is a monocotyledenous plant. In another embodiment, the plant comprising the regulatory sequences described in the present invention is a maize plant.

BRIEF DESCRIPTION OF DRAWINGS AND SEQUENCE LISTINGS

[0020] The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application. The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in Nucleic Acids Research 13:3021-3030 (1985) and in the Biochemical Journal 219 (No. 2): 345-373 (1984), which are herein incorporated by reference in their entirety. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. § 1.822.

[0021] FIG. 1 is a schematic representation of the vector used for testing introns showing the location of restriction sites used to clone introns relative to the maize ubiquitin promoter, as described in Example 2.

[0022] FIG. 2 shows the map of PHP 41353, the ITVUR-2 vector used for testing intron-mediated enhancement of gene expression.

[0023] FIG. 3 shows quantitative analysis of GUS reporter gene expression in Maize Embryos infected with the respective constructs.

[0024] FIG. 4 shows the fold enhancement of GUS reporter gene expression in rice calli infected with intron constructs when compared with the control vector ITVUR-2.

[0025] FIG. 5 shows the map of PHP38808, the vector with CYMV promoter and ADH1 intron, used for testing intron-mediated enhancement of gene expression, as described in Example 7.

[0026] FIG. 6 shows the results of Northern blot of RNA extracted from infiltrated maize tissue culture material and probed with a digoxigenin-labeled DNA probe for the insecticidal gene used. Samples were loaded based on ELISA data to contain equal amounts of PAT.

[0027] FIG. 7 shows the map of PHP34651, vector containing GATEWAY® attR recombination sites and a PAT expression cassette used for LR reactions to generate the final expression vectors for introns, as described in Example 7.

[0028] FIG. 8 shows the map of PHP42365, vector containing ZmUbi promoter and ZmUbi intron, for testing in stable transgenic rice plants, as described in Example 11.

[0029] FIG. 9 shows MUG data from stable transgenic lines transformed with different constructs. Data represents the average of 5-8 independent single copy events+SE.

[0030] FIG. 10 shows MUG data from stable transgenic lines transformed with different constructs. Data represents the average of 5-8 independent single copy events+SE.

[0031] FIG. 11 shows histochemical data from leaves and panicles collected from stable transgenic lines transformed with different constructs. Representative images are shown for each construct analyzed.

[0032] FIG. 12 shows histochemical data from leaves and panicles collected from stable transgenic lines transformed with different constructs. Representative images are shown for each construct analyzed.

[0033] FIG. 13 is the schematic representation of the PHP49597 vector (terminator test vector).

[0034] SEQ ID NO: 1 is the sequence of the maize ubiquitin promoter.

[0035] SEQ ID NO: 2 is the sequence of the first intron from maize ubiquitin gene.

[0036] SEQ ID NO: 3 is the nucleotide sequence of PHP41353, ITVUR-2 vector.

[0037] SEQ ID NOS: 4-19 and SEQ ID NOS: 52-58, SEQ ID NO: 118, SEQ ID NOS: 137 and 138 are sequences of introns that were tested to identify expression-enhancing introns, and are described in Table 1 below.

[0038] SEQ ID NOS: 105-113, SEQ ID NO: 119 and SEQ ID NOS: 136 and 139 are the sequences of promoters identified for the enhancing introns as described in Example 10 and Example 11, and are described in Table 1 below.

[0039] SEQ ID NOS: 140-143 given in Table 1 are the sequences of the endogenous terminators for the introns TS1, TS2, TS13 and TS27, identified as explained in Example 13.

TABLE 1

SEQ ID NO	Name	Intron/ Promoter	Enhancing/Non-Enhancing Intron
4	TS1	Intron	Enhancing
5	TS4	Intron	Non-Enhancing
6	TS5	Intron	Non-Enhancing
7	TS6	Intron	Non-Enhancing
8	TS7	Intron	Enhancing*
9	TS8	Intron	Non-Enhancing
10	TS10	Intron	Non-Enhancing
11	TS11	Intron	Non-Enhancing
12	TS12	Intron	Non-Enhancing
13	TS13	Intron	Enhancing
14	TS14	Intron	Non-Enhancing
15	TS15	Intron	Non-Enhancing
16	TS16	Intron	Non-Enhancing
17	TS17	Intron	Non-Enhancing
18	TS24	Intron	Non-Enhancing
19	TS27	Intron	Enhancing*
52	i1	Intron	Enhancing
53	i2	Intron	Enhancing
54	i3	Intron	Non-Enhancing
55	i4	Intron	Non-Enhancing
56	i5	Intron	Enhancing
57	i6	Intron	Enhancing
58	i7	Intron	Enhancing
105	pTS1	Promoter	Promoter identified for SEQ ID NO: 4
106	pTS7	Promoter	Promoter identified for SEQ ID NO: 8
107	pTS13	Promoter	Promoter identified for SEQ ID NO: 13
108	pTS27	Promoter	Promoter identified for SEQ ID NO: 19
109	pi1	Promoter	Promoter identified for SEQ ID NO: 52
110	pi2	Promoter	Promoter identified for SEQ ID NO: 53
111	pi5	Promoter	Promoter identified for SEQ ID NO: 56
112	pi6	Promoter	Promoter identified for SEQ ID NO: 57
113	pi7	Promoter	Promoter identified for SEQ ID NO: 58

TABLE 1-continued

SEQ ID NO	Name	Intron/ Promoter	Enhancing/Non-Enhancing Intron
118	TS2	Intron	Enhancing
119	pTS2	Promoter	Promoter identified for SEQ ID NO: 118
136	pTS1v	Promoter	Promoter sequence cloned for SEQ ID NO: 4
137	TS7v	Intron	Enhancing
138	TS27v	Intron	Enhancing
139	pTS27v	Promoter	Promoter sequence cloned for SEQ ID NO: 19
140	tTS1	Terminator	Terminator identified for SEQ ID NO: 4
141	tTS2	Terminator	Terminator identified for SEQ ID NO: 118
142	tTS13	Terminator	Terminator identified for SEQ ID NO: 13
143	tTS27	Terminator	Terminator identified for SEQ ID NO: 19

*based on results from variants

[0040] SEQ ID NOS: 20-51 are the primers used for cloning introns as described in Table 2 in Example 3.

[0041] SEQ ID NO: 59 is the sequence of the vector PHP38808, used for testing intron-mediated enhancement of gene expression as described in Example 7.

[0042] SEQ ID NO: 60 is the sequence of PHP34651, the vector containing GATEWAY® attR recombination sites and a PAT expression cassette used for LR reactions to generate the final expression vectors for introns, as described in Example 7.

[0043] SEQ ID NOS: 61-94 are the oligonucleotides used for generating introns by oligonucleotide stacking as described in Table 4 in Example 7.

[0044] SEQ ID NO: 95 is the sequence for first intron of adh1 gene.

[0045] SEQ ID NO: 96 is the sequence for intron 6 for adh1 gene.

[0046] SEQ ID NO: 97 is the sequence for intron 1 for shrunken1 (Sh-1) gene

[0047] SEQ ID NO: 98 is the sequence for ubi intron 1 used for computational analyses as described in Example 8.

[0048] SEQ ID NO: 99 is the sequence of the 8-bp motif identified as described in Example 8.

[0049] SEQ ID NO: 100 is the sequence of the 5-bp motif identified as described in Example 8.

[0050] SEQ ID NOS: 101-104 are the intron sequences containing the 8-bp motif (SEQ ID NO: 99), as described in Example 9.

[0051] SEQ ID NOS: 114-117 are the promoter sequences identified from the introns of SEQ ID NOS: 101-104 respectively, as described in Examples 9 and 10.

[0052] SEQ ID NOS: 120-128 are the sequences of the primers used for cloning the promoters and introns, as described in Table 7.

[0053] SEQ ID NOS: 129-134 are the primer and probe sequences for qPCR, as described in Table 9 and Table 10.

[0054] SEQ ID NO: 135 is the sequence of the PHP42365 vector that contains ZmUbi promoter and ZmUbi intron.

[0055] SEQ ID NO: 144 is the sequence of the PHP49597 vector (terminator test vector or TTV).

[0056] SEQ ID NO: 145 corresponds to the nucleotide sequence

[0057] GATCAAAAAAA of a ‘promiscuous’ MPSS tags.

[0058] SEQ ID NO: 146 corresponds to the nucleotide sequence of a consensus motif sequence, which encompasses variations of the motif sequence given in SEQ ID NO: 99.

[0059] The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. § 1.821-1.825. The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res.* 13:3021-3030 (1985) and in the *Biochemical J.* 219 (2): 345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. § 1.822.

DETAILED DESCRIPTION OF THE INVENTION

[0060] The disclosure of each reference set forth herein is hereby incorporated by reference in its entirety.

[0061] As used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to “a plant” includes a plurality of such plants, reference to “a cell” includes one or more cells and equivalents thereof known to those skilled in the art, and so forth.

[0062] As used herein:

[0063] The terms “monocot” and “monocotyledonous plant” are used interchangeably herein. A monocot of the current invention includes the Gramineae.

[0064] The terms “dicot” and “dicotyledonous plant” are used interchangeably herein. A dicot of the current invention includes the following families: Brassicaceae, Leguminosae, and Solanaceae.

[0065] The terms “full complement” and “full-length complement” are used interchangeably herein, and refer to a complement of a given nucleotide sequence, wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary.

[0066] “Transgenic” refers to any cell, cell line, callus, tissue, plant part or plant, the genome of which has been altered by the presence of a heterologous nucleic acid, such as a recombinant DNA construct, including those initial transgenic events as well as those created by sexual crosses or asexual propagation from the initial transgenic event. The term “transgenic” as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

[0067] “Genome” as it applies to plant cells encompasses not only chromosomal DNA found within the nucleus, but organelle DNA found within subcellular components (e.g., mitochondrial, plastid) of the cell.

[0068] “Plant” includes reference to whole plants, plant organs, plant tissues, seeds and plant cells and progeny of same. Plant cells include, without limitation, cells from seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores.

[0069] “Progeny” comprises any subsequent generation of a plant.

[0070] “Transgenic plant” includes reference to a plant which comprises within its genome a heterologous polynucleotide. For example, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant DNA construct.

[0071] “Heterologous” with respect to sequence means a sequence that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention.

[0072] “Polynucleotide”, “nucleic acid sequence”, “nucleotide sequence”, or “nucleic acid fragment” are used interchangeably to refer to a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. Nucleotides (usually found in their 5'-monophosphate form) are referred to by their single letter designation as follows: “A” for adenylate or deoxyadenylate (for RNA or DNA, respectively), “C” for cytidylate or deoxycytidylate, “G” for guanylate or deoxyguanylate, “U” for uridylate, “T” for deoxythymidylate, “R” for purines (A or G), “Y” for pyrimidines (C or T), “K” for G or T, “H” for A or C or T, “I” for inosine, and “N” for any nucleotide.

[0073] “Polypeptide”, “peptide”, “amino acid sequence” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The terms “polypeptide”, “peptide”, “amino acid sequence”, and “protein” are also inclusive of modifications including, but not limited to, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation.

[0074] “Messenger RNA (mRNA)” refers to the RNA that is without introns and that can be translated into protein by the cell.

[0075] “cDNA” refers to a DNA that is complementary to and synthesized from an mRNA template using the enzyme reverse transcriptase. The cDNA can be single-stranded or converted into the double-stranded form using the Klenow fragment of DNA polymerase I.

[0076] An “Expressed Sequence Tag” (“EST”) is a DNA sequence derived from a cDNA library and therefore is a sequence which has been transcribed. An EST is typically obtained by a single sequencing pass of a cDNA insert. The sequence of an entire cDNA insert is termed the “Full-Insert Sequence” (“FIS”). A “Contig” sequence is a sequence assembled from two or more sequences that can be selected from, but not limited to, the group consisting of an EST, FIS and PCR sequence. A sequence encoding an entire or functional protein is termed a “Complete Gene Sequence” (“CGS”) and can be derived from an FIS or a contig.

[0077] “Mature” protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or pro-peptides present in the primary translation product has been removed.

[0078] “Precursor” protein refers to the primary product of translation of mRNA; i.e., with pre- and pro-peptides still

present. Pre- and pro-peptides may be and are not limited to intracellular localization signals.

[0079] "Isolated" refers to materials, such as nucleic acid molecules and/or proteins, which are substantially free or otherwise removed from components that normally accompany or interact with the materials in a naturally occurring environment. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant polynucleotides and chemically synthesized polynucleotides.

[0080] "Recombinant" refers to an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated segments of nucleic acids by genetic engineering techniques.

[0081] "Recombinant" also includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid or a cell derived from a cell so modified, but does not encompass the alteration of the cell or vector by naturally occurring events (e.g., spontaneous mutation, natural transformation/transduction/transposition) such as those occurring without deliberate human intervention.

[0082] "Recombinant DNA construct" refers to a combination of nucleic acid fragments that are not normally found together in nature. Accordingly, a recombinant DNA construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that normally found in nature.

[0083] The terms "entry clone" and "entry vector" are used interchangeably herein.

[0084] The term "insecticidal gene" and "insect resistance gene" are used interchangeably herein.

[0085] "Operably linked" refers to the association of nucleic acid fragments in a single fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a nucleic acid fragment when it is capable of regulating the transcription of that nucleic acid fragment.

[0086] "Expression" refers to the production of a functional product. For example, expression of a nucleic acid fragment may refer to transcription of the nucleic acid fragment (e.g., transcription resulting in mRNA or functional RNA) and/or translation of mRNA into a precursor or mature protein.

[0087] "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in a null segregating (or non-transgenic) organism from the same experiment.

[0088] "Phenotype" means the detectable characteristics of a cell or organism.

[0089] "Introduced" in the context of inserting a nucleic acid fragment (e.g., a recombinant DNA construct) into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid fragment into a eukaryotic or prokaryotic cell where the nucleic acid fragment may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

[0090] A "transformed cell" is any cell into which a nucleic acid fragment (e.g., a recombinant DNA construct) has been introduced.

[0091] "Transformation" as used herein refers to both stable transformation and transient transformation.

[0092] "Stable transformation" refers to the introduction of a nucleic acid fragment into a genome of a host organism resulting in genetically stable inheritance. Once stably transformed, the nucleic acid fragment is stably integrated in the genome of the host organism and any subsequent generation.

[0093] "Transient transformation" refers to the introduction of a nucleic acid fragment into the nucleus, or DNA-containing organelle, of a host organism resulting in gene expression without genetically stable inheritance.

[0094] The term "crossed" or "cross" means the fusion of gametes via pollination to produce progeny (e.g., cells, seeds or plants). The term encompasses both sexual crosses (the pollination of one plant by another) and selfing (self-pollination, e.g., when the pollen and ovule are from the same plant). The term "crossing" refers to the act of fusing gametes via pollination to produce progeny.

[0095] A "favorable allele" is the allele at a particular locus that confers, or contributes to, a desirable phenotype, e.g., increased cell wall digestibility, or alternatively, is an allele that allows the identification of plants with decreased cell wall digestibility that can be removed from a breeding program or planting ("counterselection"). A favorable allele of a marker is a marker allele that segregates with the favorable phenotype, or alternatively, segregates with the unfavorable plant phenotype, therefore providing the benefit of identifying plants.

[0096] The term "introduced" means providing a nucleic acid (e.g., expression construct) or protein into a cell. Introduced includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell, and includes reference to the transient provision of a nucleic acid or protein to the cell. Introduced includes reference to stable or transient transformation methods, as well as sexually crossing. Thus, "introduced" in the context of inserting a nucleic acid fragment (e.g., a recombinant DNA construct/ expression construct) into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid fragment into a eukaryotic or prokaryotic cell where the nucleic acid fragment may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

[0097] Sequence alignments and percent identity calculations may be determined using a variety of comparison methods designed to detect homologous sequences including, but not limited to, the MEGALIGN® program of the LASERGENE bioinformatics computing suite (DNASTAR® Inc., Madison, WI). Unless stated otherwise, multiple alignment of the sequences provided herein were performed using the Clustal V method of alignment (Higgins and Sharp, *CABIOS*. 5:151-153 (1989)) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal V method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are KTUPLE=2, GAP PENALTY=5,

WINDOW=4 and DIAGONALS SAVED-4. After alignment of the sequences, using the Clustal V program, it is possible to obtain "percent identity" and "divergence" values by viewing the "sequence distances" table on the same program; unless stated otherwise, percent identities and divergences provided and claimed herein were calculated in this manner.

[0098] The present invention includes a polynucleotide comprising: (i) a nucleic acid sequence of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity, based on the Clustal V method of alignment, when compared to SEQ ID NOS: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101-119, 136-143; or (ii) a full complement of the nucleic acid sequence of (i), wherein the polynucleotide acts as a regulator of gene expression in a plant cell.

[0099] Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Sambrook").

Regulatory Sequences:

[0100] A recombinant DNA construct (including a suppression DNA construct) of the present invention may comprise at least one regulatory sequence.

[0101] "Regulatory sequences" or "regulatory elements" are used interchangeably and refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include, but are not limited to, promoters, translation leader sequences, introns, and polyadenylation recognition sequences. The terms "regulatory sequence" and "regulatory element" are used interchangeably herein.

[0102] "Promoter" refers to a nucleic acid fragment capable of controlling transcription of another nucleic acid fragment.

[0103] "Promoter functional in a plant" is a promoter capable of controlling transcription in plant cells whether or not its origin is from a plant cell.

[0104] "Tissue-specific promoter" and "tissue-preferred promoter" are used interchangeably to refer to a promoter that is expressed predominantly but not necessarily exclusively in one tissue or organ, but that may also be expressed in one specific cell.

[0105] "Developmentally regulated promoter" refers to a promoter whose activity is determined by developmental events.

[0106] Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters".

[0107] High level, constitutive expression of the candidate gene under control of the 35S or UBI promoter may have pleiotropic effects, although candidate gene efficacy may be estimated when driven by a constitutive promoter. Use of tissue-specific and/or stress-specific promoters may eliminate undesirable effects but retain the ability to enhance drought tolerance. This effect has been observed in *Arabidopsis* (Kasuga et al. (1999) *Nature Biotechnol.* 17:287-91).

[0108] Suitable constitutive promoters for use in a plant host cell include, but are not limited to, the core promoter of

the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Pat. No. 6,072,050; the core CaMV 35S promoter (Odell et al., *Nature* 313:810-812 (1985)); rice actin (McElroy et al., *Plant Cell* 2:163-171 (1990)); ubiquitin (Christensen et al., *Plant Mol. Biol.* 12:619-632 (1989) and Christensen et al., *Plant Mol. Biol.* 18:675-689 (1992)); pEMU (Last et al., *Theor. Appl. Genet.* 81:581-588 (1991)); MAS (Velten et al., *EMBO J.* 3:2723-2730 (1984)); ALS promoter (U.S. Pat. No. 5,659,026), and the like. Other constitutive promoters include, but are not limited to, for example, those discussed in U.S. Pat. Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611.

[0109] In choosing a promoter to use in the methods of the invention, it may be desirable to use a tissue-specific or developmentally regulated promoter.

[0110] A tissue-specific or developmentally regulated promoter is a DNA sequence which regulates the expression of a DNA sequence selectively in the cells/tissues of a plant critical to tassel development, seed set, or both, and limits the expression of such a DNA sequence to the period of tassel development or seed maturation in the plant. Any identifiable promoter may be used in the methods of the present invention which causes the desired temporal and spatial expression.

[0111] Promoters which are seed or embryo-specific and may be useful in the invention include, but are not limited to, soybean Kunitz trypsin inhibitor (Kti3, Jofuku and Goldberg, *Plant Cell* 1:1079-1093 (1989)), patatin (potato tubers) (Rocha-Sosa, M., et al. (1989) *EMBO J.* 8:23-29), convicilin, vicilin, and legumin (pea cotyledons) (Rerie, W. G., et al. (1991) *Mol. Gen. Genet.* 259:149-157; Newbiggin, E. J., et al. (1990) *Planta* 180:461-470; Higgins, T. J. V., et al. (1988) *Plant. Mol. Biol.* 11:683-695), zein (maize endosperm) (Schemthaner, J. P., et al. (1988) *EMBO J.* 7:1249-1255), phaseolin (bean cotyledon) (Segupta-Gopalan, C., et al. (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82:3320-3324), phytohemagglutinin (bean cotyledon) (Voelker, T. et al. (1987) *EMBO J.* 6:3571-3577), B-conglycinin and glycinin (soybean cotyledon) (Chen, Z-L, et al. (1988) *EMBO J.* 7:297-302), glutelin (rice endosperm), hordein (barley endosperm) (Marris, C., et al. (1988) *Plant Mol. Biol.* 10:359-366), glutenin and gliadin (wheat endosperm) (Colot, V., et al. (1987) *EMBO J.* 6:3559-3564), and sporamin (sweet potato tuberous root) (Hattori, T., et al. (1990) *Plant Mol. Biol.* 14:595-604). Promoters of seed-specific genes operably linked to heterologous coding regions in chimeric gene constructions maintain their temporal and spatial expression pattern in transgenic plants. Such examples include, but are not limited to, *Arabidopsis thaliana* 2S seed storage protein gene promoter to express enkephalin peptides in *Arabidopsis* and *Brassica napus* seeds (Vanderkerckhove et al., *Bio/Technology* 7: L929-932 (1989)), bean lectin and bean beta-phaseolin promoters to express luciferase (Riggs et al., *Plant Sci.* 63:47-57 (1989)), and wheat glutenin promoters to express chloramphenicol acetyl transferase (Colot et al., *EMBO J.* 6:3559-3564 (1987)).

[0112] Inducible promoters selectively express an operably linked DNA sequence in response to the presence of an endogenous or exogenous stimulus, for example by chemical compounds (chemical inducers) or in response to environmental, hormonal, chemical, and/or developmental signals. Inducible or regulated promoters include, but are not limited to, for example, promoters regulated by light, heat,

stress, flooding or drought, phytohormones, wounding, or chemicals such as ethanol, jasmonate, salicylic acid, or safeners.

[0113] For instance, introns of the present invention can be combined with inducible promoters to enhance their activity without affecting their inducibility characteristics.

[0114] A minimal or basal promoter is a polynucleotide molecule that is capable of recruiting and binding the basal transcription machinery. One example of basal transcription machinery in eukaryotic cells is the RNA polymerase II complex and its accessory proteins.

[0115] Plant RNA polymerase II promoters, like those of other higher eukaryotes, are comprised of several distinct "cis-acting transcriptional regulatory elements," or simply "cis-elements," each of which appears to confer a different aspect of the overall control of gene expression. Examples of such cis-acting elements include, but are not limited to, such as TATA box and CCAAT or AGGA box. The promoter can roughly be divided in two parts: a proximal part, referred to as the core, and a distal part. The proximal part is believed to be responsible for correctly assembling the RNA polymerase II complex at the right position and for directing a basal level of transcription, and is also referred to as "minimal promoter" or "basal promoter". The distal part of the promoter is believed to contain those elements that regulate the spatio-temporal expression. In addition to the proximal and distal parts, other regulatory regions have also been described, that contain enhancer and/or repressors elements. The latter elements can be found from a few kilobase pairs upstream from the transcription start site, in the introns, or even at the 3' side of the genes they regulate (Rombauts, S. et al. (2003) *Plant Physiology* 132:1162-1176, Nikolov and Burley, (1997) *Proc Natl Acad Sci USA* 94:15-22), Tjian and Maniatis (1994) *Cell* 77:5-8; Fessele et al., 2002 *Trends Genet* 18:60-63, Messing et al., (1983) *Genetic Engineering of Plants: an Agricultural Perspective*, Plenum Press, NY, pp 211-227).

[0116] When operably linked to a heterologous polynucleotide sequence, a promoter controls the transcription of the linked polynucleotide sequence.

[0117] In an embodiment of the present invention, the "cis-acting transcriptional regulatory elements" from the promoter sequence disclosed herein can be operably linked to "cis-acting transcriptional regulatory elements" from any heterologous promoter. Such a chimeric promoter molecule can be engineered to have desired regulatory properties. In an embodiment of this invention a fragment of the disclosed promoter sequence that can act either as a cis-regulatory sequence or a distal-regulatory sequence or as an enhancer sequence or a repressor sequence, may be combined with either a cis-regulatory or a distal regulatory or an enhancer sequence or a repressor sequence or any combination of any of these from a heterologous promoter sequence.

[0118] In a related embodiment, a cis-element of the disclosed promoter may confer a particular specificity such as conferring enhanced expression of operably linked polynucleotide molecules in certain tissues and therefore is also capable of regulating transcription of operably linked polynucleotide molecules. Consequently, any fragment, portion, or region of the promoter comprising the polynucleotide sequence shown in SEQ ID NO: 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 119, 136 or 139 can be used as a regulatory polynucleotide molecule.

[0119] Promoter fragments that comprise regulatory elements can be added, for example, fused to the 5' end of, or inserted within, another promoter having its own partial or complete regulatory sequences (Fluhr et al., *Science* 232: 1106-1112, 1986; Ellis et al., *EMBO J.* 6:11-16, 1987; Strittmatter and Chua, *Proc. Nat. Acad. Sci. USA* 84:8986-8990, 1987; Poulsen and Chua, *Mol. Gen. Genet.* 214:16-23, 1988; Comai et al., *Plant Mol. Biol.* 15:373-381, 1991; 1987; Aryan et al., *Mol. Gen. Genet.* 225:65-71, 1991).

[0120] Cis elements can be identified by a number of techniques, including deletion analysis, i.e., deleting one or more nucleotides from the 5' end or internal to a promoter; DNA binding protein analysis using DNase I footprinting; methylation interference; electrophoresis mobility-shift assays, in vivo genomic footprinting by ligation-mediated PCR; and other conventional assays; or by sequence similarity with known cis element motifs by conventional sequence comparison methods. The fine structure of a cis element can be further studied by mutagenesis (or substitution) of one or more nucleotides or by other conventional methods (see for example, *Methods in Plant Biochemistry and Molecular Biology*, Dashek, ed., CRC Press, 1997, pp. 397-422; and *Methods in Plant Molecular Biology*, Maliga et al., eds., Cold Spring Harbor Press, 1995, pp. 233-300).

[0121] Cis elements can be obtained by chemical synthesis or by cloning from promoters that include such elements, and they can be synthesized with additional flanking sequences that contain useful restriction enzyme sites to facilitate subsequent manipulation. Promoter fragments may also comprise other regulatory elements such as enhancer domains, which may further be useful for constructing chimeric molecules.

[0122] Methods for construction of chimeric and variant promoters of the present invention include, but are not limited to, combining control elements of different promoters or duplicating portions or regions of a promoter (see for example, U.S. Pat. Nos. 4,990,607; 5,110,732; and 5,097,025). Those of skill in the art are familiar with the standard resource materials that describe specific conditions and procedures for the construction, manipulation, and isolation of macromolecules (e.g., polynucleotide molecules and plasmids), as well as the generation of recombinant organisms and the screening and isolation of polynucleotide molecules.

[0123] In an embodiment of the present invention, the promoters disclosed herein can be modified. Those skilled in the art can create promoters that have variations in the polynucleotide sequence. The polynucleotide sequence of the promoters of the present invention as shown in SEQ ID NOS: 105-113, 119, 136 or 139, may be modified or altered to enhance their control characteristics. As one of ordinary skill in the art will appreciate, modification or alteration of the promoter sequence can also be made without substantially affecting the promoter function. The methods are well known to those of skill in the art. Sequences can be modified, for example by insertion, deletion, or replacement of template sequences in a PCR-based DNA modification approach.

[0124] The present invention encompasses functional fragments and variants of the promoter sequences disclosed herein.

[0125] A "functional fragment" of a regulatory sequence herein is defined as any subset of contiguous nucleotides of any of the regulatory sequences disclosed herein, that can

perform the same, or substantially similar function as the full length promoter sequences disclosed herein.

[0126] A “functional fragment of a promoter” with substantially similar function to a full length promoter disclosed herein refers to a functional fragment that retains largely the same level of activity as the full length promoter sequence and exhibits the same pattern of expression as the full length promoter sequence.

[0127] A “variant promoter”, as used herein, is the sequence of the promoter or the sequence of a functional fragment of a promoter containing changes in which one or more nucleotides of the original sequence is deleted, added, and/or substituted, while substantially maintaining promoter function. One or more base pairs can be inserted, deleted, or substituted internally to a promoter. In the case of a promoter fragment, variant promoters can include changes affecting the transcription of a minimal promoter to which it is operably linked. Variant promoters can be produced, for example, by standard DNA mutagenesis techniques or by chemically synthesizing the variant promoter or a portion thereof.

[0128] Enhancer sequences refer to the sequences that can increase gene expression. These sequences can be located upstream, within introns or downstream of the transcribed region. The transcribed region is comprised of the exons and the intervening introns, from the promoter to the transcription termination region. The enhancement of gene expression can be through various mechanisms which include, but are not limited to, increasing transcriptional efficiency, stabilization of mature mRNA and translational enhancement.

[0129] Recombinant DNA constructs of the present invention may also include other regulatory sequences, including but not limited to, translation leader sequences, introns, and polyadenylation recognition sequences. In another embodiment of the present invention, a recombinant DNA construct of the present invention further comprises an enhancer or silencer.

[0130] An “intron” is an intervening sequence in a gene that is transcribed into RNA and then excised in the process of generating the mature mRNA. The term is also used for the excised RNA sequences. An “exon” is a portion of the sequence of a gene that is transcribed and is found in the mature messenger RNA derived from the gene, and is not necessarily a part of the sequence that encodes the final gene product.

[0131] Many genes exhibit enhanced expression on inclusion of an intron in the transcribed region, especially when the intron is present within the first 1 kb of the transcription start site. The increase in gene expression by presence of an intron can be at both the mRNA (transcript abundance) and protein levels. The mechanism of this Intron Mediated Enhancement (IME) in plants is not very well known (Rose et al., *Plant Cell*, 20:543-551 (2008) Le-Hir et al., *Trends Biochem Sci* . . . 28:215-220 (2003), Buchman and Berg, *Mol. Cell Biol.* (1988) 8:4395-4405; Callis et al., *Genes Dev* 1 (1987): 1183-1200).

[0132] An “enhancing intron” is an intronic sequence present within the transcribed region of a gene which is capable of enhancing expression of the gene when compared to an intronless version of an otherwise identical gene. An enhancing intronic sequence might also be able to act as an enhancer when located outside the transcribed region of a gene, and can act as a regulator of gene expression independent of position or orientation (Chan et. al. (1999) *Proc.*

Natl. Acad. Sci. 96:4627-4632; Flodby et al. (2007) *Biochem. Biophys. Res. Commun.* 356:26-31).

[0133] Short consensus sequences or motifs can be identified from the intron sequences experimentally identified to be enhancing introns. These motifs can be used to scan and help identify more gene-expression enhancing introns. A motif capable of conferring transgene expression in male reproductive tissue in dicot plants has been described in US application No. US2007/020436.

[0134] An 8-bp sequence (SEQ ID NO: 99) and a 5-bp sequence (SEQ ID NO: 100) that can be used for identifying novel enhancing introns have been described in this application. Some variations of the 8-bp sequence can also be useful for identifying enhancing introns. The useful variations from the 8-bp motif (SEQ ID NO: 99) described herein can occur mainly at the first three positions. The last 5 bp of the sequence are highly conserved. Also, the variations from the 8-bp consensus (SEQ ID NO: 99) occur at maximum two out of 8 positions at any one time. In the event of more than 2 bp being different than the consensus, the enhancing intron might have additional copies of either the 5-bp (SEQ ID NO: 100) or the 8-bp motif (SEQ ID NO: 99).

[0135] The motif variations can be represented as a consensus motif sequence, Y[R/T]RATCYG (SEQ ID NO: 146). The first position can be any of the two pyrimidine bases, C or T. The second position can be substituted by an A, G or T. The third position can be a purine. The ATC core is the most highly conserved region, and does not exhibit any variability.

[0136] An intron sequence can be added to the 5' untranslated region, the protein-coding region or the 3' untranslated region to increase the amount of the mature message that accumulates in the cytosol.

[0137] The intron sequences can be operably linked to a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments.

[0138] Sequences orthologous to an intron are sequences that are present in orthologous genes at the same position as the intron in the original gene sequence.

[0139] The tissue expression patterns of the genes can be determined using the RNA profile database of the Massively Parallel Signature Sequencing (MPSS™). This proprietary database contains deep RNA profiles of more than 250 libraries and from a broad set of tissue types. The MPSS™ transcript profiling technology is a quantitative expression analysis that typically involves 1-2 million transcripts per cDNA library (Brenner S. et al., (2000). *Nat Biotechnol* 18:630-634, Brenner S. et al. (2000) *Proc Natl Acad Sci USA* 97:1665-1670). It produces a 17-base high quality usually gene-specific sequence tag usually captured from the 3'-most DpnII restriction site in the transcript for each expressed gene. The use of this MPSS data including statistical analyses, replications, etc, has been described previously (Guo M et al. (2008) *Plant Mol Biol* 66:551-563).

[0140] IMETER is a word-based discriminator that can do a computational analysis as to whether an intron can act as an enhancer of gene expression or not. The IMETER scoring system is described in Rose, A. B. (2004). *Plant J.* 40_744-751, and Rose et al (2008) *Plant Cell* 20:543-551.

[0141] “Transcription terminator”, “termination sequences”, or “terminator” as described herein refer to DNA sequences located downstream of a coding sequence,

including polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht, I. L., et al., *Plant Cell* 1:671-680 (1989). A polynucleotide sequence with "terminator activity" refers to a polynucleotide sequence that, when operably linked to the 3' end of a second polynucleotide sequence that is to be expressed, is capable of terminating transcription from the second polynucleotide sequence and facilitating efficient 3' end processing of the messenger RNA resulting in addition of poly A tail. Transcription termination is the process by which RNA synthesis by RNA polymerase is stopped and both the processed messenger RNA and the enzyme are released from the DNA template.

[0142] Improper termination of an RNA transcript can affect the stability of the RNA, and hence can affect protein expression. Variability of transgene expression is sometimes attributed to variability of termination efficiency (Bieri et al (2002) *Molecular Breeding* 10:107-117). As used herein, the terms "bidirectional transcriptional terminator" and "bidirectional terminator" refer to a transcription terminator sequence that has the capability of terminating transcription in both 5' to 3', and 3' to 5' orientations. A single sequence element that acts as a bidirectional transcriptional terminator can terminate transcription from two convergent genes.

[0143] The present invention encompasses functional fragments and variants of the terminator sequences disclosed herein.

[0144] A "functional fragment of a terminator" with substantially similar function to the full length terminator disclosed herein refers to a functional fragment that retains the ability to terminate transcription largely to the same level as the full length terminator sequence. A recombinant construct comprising a heterologous polynucleotide operably linked to a "functional fragment" of the terminator sequence disclosed herein exhibits levels of heterologous polynucleotide expression substantially similar to a recombinant construct comprising a heterologous polynucleotide operably linked to the full length terminator sequence.

[0145] A "variant terminator", as used herein, is the sequence of the terminator or the sequence of a functional fragment of a terminator containing changes in which one or more nucleotides of the original sequence is deleted, added, and/or substituted, while substantially maintaining terminator function. One or more base pairs can be inserted, deleted, or substituted internally to a terminator, without affecting its activity. Fragments and variants can be obtained via methods such as site-directed mutagenesis and synthetic construction.

[0146] These terminator functional fragments will comprise at least about 20 contiguous nucleotides, preferably at least about 50 contiguous nucleotides, more preferably at least about 75 contiguous nucleotides, even more preferably at least about 100 contiguous nucleotides of the particular terminator nucleotide sequence disclosed herein. Such fragments may be obtained by use of restriction enzymes to cleave the naturally occurring terminator nucleotide sequences disclosed herein; by synthesizing a nucleotide sequence from the naturally occurring terminator DNA sequence; or may be obtained through the use of PCR technology. See particularly, Mullis et al., *Methods Enzymol.* 155:335-350 (1987), and Higuchi, R. In PCR Technology:

Principles and Applications for DNA Amplifications; Erlich, H. A., Ed.; Stockton Press Inc.: New York, 1989. Again, variants of these terminator fragments, such as those resulting from site-directed mutagenesis, are encompassed by the compositions of the present invention.

[0147] The terms "substantially similar" and "corresponding substantially" as used herein refer to nucleic acid fragments, particularly regulatory sequences, wherein changes in one or more nucleotide bases do not substantially alter the ability of the regulatory sequence to perform the same function as the corresponding full length sequence disclosed herein. These terms also refer to modifications, including deletions and variants, of the nucleic acid sequences of the instant invention by way of deletion or insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting sequence relative to the initial, unmodified sequence. It is therefore understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences.

[0148] As will be evident to one of skill in the art, any heterologous polynucleotide of interest can be operably linked to the regulatory sequences described in the current invention. Examples of polynucleotides of interest that can be operably linked to the regulatory sequences described in this invention include, but are not limited to, polynucleotides comprising other regulatory elements such as introns, enhancers, promoters, translation leader sequences, protein coding regions such as disease and insect resistance genes, genes conferring nutritional value, genes conferring yield and heterosis increase, genes that confer male and/or female sterility, antifungal, antibacterial or antiviral genes, and the like. Likewise, the regulatory sequences described in the current invention can be used to regulate transcription of any nucleic acid that controls gene expression. Examples of nucleic acids that could be used to control gene expression include, but are not limited to, antisense oligonucleotides, suppression DNA constructs, or nucleic acids encoding transcription factors.

[0149] Embodiments of the invention are:

[0150] The present invention relates to regulatory sequences for modulating gene expression in plants. Recombinant DNA constructs comprising regulatory sequences are provided. Recombinant DNA constructs comprising intron sequences acting as enhancers of gene expression and endogenous promoter and terminator sequences corresponding to these intron sequences are provided.

[0151] Another embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the intron comprises a nucleotide sequence that has at least 95% sequence identity to SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138. In another embodiment, the intron comprises the nucleotide sequence of SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138.

[0152] One embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the promoter comprises a nucleotide sequence that has at least 95% sequence identity to SEQ ID NO: 105-117, 119, 136 or 139. In another embodiment, the promoter comprises the nucleotide sequence of SEQ ID NO: 105-117, 119, 136 or 139.

[0153] One embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the terminator comprises

a nucleotide sequence that has at least 95% sequence identity to SEQ ID NOS: 140, 141, 142 or 143. In another embodiment, the terminator comprises the nucleotide sequence of SEQ ID NO: 140, 141, 142 or 143.

[0154] One embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the intron comprises a nucleotide sequence that has at least 95% identity to SEQ ID NOS: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138; and the promoter comprises a nucleotide sequence that has at least 95% identity to SEQ ID NOS: 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 119, 136 or 139.

[0155] One embodiment of the invention is a recombinant DNA construct comprising an intron operably linked to a promoter and a terminator wherein the intron comprises a nucleotide sequence that has at least 95% identity to SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138; the promoter sequence has at least 95% identity to SEQ ID NO: 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 119, 136 or 139; and the terminator has at least 95% sequence identity to SEQ ID NO: 140, 141, 142 or 143.

[0156] In one embodiment of the current invention, the intron is operably linked to the promoter, and is present downstream of the promoter, in the recombinant DNA constructs described herein. One embodiment of the present invention includes a recombinant DNA construct comprising an intron described in the present invention, operably linked to a promoter and a heterologous polynucleotide, wherein the intron can act as enhancer of expression of the heterologous polynucleotide.

[0157] Another embodiment of the invention encompasses a recombinant DNA construct comprising an intron wherein the intron sequence comprises at least one copy of the 8-bp sequence motif of SEQ ID NO: 99; or contains at least one copy of the 8-bp sequence motif of SEQ ID NO: 99 and at least one copy of the 5-bp sequence motif of SEQ ID NO: 100, wherein the intron is capable of enhancing expression of a heterologous polynucleotide in a transgenic plant. The intron sequence can also comprise more than one copy of SEQ ID NO: 99, or can comprise one or more than one copy of SEQ ID NO: 99 and more than one copy of SEQ ID NO: 100.

[0158] Another embodiment of this invention is a method to identify novel introns that are useful for enhancing expression of a heterologous polynucleotide in a plant cell, the method comprising the steps of scanning a plurality of introns from plants for presence of SEQ ID NO: 99, selecting a sequence that contains at least one copy of SEQ ID NO: 99, measuring the efficacy of the identified intron to enhance expression of a heterologous polynucleotide in a plant.

[0159] Another embodiment of the invention is a method for identifying novel intronic sequences for enhancing transgene expression in monocotyledenous plants by identifying sequences orthologous to SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138; and measuring the enhancing effect of the identified intron on the expression of an operably linked heterologous polynucleotide.

[0160] Another embodiment of the current invention includes the promoter and the terminator sequences that are

endogenously linked to the introns identified using the methods described in the current invention.

[0161] Another embodiment of the current invention is a method for modulating expression of a heterologous polynucleotide in a monocotyledonous plant comprising the steps of: (a) introducing into a regenerable plant cell a recombinant DNA construct comprising a promoter and a heterologous polynucleotide wherein each is operably linked to an intron, wherein the intron comprises either (i) a nucleotide sequence that is orthologous to SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138; or (ii) a nucleotide sequence that contains least one copy of a sequence motif identical to SEQ ID NO: 99; and; (b) regenerating a transgenic plant from a regenerable monocotyledonous plant cell after step (a) wherein the transgenic plant comprises the recombinant DNA construct; and (c) obtaining a progeny plant derived from the transgenic plant of step (b), wherein said progeny plant comprises the recombinant DNA construct and exhibits enhanced expression of the heterologous polynucleotide when compared to a plant comprising a corresponding recombinant DNA construct without the intron sequence.

[0162] In another embodiment, this invention concerns a vector, cell, plant, or seed comprising a recombinant DNA construct comprising the regulatory sequences described in the present invention.

[0163] The invention encompasses regenerated, mature and fertile transgenic plants comprising the recombinant DNA constructs described above, transgenic seeds produced therefrom, T1 and subsequent generations. The transgenic plant cells, tissues, plants, and seeds may comprise at least one recombinant DNA construct of interest.

[0164] In one embodiment, the plant comprising the regulatory sequences described in the present invention is a monocotyledenous plant. In another embodiment, the plant comprising the regulatory sequences described in the present invention is a maize plant.

EXAMPLES

[0165] The present invention is further illustrated in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these examples, while indicating embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Furthermore, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Example 1

Identification of Candidate Gene Expression/Transcript-Enhancing First Introns

[0166] Introns that may enhance transcript abundance were sought from among a set of maize genes which (a) had first introns near the N-terminus of the transcript, and (b) had high level transcript abundance. A subset of maize genes

were identified whose models were deemed to be complete. This assessment was done using a combination of maize public B73 BAC sequences plus a proprietary EST transcript assembly in an analysis comparing the predicted gene structures and the predicted transcript open reading frames (ORFs) in relation to public reference proteins plus some manual curations. Only full-length transcripts were considered; that is, those with complete protein coding regions. This set did not represent all maize genes, and there was some redundancy in the list.

[0167] This set of gene models was then analyzed versus a body of over 250 MPSS mRNA transcript profiling samples produced from a variety of maize tissues and treatments. The MPSS profiling technology produces a 17-bp tag sequence beginning with GATC. These tags were matched to the gene set via the full-length transcript, and those genes which (a) had an MPSS tag matching the plus strand of the transcript, and (b) had a measured expression level of at least 1000 ppm (parts per million) in at least one of the MPSS samples, were retained. In this way a working set of 3131 genes was produced. Using the maize BAC genomic sequence to analyze these 3131 genes, a subset of genes was produced that (a) contained an intron, and (b) contained an intron which was located within the 5'UTR or within the first 300 nucleotides of the ORF. This resulted in a subset of 1185 genes for further consideration.

[0168] This set of 1185 candidate genes was then filtered down by a number of criteria. Duplicates were removed. Introns without canonical GT-AG rules were excluded. Genes whose expression was defined by 'promiscuous' MPSS tags, such as GATCAAAAAAA (SEQ ID NO: 145), and also MPSS tags matching repetitive elements, were removed. Genes whose first introns were greater than 2 kb were dropped. In addition, genes whose first introns' GC content were higher than 50% GC and/or the intron T (=U) content was below 25% were removed. In addition, the IMeter score for the first intron had to be positive. The IMeter scoring system is described in Rose, A. B. (2004) *Plant J.* 40:744-751. This resulted in an interim set of remaining 331 candidates. This set was then further manually winnowed down to 86 by positively considering a combinations of factors but chiefly: (a) the breadth of diverse tissue expression and (b) the ratio of the IMeter score to intron length.

[0169] This set of 86 introns was one prioritized pool from which introns were drawn for functional testing of whether they enhance transcript abundance. Seventeen of these 86 were tested.

Example 2

Creation of an Intron Testing Vector with Maize Ubiquitin Promoter

[0170] Maize ubi promoter (SEQ ID NO: 1) along with its intron (SEQ ID NO: 2) in the 5' UTR confers high level constitutive expression in monocot plants (Christensen, A. H., Sharrock, R. A. and Quail, P. H., *Plant Mol. Biol.* 18, 675-89, 1992). This high-level expression is dependent on the first intron in the 5' UTR. Removal of this intron results in a >4-fold reduction in expression measured by transient assays (FIG. 3). We created a plant transformation vector where the maize ubiquitin promoter together with its endogenous intron drives *E. coli* β-glucuronidase (GUS) reporter gene expression. We then replaced the maize ubiquitin intron with two restriction sites, AsiS1 and Acc651 to allow

the insertion of novel introns and test their ability to enhance reporter gene expression driven by the ubiquitin promoter (SEQ ID NO: 1) (FIG. 1).

Example 3

Intron Amplification and Cloning

[0171] *Zea mays* B73 seeds were germinated in Petri plates and genomic DNA was made from seedling leaf tissue using the QIAGEN® DNEASY® Plant Maxi Kit (QIA-GEN® Inc.) according to the manufacturer's instructions. DNA products were amplified with primers shown in Table 2 using genomic DNA as template with PHUSION™ DNA polymerase (New England Biolabs Inc.). The resulting DNA fragments were cloned into the intron testing vector ITVUR-2 (SEQ ID NO: 3), using standard molecular biology techniques (Sambrook et al.) or using INFUSION™ from (Clontech Inc.), and sequenced completely.

TABLE 2

Name	Intron SEQ ID NO	Length (nt)	Forward Primer	Reverse Primer
			(SEQ ID NO)	(SEQ ID NO)
TS1	4	814	20	21
TS4	5	727	22	23
TS5	6	834	24	25
TS6	7	982	26	27
TS7v	137	856	28	29
TS8	9	1020	30	31
TS10	10	841	32	33
TS11	11	1044	34	35
TS12	12	648	36	37
TS13	13	632	38	39
TS14	14	1405	40	41
TS15	15	1361	42	43
TS16	16	703	44	45
TS17	17	1341	46	47
TS24	18	1125	48	49
TS27v	138	884	50	51

[0172] All the constructs were mobilized into the *Agrobacterium* strain LBA4404/pSB1 and selected on Spectinomycin and tetracycline. *Agrobacterium* transformants were isolated and the integrity of the plasmid was confirmed by retransforming to *E. coli* or PCR analysis.

Example 4

Transient Transformation and Expression of Intron Constructs in Maize Embryos Infected with *Agrobacterium*

Preparation of *Agrobacterium* Suspension:

[0173] *Agrobacterium* was streaked out from -80° C. frozen aliquot onto a plate containing PHI-L medium and was cultured at 28° C. in the dark for 2 days. The PHI-L medium comprises 50 ml Stock Solution A, 50 ml/L stock Solution B, 900 ml Stock Solution C and spectinomycin (Sigma chemicals) was added to a concentration of 50 mg/L in sterile ddH2O (Stock Solution A: K2HPO4 60 g/l, NaH2PO4 20 g/l, pH adjusted to 7.0 w/KOH and autoclaved; stock solution B: NH4Cl 20 g/l, MgSO4·7H2O 6 g/l, KCl 3 g/l, CaCl2 0.2 g/l, FeSO4·7H2O 50 mg/l; stock solution C: glucose 5 g/l, agar 15 g/l (#A-7049, Sigma Chemicals, St. Louis, Mo.) and was autoclaved.

[0174] The plate can be stored at 4° C. and used usually for about 1 month. A single colony was picked from the

master plate and was streaked onto a plate containing PHI-M medium [Yeast Extract 5 g/l (Difco); Peptone 10 g/l (Difco); NaCl 5 g/l (Hi-Media); agar (Sigma Chemicals) 15 g/l; pH 6.8, containing 50 mg/l spectinomycin] and incubated at 28° C. in the dark for overnight.

[0175] Five ml of PHI-A, [CHU (N6) Basal salts (Sigma C-1416) 4 g/l; Erikson's vitamin solution (1000 \times , Sigma-1511) 1 ml/l; Thiamine-HCl (Sigma) 0.5 mg/l; 2,4-Dichlorophenoxyacetic acid (2,4-D, Sigma) 1.5 mg/l; L-Proline (Sigma) 0.69 g/l; Sucrose (Sigma) 68.5 g/l; Glucose (Sigma) 36 g/l; pH adjusted to 5.2 with KOH] was added to a 14 ml FALCON™ tube in a hood. About 3 full loops (5 mm loop size) *Agrobacterium* was collected from the plate and suspended in the tube, then the tube vortexed to make an even suspension. One ml of the suspension was transferred to a spectrophotometer tube and the OD of the suspension was adjusted to 0.72 at 550 nm by adding either more *Agrobacterium* or more of the same suspension medium, for an *Agrobacterium* concentration of approximately 0.5×10^9 cfu/ml. The final *Agrobacterium* suspension was aliquoted into 2 ml microcentrifuge tubes, each containing 1 ml of the suspension. The suspension was then used as soon as possible.

Embryo Isolation, Infection and Co-Cultivation:

[0176] About 2 ml of the same medium (PHI-A) which is used for the *Agrobacterium* suspension was added into a 2 ml microcentrifuge tube. Immature embryos were isolated from a sterilized ear with a sterile spatula and dropped directly into the medium in the tube. A total of 25 embryos are placed in the tube. The optimal size of the embryos was about 1.7-2.0 mm. The entire medium was drawn off and 1 ml of *Agrobacterium* suspension was added to the embryos and the tube was vortexed for 30 sec. The tube was allowed to stand for 5 min in the hood. The suspension of *Agrobacterium* and embryos was poured into a Petri plate containing co-cultivation medium PHI-B [CHU (N6) Basal salts (Sigma C-1416) 4 g/l; Eriksson's vitamin solution (1000 \times , Sigma-1511) 1 ml/l; Thiamine-HCl 0.5 mg/l; 2,4-D 1.5 mg/l; L-Proline 0.69 g/l; GELRITE® (Sigma) 3 g/l; Sucrose 30 g/l; pH adjusted to 5.8 with KOH; Post sterilization, Silver nitrate (0.85 mg/l) and acetosyringone (100 mM) were added after cooling the medium to 45° C.]. Any embryos left in the tube were transferred to the plate using a sterile spatula. The *Agrobacterium* suspension was drawn off and the embryos placed axis side down on the media. The plate was sealed with PARAFILM® and was incubated in the dark at 23-25° C. for about 3 days of co-cultivation.

Resting of Co-Cultivated Embryos:

[0177] For the resting step, all the embryos were transferred to a new plate containing PHI-C medium [CHU (N6) Basal salts (Sigma C-1416) 4 g/l; Eriksson's vitamin solution (1000 \times , Sigma-1511) 1 ml/l; Thiamine-HCl 0.5 mg/l; 2,4-D 2.0 mg/l; L-Proline 0.69 g/l; Casein hydrolysate (Sigma) 300 mg/l; Sucrose (Sigma) 30 g/l; GELRITE® (Sigma) 4 g/l; pH adjusted to 5.8 with KOH]. Coleoptile of the rice calli was removed and calli were spliced to the size of approximately 2 to 3 mm. Spliced calli were transferred to the FALCON™ tubes containing *Agrobacterium* cultures and infected for 15 minutes with gentle intermittent shaking. The liquid *Agrobacterium* culture was decanted and the wet calli were taken out and blotted on sterile WHATMAN® filter paper No 4. Subsequently, the calli were transferred onto co-cultivation medium, PHI-R supplemented with Acetosyringone (Sigma) at 100 μ M. The infected calli were co-cultivated in dark at 21° C. for 72 hours.

Histochemical and Fluorometric GUS Analysis:

[0178] Transformed embryos were taken for expression analysis after 3 days of resting. Ten embryos for each

construct were used for histochemical GUS staining using standard protocols (Janssen and Gardner, *Plant Mol. Biol.* (1989) 14:61-72,) and two pools of 5 each were used to do quantitative assays using MUG substrate using standard protocols [Jefferson, R. A., *Nature*. 342:837-838 (1989); Jefferson, R. A., Kavanagh, T. A. & Bevan, M. W. *EMBO J.* 6:3901-3907 (1987)] (FIG. 3). Introns TS1 (SEQ ID NO: 4), TS7v (SEQ ID NO: 137), TS13 (SEQ ID NO: 13) and TS27v (SEQ ID NO: 138) all enhanced the GUS reporter gene expression between 3 to 5 fold when compared to the ubiquitin promoter alone without any intron. The level of enhancement is comparable to that of the maize ubiquitin first intron. Introns TS4, TS5, TS6, TS8, TS10, TS11, TS12, TS14, TS15, TS16, TS17 and TS24 did not enhance expression (Data shown for TS5, TS6, TS10 and TS14 in FIG. 3).

Example 5

Transient Transformation and Expression of Intron Constructs in Rice Calli via *Agrobacterium*

Preparation of *Agrobacterium* Suspension:

[0179] *Agrobacterium* was streaked out from -80° C. frozen aliquot onto a plate containing YEB medium and was cultured at 28° C. in the dark for 2 days. The YEB medium comprises (MgSO4 (Hi-Media) 0.2 g/l; K2HPO4 (Fisher Scientific) 0.5 g/l; Mannitol 10 g/l; NaCl 0.1 g/l; Yeast Extract 0.4 g/l; Agar 15 g/l). *Agrobacterium* cultures harboring the intron constructs were cultured one day prior to rice calli infection in YEB broth. A large swipe of *Agrobacterium* growth was inoculated into 7.5 ml of YEB broth in FALCON™ tubes. Then in the next morning OD of each culture was measured at 550 nm. Cultures were centrifuged at 4000 rpm for 10 minutes. Supernatant was discarded and the pellet was resuspended in PHI-L supplemented with Acetosyringone at 100 μ M. Another spin was given to *Agrobacterium* cultures at 4000 rpm for 10 min and the pellets were resuspended in PHI-L supplemented with Acetosyringone at 100UM and the OD was adjusted to 1.0 by adding either more *Agrobacterium* or more of the same suspension medium, for an *Agrobacterium* concentration of approximately 0.5×10^9 cfu/ml.

Rice Callus Induction, Infection and Co-Cultivation:

[0180] 15 to 21 days old Rice calli which were grown on callus induction medium, PHI-R [CHU (N6) Basal salts (Sigma C-1416) 4 g/l; Eriksson's vitamin solution (1000 \times , Sigma-1511) 1 ml/l; Thiamine-HCl 0.5 mg/l; 2,4-D 2.0 mg/l; L-Proline 0.69 g/l; Casein hydrolysate (Sigma) 300 mg/l; Sucrose (Sigma) 30 g/l; GELRITE® (Sigma) 4 g/l; pH adjusted to 5.8 with KOH]. Coleoptile of the rice calli was removed and calli were spliced to the size of approximately 2 to 3 mm. Spliced calli were transferred to the FALCON™ tubes containing *Agrobacterium* cultures and infected for 15 minutes with gentle intermittent shaking. The liquid *Agrobacterium* culture was decanted and the wet calli were taken out and blotted on sterile WHATMAN® filter paper No 4. Subsequently, the calli were transferred onto co-cultivation medium, PHI-R supplemented with Acetosyringone (Sigma) at 100 μ M. The infected calli were co-cultivated in dark at 21° C. for 72 hours.

Resting of Co-Cultivated Rice Calli:

[0181] The co-cultivation was terminated by washing in sterile water containing carbenicillin (Sigma, 400 mg/l). Calli were washed with gentle intermittent shaking in the antibiotic solution for 15 minutes. The wet calli were blotted on WHATMAN® filter paper No 4. The dried calli were transferred to resting/callusing medium, PHI-R in which carbenicillin (400 mg/l) was added after cooling the medium to 45° C. after sterilization. The plates were sealed with PARAFILM® and incubated in the dark at 28° C. for 3-5 days.

Histochemical and Fluorometric GUS Analysis:

[0182] After 3 days, calli were taken for expression analysis. For each construct 20 calli were infected and 8 calli were used for histochemical GUS staining using X-Gluc solution and another eight calli were taken for GUS quantitation using standard protocol (Jefferson et al., EMBO J. 6:3901-3907, 1987). TS7v (SEQ ID NO: 137) and TS27v (SEQ ID NO: 138) were able to enhance GUS reporter expression from the maize ubiquitin promoter (SEQ ID NO: 1) (FIG. 4).

Example 6

Description of Constitutive Promoter Selection Via MPSS Samples

[0183] Promoter candidates were identified using a set of 241 proprietary expression profiling experiments run on the MPSS (Massively Parallel Signature Sequencing) technology platform provided by Lynx Therapeutics. The 241 samples from corn consisted of various tissue samples spanning most of the range of corn tissues and developmental stages. Each experiment resulted in approximately 20,000 unique sequence tags of 17 bp length from a single tissue sample. Typically these tags could be matched to one or a few transcript sequences from the proprietary “Unicorn” EST assembly set. A query of the MPSS database was performed looking for tags that were observed in 240 or more of the 241 samples. We identified 111 tags that met the criteria and chose 22 that were observed at an expression level of 1 or greater PPM (Parts Per Million tags) in all 241 experiments for further development. 21 of these 22 tags mapped to a single gene based on the transcript set. We took the top 6 candidates from this list and identified the 1500 bp of promoter regions and the first intron, defined as the first intron in the transcript from the 5' end, (i1 (SEQ ID NO: 52), i2 (SEQ ID NO: 53), i3 (SEQ ID NO: 54), i5 (SEQ ID NO: 56), i6 (SEQ ID NO: 57) and i7 (SEQ ID NO: 58). In addition we also included one second intron (i4; SEQ ID NO: 55) to the list. All introns were evaluated for intron-mediated enhancement of expression from CYMV promoter.

Example 7

Enhancement Activity of Introns in Transient Expression System

[0184] To determine whether the experimental introns function to enhance promoter activity in plant tissue, transient infiltration assays using the maize suspension cell line, BMS (Black Mexican Sweet), were performed. These *Agro-*

bacterium-mediated assays, known in the art, provide a rapid screening method to evaluate the enhancement capability of the introns.

[0185] The introns were cloned into an expression vector downstream of the Citrus Yellow Mosaic virus promoter and upstream of the coding region of an insecticidal gene described in US2007/0202089 A1. The insecticidal gene acted as a reporter for expression. A vector with no intron between the promoter and coding region was included to provide a baseline control for expression. A vector (SEQ ID NO: 59; PHP38808) with the Adh1 intron1 was also included to provide a comparison for the level of increased expression by each experimental intron. The Adh1 intron has been shown to enhance the expression of foreign genes in plant tissue (Callis et al. (1987) *Genes and Development*: 1183-1200; Kyozuka et al. (1990) *Maydica* 35:353-357). Each expression vector also contained an expression cassette for phosphinothricin acetyl transferase (PAT).

[0186] Transiently transformed BMS cells were evaluated for expression by both northern blot analysis for RNA accumulation and ELISA analysis for protein accumulation. If the experimental introns, particularly introns i1 (SEQ ID NO: 52), i2 (SEQ ID NO: 53), i5 (SEQ ID NO: 56), i6 (SEQ ID NO: 57), and i7 (SEQ ID NO: 58), exhibited intron mediated enhancement of expression, the increased expression would be reflected at both the RNA and protein levels.

[0187] The ratio of expression for each intron cassette showed that introns i1, i2, i5, i6, and i7 had expression levels that were between 2.3 and 4.8 fold higher than the intronless control (Table 3). These increased expression levels were comparable to the control cassette (SEQ ID NO: 59, PHP38808; FIG. 5) containing the Adh1 intron. The ELISA values were standardized for differences in transformation efficiency between vectors by normalizing against PAT gene expression.

TABLE 3

ELISA Results Indicating Expression Levels of Insecticidal Gene (IG) and PAT in Constructs Containing Experimental Introns				
Intron	IG (ppm)	PAT (ppm)	IG/PAT	Fold difference from no intron
none	38.8	179.0	0.22	N/A
ADH1	104.3	117.4	0.89	4.05
i1	98.3	136.5	0.72	3.27
i2	118.7	154.0	0.77	3.50
i5	115.5	108.5	1.06	4.82
i6	107.6	209.0	0.51	2.32
i7	104.3	117.4	0.89	4.05

[0188] To determine whether introns i1 (SEQ ID NO: 52), i2 (SEQ ID NO: 53), i5 (SEQ ID NO: 56), i6 (SEQ ID NO: 57), and i7 (SEQ ID NO: 58) resulted in increased mRNA levels, northern blot analysis was performed. RNA amounts for each vector were normalized against PAT expression prior to electrophoresis. The results of the analysis mirrored the ELISA results. Introns i1, i2, i5, i6, and i7 facilitated levels of reporter mRNA accumulation that were above that of the intronless cassette and comparable to the ADH1 cassette (see FIG. 6). These results show that i1, i2, i5, i6, and i7 (SEQ ID NOS: 52-53, 56-58 respectively) display intron-mediated enhancement of expression in this system.

Materials and Methods

[0189] Introns i1 (SEQ ID NO: 52), i2 (SEQ ID NO: 53), i3 (SEQ ID NO: 54), i4 (SEQ ID NO: 55) and i5 (SEQ ID NO: 56) were generated using a method known in the art as oligonucleotide stacking. Oligos and primers (Table 4) synthesized by IDT

[0190] (Integrated DNA Technologies, Inc. Coralville, IA) were resuspended in distilled water to a concentration of 100 μ M. Equal amounts of each oligonucleotide were mixed to create a total volume of 10 μ l. The flanking primers for PCR amplification were also mixed equally to a volume of 10 μ l. Two microliters of the oligonucleotide mix and 10 μ l of the primer mix were combined for PCR using the HotStart Herculase system from Stratagene. PCR was performed using 10 μ l Herculase buffer, 2 μ l of 25 nM dNTPs, 1.2 μ l of the oligo and primer mixture, 1 μ l 100 mM MgSO₄, 2 μ l DMSO, 1 μ l HotStart Herculase enzyme, and 82.8 μ l of distilled water. PCR conditions were 96° C. for 3 minutes, then 35 cycles at 94° C. for 30 s, 60° C. for 30 s, and 72° C. for 1 min., followed by 72° C. for 10 min. Reactions were stored at 4° C. Introns i6 and i7 were synthesized by GENEART, Inc., Burlingame, CA. To clone introns i1 (SEQ ID NO: 52), i2 (SEQ ID NO: 53), i6 (SEQ ID NO: 57), and i7 (SEQ ID NO: 58), the starting product was cut with the restriction enzymes EcoRV (5' end) and BamHI (3' end). Intron i5 (SEQ ID NO: 56), was cut with EcoRV (5' end) and BgIII (3' end). A plasmid containing a cassette (SEQ ID NO: 59, PHP38808; FIG. 5) with the CYMV promoter the ADH1 intron and an insecticidal gene flanked by GATEWAY® (INVITROGEN™) attL recombination sites was cut with EcoRV and BamHI to remove the ADH1 intron and allow the experimental introns to be ligated into the cut plasmid. The resulting vectors (entry vectors, PHP38811, PHP38813, PHP38815, PHP38817, PHP38819, PHP38821, PHP38823 for i1, i2, i3, i4, i5, i6, i7 respectively) were used in LR reactions with a larger plasmid (PHP34651, FIG. 7, SEQ ID NO: 60) containing GATEWAY® attR recombination sites and a PAT expression cassette to generate the final expression vectors (destination vectors PHP38812, PHP38814, PHP38816, PHP38818, PHP38820, PHP38822 and PHP38824 respectively for introns i1, i2, i3, i4, i5, i6, i7, i8 and i9). These vectors were used to transform competent *Agrobacterium tumefaciens* cells, which were then used to transiently transform BMS cells.

TABLE 4

Primers and Oligonucleotides Used for Oligonucleotide Stacking			
Oligo/Primer SEQ ID NO:	(Used for) Intron	Sense/ Antisense	Flanking Primer/Oligonucleotide
61	i1	Sense	Flanking Primer
62	i1	Sense	Oligonucleotide
63	i1	Sense	Oligonucleotide
64	i1	Sense	Oligonucleotide
65	i1	Antisense	Oligonucleotide
66	i1	Antisense	Oligonucleotide
67	i1	Antisense	Oligonucleotide
68	i1	Antisense	Flanking Primer
69	i2	Sense	Flanking Primer
70	i2	Sense	Oligonucleotide
71	i2	Sense	Oligonucleotide
72	i2	Sense	Oligonucleotide
73	i2	Antisense	Oligonucleotide
74	i2	Antisense	Oligonucleotide
75	i2	Antisense	Oligonucleotide

TABLE 4-continued

Primers and Oligonucleotides Used for Oligonucleotide Stacking			
Oligo/Primer SEQ ID NO:	(Used for) Intron	Sense/ Antisense	Flanking Primer/Oligonucleotide
76	i2	Antisense	Flanking Primer
77	i3	Sense	Flanking Primer
78	i3	Sense	Oligonucleotide
79	i3	Sense	Oligonucleotide
80	i3	Antisense	Oligonucleotide
81	i3	Antisense	Oligonucleotide
82	i3	Antisense	Flanking Primer
83	i4	Sense	Flanking Primer
84	i4	Sense	Oligonucleotide
85	i4	Sense	Oligonucleotide
86	i4	Antisense	Oligonucleotide
87	i4	Antisense	Oligonucleotide
88	i4	Antisense	Flanking Primer
89	i5	Sense	Flanking Primer
90	i5	Sense	Oligonucleotide
91	i5	Sense	Oligonucleotide
92	i5	Antisense	Oligonucleotide
93	i5	Antisense	Oligonucleotide
94	i5	Antisense	Flanking Primer

[0191] RNA was extracted from infiltrated tissue culture material using the QIAGEN® RNA Maxiprep kit. Based on ELISA data for PAT, RNA samples were loaded on an agarose gel (1% Lonza SeaKem LE agarose) to contain equal parts per million of PAT to normalize for variations in transformation efficiency. After electrophoresis, samples on the gel were transferred to a nylon membrane via capillary transfer overnight using the WHATMAN® TurboBlotter system standard protocol. RNA was crosslinked to the membrane by UV light. Prehybridization and hybridization steps were performed following the manufacturer's protocol for Roche DIG Easy Hyb solution (catalog #11603558001). The blot was prehybridized at 50° C. in Roche DIG Easy Hyb solution, then was probed overnight at 50° C. with a mixture of digoxigenin-labeled DNA probes for the insecticidal and PAT gene in Roche DIG Easy Hyb solution. Probes were generated using Roche PCR DIG Probe Synthesis Kit (Roche catalog #11636090910). The blot was washed twice for five minutes each at room temperature in low stringency buffer (2 \times SSC+0.1% SDS), then washed twice for 15 minutes each at 50° C. in high stringency buffer (0.1 \times SSC+0.1% SDS).

[0192] For detection, the Roche DIG Wash and Block Buffer Set (catalog #11585762001) was used. The membrane was washed for 2 minutes at room temperature in wash buffer, and then blocked in block solution for 30 minutes at room temperature. A 1:10,000 dilution of anti-digoxigenin-AP antibody (Roche catalog #11093274910, 0.75 U/ μ l) in 50 ml block solution was added to the blot for 30 minutes. The blot was washed twice for 15 minutes each at room temperature in wash buffer, and then equilibrated in 50 ml of detection buffer for 3 minutes. Blot was incubated at room temperature for 5 minutes with 3 ml of CSPD (Roche catalog #1755633001), and then incubated at 37° C. for 10 minutes. Detection was done with film at 37° C.

Example 8

Identification of Unique Motif from Maize First Introns Using the Experimental Dataset of Tested Enhancing Introns

[0193] Computational analysis was performed to identify unique motifs that were present in the 9 enhancing introns

identified as explained in Examples 4 and 7 and Table 1 (TS1, TS7, TS13, TS27, 11, 12, i5, 16, 17 (SEQ ID NOS: 4, 8, 13, 19, 52, 53, 56, 57, and 58 respectively)). The proprietary promoter REAPER tool was adapted to look for possibly conserved motifs. The promoter REAPER tool is a regulatory element identification tool that relies on the conserved word approach. It is described in the U.S. patent application Ser. No. 12/534,471. The introns were searched in both directions using sets of 3-6 introns at a time. When candidates were found, they were used to search all the introns.

[0194] The introns were divided into the following categories. "All Enhancing Introns" are the 9 introns (new enhancing introns) described in Table 1 and experimentally shown to be enhancing gene expression (TS1, TS7, TS13, TS27, i1, 12, i5, i6, and i7 (SEQ ID NOS: 4, 8, 13, 19, 52, 53, 56, 57, and 58 respectively), plus four known enhancing introns (Adh1_intron1 (SEQ ID NO: 95), Adh1_intron 6 (SEQ ID NO: 96), Sh-1_intron 1 (SEQ ID NO: 97) and Ubi1ZM_intron (SEQ ID NO: 98) Callis, J. et al (1987) *Genes Dev.* 1:1183-1200, Vasil, V. et al (1989) *Plant Physiol.* 91: 1575-1579, Christensen, A. H. et al (1992) *Plant Mol. Biol.* 18:675-689, Jeong, Y.-M. et al (2009) *Plant Sci.* 176:58-65). The 10 "non-enhancing introns" are 10 introns found not to enhance gene expression in transient maize assays as explained in Examples 4 and 7 and Table 1 (SEQ ID NOS: 5-7, 9, 11, 12, 17, 18, 54, and 55).

[0195] The 8-bp sequence CAGATCTG (SEQ ID NO: 99) or its variations were found in all the enhancing introns except TS27. The exact 8-bp sequence CAGATCTG was found in 2 out of the 9 enhancing introns identified (SEQ ID NOS: 52 and 53), but was not found in any of the 10

non-enhancing introns (SEQ ID NOS: 5-79, 11, 12, 17, 18, 54, and 55). A subset of this sequence ATCTG (SEQ ID NO: 100) was also present in 8 out of 9 enhancing introns (SEQ ID NOS: 4, 8, 13, 52, 53, 56, 57 and 58), and was also found to be present in the four known enhancing introns (SEQ ID NOS: 95-98). The frequency of occurrence of these motifs was normalized to the intron length (Table 6).

[0196] The variations of the 8-bp sequence CAGATCTG are mainly in the first 3 base pairs. The motif variations can be represented as the consensus sequence, Y[R/T]RATCYG (SEQ ID NO: 146). The first position can be any of the two pyrimidine bases, C or T. The second position can be substituted by an A, G or T and the third position can any purine. The last 5 base pairs of the sequence, that is the sequence ATCTG is highly conserved.

Statistical Analyses of Motif Frequencies:

[0197] A number of simple frequency statistics were determined for the introns. The statistics are shown in Tables 5 and 6.

TABLE 5

Intron Classification	Intron Count	Aggregate Nts	Average Intron Length
All Enhancing Introns	13	7716	594
New Enhancing Introns	9	4813	535
Other Enhancing Introns	4	2903	726
Non-Enhancing Introns	10	7888	789
Non-Tested Introns	1066	933097	875

TABLE 6

Intron Classification	Total Introns Containing CAGATCTG	Total Introns Containing ATCTG	Frequency Intron Contains CAGATCTG	Frequency Intron Contains ATCTG
All Enhancing Introns	2	12	0.15	0.92
New Enhancing Introns	2	8	0.22	0.89
Other Enhancing Introns	0	4	0.00	1.00
Non-Enhancing Introns	0	7	0.00	0.70
Non-Tested Introns	15	502	0.01	0.47
Ratio All Enhancing/Non-Enhancing		1.71		1.32
Ratio New Enhancing/Non-Enhancing		1.14		1.27

Intron Classification	Total Occurrences CAGATCTG Either Strand	Total Occurrences ATCTG Either Strand	Gross Frequency CAGATCTG	Gross Frequency ATCTG
All Enhancing Introns	6	29	0.0008	0.0038
New Enhancing Introns	6	23	0.0012	0.0048

TABLE 6 -continued

Other Enhancing Introns	0	6	0	0.00207
Non-Enhancing Introns	0	18	0	0.00228
Non-Tested Introns	15	1391	1.6075E-05	0.00149
Ratio All Enhancing/Non-Enhancing		1.61		1.647
Ratio New Enhancing/Non-Enhancing		1.28		2.094
Intron Classification	Average Individual Frequency of CAGATCTG/ kb	Average of Individual Intron Frequency of ATCTG/kb	SE Frequency CAGATCTG/ kb	SE Frequency ATCTG/kb
All Enhancing Introns	0.0036	0.0094	0.0025	0.0004
New Enhancing Introns	0.0052	0.0124	0.0035	0.0050
Other Enhancing Introns	0.00000	0.00266	0.00000	0.00107
Non-Enhancing Introns	0.00000	0.00203	0.00000	0.00057
Non-Tested Introns	0.00013	0.00271	0.00005	0.00013
Ratio All Enhancing/Non-Enhancing		4.62		
Ratio New Enhancing/Non-Enhancing		6.10		

[0198] SE frequency is standard error of frequency. Gross frequency is simply the total occurrences divided by the aggregate nucleotides of all the introns in the set.

[0199] The ‘all’ 13 enhancing introns have 4.6-fold higher, and the 9 ‘new’ enhancing introns have 6.1-fold higher frequencies of ATCTG relative to the non-enhancing introns on a mean frequency per kb of intron basis (See Tables 5 and 6 above).

Example 9

Identification of Novel Maize Introns with 8-bp Motif

[0200] From the initial set of 1085 introns explained in Example 1, 1066 introns that were still not tested experimentally were scanned computationally to identify the ones with the 8-bp motif. Four introns (SEQ ID NOS: 101-104) were found to contain the exact 8-bp motif and these are good candidates for being enhancing introns.

Example 10

Identifying Promoters of Expression-Enhancing Introns

[0201] It is likely that the expression enhancing introns from Examples 4, 7 and 9 perform optimally along with their endogenous promoters. To test this 1000 bp-2000 bp of promoter regions upstream of the start codon from the

respective genes (SEQ ID NOS: 105-117, SEQ ID NOS: 136 and 139) were identified and these can be tested with the respective introns.

Cloning Endogenous Promoters of Expression Enhancing Introns

[0202] We amplified 1000 base pairs region of endogenous promoter, (using the primers given in Table 7) upstream of the start codon of the gene that carries TS1 intron as its first intron and cloned the pTS1v sequence (SEQ ID NO: 136) in ITVUR-2 vector (SEQ ID NO: 3, PHP41353) between Ascl-AsiS1 restriction sites, followed by the TS1 intron (SEQ ID NO: 4) at AsiSI-Acc65I sites to create an endogenous promoter and intron combination (PHP50061). Similarly, we amplified a 1487 base pair region of endogenous promoter (pTS27v; SEQ ID NO: 139) upstream of the TS27 intron and cloned it in ITVUR-2 vector (SEQ ID NO: 3, PHP41353) at Ascl-AsiS1 restriction sites, followed by the TS27v intron (SEQ ID NO: 138) at AsiSI-Acc65I sites to give us an endogenous promoter and intron combination (PHP52322).

Example 11

Cloning and Testing of TS2 Enhancing Intron and Corresponding Endogenous Promoter

[0203] We tested another intron with potential gene expression enhancing properties. TS2 intron (SEQ ID NO:

118) was cloned into ITVUR-2 vector (SEQ ID NO: 3, PHP41353) using the same procedure as explained in Example 3 to create PHP50062. We created 2 more constructs to test the ability of the endogenous promoter upstream of the start codon of the gene that carries TS2 as its first intron to drive gene expression and ability of TS2 intron to enhance gene expression. We amplified 1077-bp of endogenous TS2 promoter (pTS2; SEQ ID NO: 119), as defined by the sequence upstream of the TS2 intron at the genomic location, and cloned that in ITVUR-2 vector (SEQ ID NO: 3) between Ascl and Ncol sites (PHP50063). We also amplified the pTS2 promoter and TS2 intron sequence from the endogenous locus (1077 bp promoter (SEQ ID NO: 118)+1329 bp intron (SEQ ID NO: 119)) and cloned that between Ascl and Ncol sites (PHP50111). The primers for these amplifying promoter and intron sequences to make these constructs are given in Table 2 and Table 7.

TABLE 7

Cloned sequence	For-ward Primer		Reverse Primer
	Intron	(SEQ ID NO)	(SEQ ID NO)
Promoter			
-	TS2 (SEQ ID NO: 118)	120	121
pTS2 (SEQ ID NO: 119)	TS2 (SEQ ID NO: 118)	122	123
pTS2 (SEQ ID NO: 119)	-	122	124
pTS1v (SEQ ID NO: 136)	-	125	126
pTS27v (SEQ ID NO: 139)	-	127	128

[0204] All the constructs were mobilized into the *Agrobacterium* strain LBA4404/pSB1 and selected on spectinomycin and tetracycline. *Agrobacterium* transformants were isolated and the integrity of the plasmid was confirmed by retransforming to *E. coli* or PCR analysis.

Example 12

Stable Transfection of Rice with Promoter and Intron Sequence Constructs

Transformation and Regeneration of Rice Callus Via *Agrobacterium* Infection

[0205] *O. sativa* spp. *japonica* rice var. Nipponbare seeds are sterilized in absolute ethanol for 10 minutes then washed 3 times with water and incubated in 70% Sodium hypochlorite [Fisher Scientific-27908] for 30 minutes. The seeds are then washed 5 times with water and dried completely. The dried seeds are inoculated into NB-CL media [CHU (N6) basal salts (PhytoTechnology-C416) 4 g/l; Eriksson's vitamin solution (1000× PhytoTechnology-E330) 1 ml/l; Thiamine HCl (Sigma-T4625) 0.5 mg/l; 2,4-Dichloro phenoxyacetic acid (Sigma-D7299) 2.5 mg/l; BAP (Sigma-B3408) 0.1 mg/l; L-Proline (PhytoTechnology-P698) 2.5 g/l; Casein acid hydrolysate vitamin free (Sigma-C7970) 0.3 g/l; Myoinositol (Sigma-13011) 0.1 g/l; Sucrose (Sigma-S5390) 30 g/l; GELRITE® (Sigma-G1101.5000) 3 g/l; pH 5.8] and kept at 28° C. in dark for callus proliferation.

[0206] A single *Agrobacterium* colony containing a desired insert with the candidate sequences from a freshly streaked plate can be inoculated in YEB liquid media [Yeast

extract (BD Difco-212750) 1 g/l; Peptone (BD Difco-211677) 5 g/l; Beef extract (Amresco-0114) 5 g/l; Sucrose (Sigma-S5390) 5 g/l; Magnesium Sulfate (Sigma-M8150) 0.3 g/l at pH-7.0] supplemented with Tetracycline (Sigma-T3383) 5 mg/l, Rifamycin 10 mg/l and Spectinomycin (Sigma-5650) 50 mg/l. The cultures are grown overnight at 28° C. in dark with continuous shaking at 220 rpm. The following day the cultures are adjusted to 0.5 Absorbance at 550 nm in PHI-A (CHU (N6) basal salts (PhytoTechnology-C416) 4 g/l; Eriksson's vitamin solution (1000× PhytoTechnology-E330) 1 ml/l; Thiamine HCl (Sigma-T4625) 0.5 mg/l; 2,4-Dichloro phenoxyacetic acid (Sigma-D7299) 2.5 mg/l; L-Proline (PhytoTechnology-P698) 0.69 mg/l; Sucrose (Sigma-S5390) 68.5 g/l; Glucose-36 g/l (Sigma-G8270); pH 5.8); media supplemented with 200 μM Acetosyringone (Sigma-D134406) and incubated for 1 hour at 28° C. with continuous shaking at 220 rpm.

[0207] 17-21 day old proliferating calli are transferred to a sterile culture flask and *Agrobacterium* solution prepared as described above was added to the flask. The suspension is incubated for 20 minutes with gentle shaking every 2 minutes. The *Agrobacterium* suspension is decanted carefully and the calli are placed on WHATMAN filter paper No—4. The calli are immediately transferred to NB-CC medium [NB-CL supplemented with 200 μM Acetosyringone (Sigma-D134406) and incubated at 21° C. for 72 hrs.

Culture Termination and Selection

[0208] The co-cultivated Calli are placed in a dry, sterile, culture flask and washed with 1 liter of sterile distilled water containing Cefotaxime (Duchefa-C0111.0025) 0.250 g/l and Carbenicillin (Sigma-C0109.0025) 0.4 g/l. The washes are repeated 4 times or until the solution appeared clear. The water is decanted carefully and the calli are placed on WHATMAN filter paper No—4 and dried for 30 minutes at room temperature. The dried calli are transferred to NB-RS medium [NB-CL supplemented with Cefotaxime (Duchefa-C0111.0025) 0.25 g/l; and Carbenicillin (Sigma-C0109.0025) 0.4 g/l and incubated at 28° C. for 4 days.

[0209] The calli are then transferred to NB-SB media [NB-RS supplemented with Bialaphos (Meiji Seika K.K., Tokyo, Japan) 5 mg/l and incubated at 28° C. and subcultured into fresh medium every 14 days. After 40-45 days on selection, proliferating, Bialaphos resistant, callus events are easily observable.

Regeneration of Stably Transformed Rice Plants from Transformed Rice Calli

[0210] Transformed callus events are transferred to NB-RG media [CHU (N6) basal salts (PhytoTechnology-C416) 4 g/l; N6 vitamins 1000×1 ml {Glycine (Sigma-47126) 2 g/l; Thiamine HCl (Sigma-T4625) 1 g/l; acid; Kinetin (Sigma-K0753) 0.5 mg/l; Casein acid hydrolysate vitamin free (Sigma-C7970) 0.5 g/l; Sucrose (Sigma-S5390) 20 g/l; Sorbitol (Sigma-S1876) 30 g/l; pH was adjusted to 5.8 and 4 g/l GELRITE® (Sigma-G1101.5000) was added. Post-sterilization 0.1 ml/l of CuSO₄ (100 mM concentration, Sigma-C8027) and 100 ml/l 10×AA Amino acids pH free {Glycine (Sigma-G7126) 75 mg/l; L-Aspartic acid (Sigma-A9256) 2.66 g/l; L-Arginine (Sigma-A5006) 1.74 g/l; L-Glutamine (Sigma-G3126) 8.76 g/l} and incubated at 32° C. in light. After 15-20 days, regenerating plantlets can be transferred to magenta boxes or tubes containing NB-RT media [MS basal salts (PhytoTechnology-M524) 4.33 g/L; B5 vitamins 1 ml/l from 1000× stock {Nicotinic acid

(Sigma-G7126) 1 g/l, Thiamine HCl (Sigma-T4625) 10 g/l}; Myo-inositol (Sigma-13011) 0.1 g/l; Sucrose (Sigma-S5390) 30 g/l; and IBA (Sigma-15386) 0.2 mg/l; pH adjusted to 5.8]. Rooted plants obtained after 10-15 days can be hardened in liquid Y media [1.25 ml each of stocks A-F and water sufficient to make 1000 ml. Composition of individual stock solutions: Stock (A) Ammonium Nitrate (HIMEDIA-RM5657) 9.14 g/l, (B) Sodium hydrogen Phosphate (HIMEDIA-58282) 4.03 g, (C) Potassium Sulphate (HIMEDIA-29658-4B) 7.14 g, (D) Calcium Chloride (HIMEDIA-C5080) 8.86 g, (E) Magnesium Sulphate (HIMEDIA-RM683) 3.24 g, (F) (Trace elements) Magnesium chloride tetra hydrate (HIMEDIA-10149) 15 mg, Ammonium Molybdate (HIMEDIA-271974B) 6.74 mg/l, Boric acid (Sigma-136768) 9.34 g/l, Zinc sulphate heptahydrate (Himedia-RM695) 0.35 mg/l, Copper Sulphate heptahydrate (HIMEDIA-C8027) 0.31 mg/l, Ferric chloride hexahydrate (Sigma-236489) 0.77 mg/l, Citric acid monohydrate (HIMEDIA-C4540) 0.119 g/l] at 28° C. for 10-15 days before transferring to greenhouse. Leaf samples are collected for histochemical GUS staining with 5-bromo-4-chloro-3-indolyl-B-D-glucuronide (X-Gluc), using standard protocols (Janssen and Gardner, *Plant Mol. Biol.* (1989) 14:61-72).

[0211] Transgenic plants are analyzed for copy number by southern blotting using standard procedure. All single copy events are transferred to individual pots and further analysis is performed only on these. For all the analysis leaf material from three independent one month old single copy T₀ events were taken.

Transgene Copy Number Determination by Quantitative PCR

[0212] Transgenic rice plants generated using different constructs were analyzed to determine the transgene copy number using TaqMan-based quantitative real-time PCR (qPCR) analysis. Genomic DNA was isolated from the leaf tissues collected from 10-day old TO rice plants using the QIAGEN® DNEASY® Plant Maxi Kit (QIAGEN® Inc.) according to the manufacturer's instructions. DNA concentration was adjusted to 100 ng/μl and was used as a template for the qPCR reaction to determine the copy number. The copy number analysis was carried out by designing PCR primers and TaqMan probes for the target gene and for the endogenous glutathione reductase 5 (GR5) gene. The endogenous GR5 gene serves as an internal control to normalize the Ct values obtained for the target gene across different samples. In order to determine the relative quantification (RQ) values for the target gene, genomic DNA from known single and two copy calibrators for a given gene were also included in the experiment. Test samples and calibrators were replicated twice for accuracy. Non-transgenic control and no template control were also included in the reaction. The reaction mixture (for a 20 μl reaction volume) comprises 10 μl of 2× TaqMan universal PCR master mix (Applied Biosystems), 0.5 μl of 10 μM PCR primers and 0.5 μl of 10 μM TaqMan probe for both target gene and endogenous gene. Volume was adjusted to 19 μl using sterile Milli Q water and the reaction components were mixed properly and spun down quickly to bring the liquid to bottom of the tube. 19 μl of the reaction mix was added into each well of reaction plate containing 1 μl of genomic DNA to achieve a final volume of 20 μl. The plate was sealed properly using MicroAmp optical adhesive tape (Applied Biosystems) and centrifuged briefly before loading onto the

Real time PCR system (7500 Real PCR system, Applied Biosystems). The amplification program used was: 1 cycle each of 50° C. for 2:00 min and 95° C. for 10:00 min followed by 40 repetitions of 95° C. for 15 sec and 58° C. for 1:00 min. After completion of the PCR reaction, the SDS v2.1 software (Applied Biosystems) was used to calculate the RQ values in the test samples with reference to single copy calibrator.

[0213] Stable transgenic rice events were generated with the constructs, PHP50063, PHP50111, PHP50062, PHP50061, PHP52322, and PHP42365 as given in Table 8. The primers used for amplifying the cloned promoter and intron sequences for these constructs are given in Table 2 and Table 7.

TABLE 8

Description of Promoter and Intron Elements in Constructs

Construct	Intron	Promoter
PHP50063	—	pTS2 (SEQ ID NO: 119)
PHP50111	TS2 (SEQ ID NO: 118)	pTS2 (SEQ ID NO: 119)
PHP50062	TS2 (SEQ ID NO: 118)	Zm Ubi promoter
PHP50061	TS1 (SEQ ID NO: 4)	pTS1v (SEQ ID NO: 136)
PHP52322	TS27v (SEQ ID NO: 138)	pTS27v (SEQ ID NO: 139)
PHP42365	Zm Ubi intron	Zm Ubi promoter

[0214] The stable transgenic rice events generated with these constructs were subjected to TaqMan-based qPCR (quantitative PCR) analysis to determine the transgene copy number as described above. PCR primers and TaqMan probes designed for the GUS reporter gene and for the endogenous GR5 gene are listed in Table 9.

TABLE 9

Primer Sequences for qPCR	
Primer ID	SEQ ID NO:
GUS F primer	129
GUS R primer	130
GR5, F primer	131
GR5, R primer	132

TABLE 10

Probe Sequences for qPCR			
SEQ ID NO	Probe	Quencher	
GUS	133	Fam	Tamra
GR5	134	Vic	MGB

[0215] All single copy events were transferred to individual pots and further analysis was performed on leaf material and panicle collected one month after transplanting in the greenhouse.

Qualitative and Quantitative Analysis of GUS Reporter Gene Expression in Stable Rice Events

[0216] Both qualitative and quantitative GUS reporter gene expression analyses were carried out in triplicates on at least 5 independent single copy events for each construct. Leaf and panicle samples were collected for histochemical GUS staining with 5-bromo-4-chloro-3-indolyl-B-D-glucuronide (X-Gluc), using standard protocols (Janssen and Gardner, *Plant Mol. Biol.* (1989) 14:61-72) and for quantitative MUG assay using standard protocols (Jefferson, R. A., *Nature*. 342, 837-8 (1989); Jefferson, R. A., *Kavanagh, T. A. & Bevan, M. W.*, *EMBO J.* 6, 3901-3907 (1987).

[0217] TS1 and TS27v when combined with their respective endogenous promoters (pTS1v+TS1 (PHP50061) and pTS27v+TS27v (PHP52322) were able to drive GUS expression in stable rice transgenic events (FIG. 9).

[0218] TS2 intron with its endogenous promoter (PHP50111) enhanced the GUS reporter gene expression by 11.6 fold in leaves and 8.9 fold in panicles compared to the TS2 promoter alone (PHP50063) driving the GUS reporter gene expression (FIG. 10) and the values obtained were comparable to the levels observed with maize ubiquitin promoter and intron (PHP42365) driving GUS in transgenic rice plants. There is a slight increase in the GUS reporter gene expression levels when the TS2 intron is cloned with maize Ubiquitin promoter (PHP50062) compared to the data obtained with maize ubiquitin intron cloned with maize ubiquitin promoter (FIG. 10).

[0219] GUS histochemical staining data were found to correlate very well with the quantitative GUS assay in all events. Representative images are shown in FIG. 10 and FIG. 11.

Example 13

Identification of Novel Terminator Sequences

[0220] Transcription terminators for the 4 genes comprising the expression enhancing introns TS1, TS2, TS13 and TS27v (SEQ ID NOS: 4, 118, 13 and 138 respectively) were identified, and were called tTS1 (SEQ ID NO: 140), tTS2 (SEQ ID NO: 141), tTS13 (SEQ ID NO: 142) and tTS27 (SEQ ID NO: 143). Terminator sequences were defined as 500-900 bp of sequence downstream of the translational stop codon of the respective genes.

Example 14

Amplification and Cloning of Terminator Sequences

[0221] We constructed a terminator test vector (TTV) (PHP49597—FIG. 13; SEQ ID NO: 144) carrying GUS (B-glucuronidase) reporter gene driven by the Maize Ubiquitin promoter using standard molecular biology techniques (Sambrook et al.). A promoterless Ds-RED coding sequence was included downstream of the GUS gene for measurement of transcription downstream of the cloned test terminator

sequences (read-through transcripts). The Ds-Red sequence was followed by a Pin1I terminator to enable termination and polyadenylation of all transcripts, so we could detect them by reverse-transcription-PCR (RT-PCR) using oligo dT primer. The Terminator test vector also carried a monocot-optimized Phosphinothricin acetyl transferase (MOPAT) gene as a plant selectable marker.

[0222] Candidate terminator sequences can be amplified from maize genomic DNA. The resulting DNA fragments can be cloned into the terminator test vector at Acc651 restriction site using IN-FUSION™ cloning (Clontech Inc.). All constructs will be transformed into *Agrobacterium* (LBA4404/pSB1)

Example 15

Rice Transformation with Candidate Terminator Sequences

[0223] The candidate maize terminator sequences tTS1, tTS2, tTS13 and tTS27 (SEQ ID NOS: 140-143 respectively) will be tested for their ability to function as transcription terminators in stable rice transgenic plants generated by *Agrobacterium* mediated transformation as described in Example 12.

Example 16

Testing of Candidate Rice Terminator Sequences in Stably Transformed Rice Tissues

[0224] Reverse Transcriptase-PCR (RT-PCR) and GUS assays can be done from stably transformed rice plant tissues, to test the ability of candidate maize terminator sequences tTS1, tTS2, tTS13 and tTS27 (SEQ ID NOS: 140-143 respectively) to prevent transcription read-through and to compare GUS expression

Reverse Transcription PCR (RT-PCR) to Determine Transcription Read-Through

[0225] RNA will be extracted from leaf tissue from multiple independent TO events for each construct. cDNA can be synthesized using SuperScript® III First-Strand Synthesis System from Invitrogen. The level of GUS gene and read-through transcripts will be assayed using specific primers within GUS gene and DS-Red respectively. Transcript levels can also be measured by quantitative RT-PCR using primers and probes within GUS and DS-Red sequences.

Histochemical and Fluorometric GUS Analysis

[0226] Tissue samples from each independent stably transformed rice line can be stained for histochemical GUS analysis, with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-Gluc), using standard protocols (Janssen and Gardner, *Plant Mol. Biol.* (1989) 14:61-72.). Tissue samples can also be used for quantitative MUG assay using standard protocols [Jefferson, R. A., *Nature*. 342:837-838 (1989); Jefferson, R. A., *Kavanagh, T. A. & Bevan, M. W.*, *EMBO J.* 6:3901-3907 (1987)].

SEQUENCE LISTING

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Sequence total quantity: 146
SEQ ID NO: 1 moltype = DNA length = 978
FEATURE Location/Qualifiers
source 1..978
```

-continued

-continued

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cgccggatga	tttgtatctt	gaatctgcct	tgttgatagc	tcttggccc	caccgagtgc	180
atcaacaaga	caacaagaaa	ccgtcccta	tacgaatggt	atgggttacc	gtgttcagt	240
cgtccggcgc	gttgtggga	aaaaaaaaatg	gacggtttag	ttaggtcggt	gcgcagccgg	300
acaggacaaa	tacaccctgt	cggtggtag	gcagcggaga	ttgacgcate	attgcacact	360
cgtttttttttt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt
cgaaatggt	ccttgcctcc	ctgtgggttgc	tatgtctgt	tccaacatcc	gttgaggaaac	420
tgcgtttttt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt
cttcgtggga	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt
gcaatcagt	agatttgggt	tgccataggg	cgccttctt	tcctgggtct	actgatttgg	600
ccttcccaat	ccccatgtgt	tgccatggaa	caacttgaca	atcaatggaa	aggttgagggg	660
gtccgaaggaa	gttatttacg	agcaataac	tttgcgttgc	agatttatgg	cactgaataa	720
cagaatgtac	acacaaatgt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt	ttttttttttt
ataacctctcc	tcttcgttaac	atagaatttct	tatctcataa	attcgttgc	gcag	834

SEQ ID NO: 7 moltype = DNA length = 982

FEATURE Location/Qualifiers
source 1..982
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 7

gtaaactctt	acgcctccct	tcgatgcctc	cagcgtctcc	ttcccgagtt	ccttagtctgt	60
agtttattact	gccttgcatt	gtactccctc	cgttttagt	gtcgctggat	agtgcacaaat	120
tgaactatcc	agcaacaact	aaaaaaac	ggagggagta	totatctttt	tgttaggcct	180
ctcaactgtt	gtgtccggaa	tacccgtact	gttcttacc	tcacgttga	aacacaggga	240
ttccgcgtgc	ctgttgtgtt	tcgtgtgtge	cgtgagccca	aatttagggc	gcatagagca	300
tgccggacgt	agattgtacc	gagcggtaa	acttgcgg	tgcaaccaa	agagggcatg	360
aggccggcgc	acgtcccccc	tcgcgggtt	ctgtgtgttgc	gggcggagatc	tcgcgttatt	420
gagtcgtcg	aaaaggcgcag	tcgcgtatca	gtcgacgtc	gttgcgtgt	atctcgctc	480
ccatccagat	gtgggtgggc	agtttgcgt	agatccggcc	ccttgcgtca	tgtccgtgt	540
gaccggatca	ggctgtgttgc	gttttttttgc	tactcgtcaaa	gtgtgtatgc	tacgatttt	600
atttatgttt	atgtatagag	tgtaaaatac	gattttttat	tatattttat	tatagaatgt	660
aaaacacaaa	tacatcgtt	tttgtgtggg	tttgtgtgg	aatttgcgtt	atagccaaaa	720
attgggtttt	tattttgttac	taattttaaa	ttaaatgtt	atgagtatgt	tgatgtgtt	780
aatggaaaact	agtgcgtttt	atatagtt	gaaaatgtt	ggagtaggtt	ggcttcatcc	840
agatcttcg	gttgcgttgc	gttttttttgc	gtcatggca	gcagggccac	atgagatttc	900
tgtacgggg	ttgaatttgc	actgcacgt	cttcgactgt	cattatgtc	tattgtatct	960
atgaacttgc	aaacttttac	ag				982

SEQ ID NO: 8 moltype = DNA length = 856

FEATURE Location/Qualifiers
source 1..856
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 8

gttaagggttt	cttccctccct	ccccctcacac	ccctgttctgt	gttccttcgg	atcgatctc	60
agtgggtatgt	tttagacgtcc	gccccgtgcct	acgtgtgttgc	attgcgcgg	gaaagggttt	120
tttaggtgg	gttagatccca	aacacggccgg	atctggatcc	tgtcccgccg	ggggccggcg	180
gacttgcgt	cgtagtgc	gtgtgcattt	ctcccttacca	gtggggaaat	ccggcgatgt	240
gacctaagg	ctaaggctt	tctgtgcct	tgaccattt	tcgcgttgc	aaaacaaat	300
gacaatcatg	ccgttctctg	tttgcgttac	tggatgttta	ttacgttgc	aatctgcga	360
atgtgttgc	atgtgtttt	cttgcgttgc	tggggcgttgc	ttacgttgc	acccagttct	420
aggtgtgttgc	actaggact	ttgtgtatgt	ttgtgtatgt	ttgtgtatgt	ttgtgtatgt	480
tggacgtttt	tttccccaat	tttgtgtatgt	tttacgttgc	aggtgtgtca	agtaattttgc	540
ctagtgtatgt	tgtgtatccat	tttcaacgtt	gaaccttgc	tttcccccata	aaaccccaaa	600
caggaaatct	tgcggccact	tctatgtca	aaatgttac	gtttagtacc	ctgattgtact	660
caatttctgt	cactaggat	gtctggatca	aaggatgtt	tttaccatct	agaaactgtcc	720
ctggccctgc	tttccacata	gtatccgtt	catttttact	actatgttac	cccccttaac	780
ttgcccgtact	atttctctt	tcagatctacta	tttacgttgc	tataatttaca	ttaatgttgc	840
tgtgtatctt	gtgcag					856

SEQ ID NO: 9 moltype = DNA length = 1020

FEATURE Location/Qualifiers
source 1..1020
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 9

gttaaatctca	attttatcat	gtattttca	atgtatataaa	attcggat	gaataattt	60
ttgacatttt	ttctcttagag	agcaataat	acataaaaaca	aattatacaa	attttatctt	120
ttatattataa	taataatgtt	aataacataa	aaaaaaatca	gcatcaattt	tcatatata	180
ctatattataa	atattttatctt	tatgttataa	atttggat	ccattttatac	atcatctt	240
tccgatattt	tattttctgc	aatcaaacac	atgcccagg	aacgcgttgc	cccgaaatgt	300
ggcgtaacc	aaccgtatgc	accttgcgc	cgtttcaaa	tgaaatgggc	cggtcaatgc	360
gccccccatc	cgcccgatgt	tatcacgacc	cagcgttgc	cctatgttac	ctctgcccc	420
gggcttgggt	ttccctccat	ccgttgc	tttacgttgc	ctacagatgc	gcggaggagac	480
gaaaaggatgt	ccacccact	ccgcgttgc	tttgcgttgc	catttgcgtt	ggccgtctaa	540
ttcgttggcc	ggcccgatct	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	600

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ctcggttcgggtt	tttgccggc	ggaggttgtct	cgcgaggcgt	agcttgtcgc	tcaggctct	660
tttgccttg	tagattccct	gcatctgc	ttactgatag	cttttgtgt	taatcttgag	720
agagtttatgc	cacgagtctc	tttgatattc	tattgggtt	atgatatgt	gggatactgt	780
aatgtttctc	atattctgc	tatggttgt	ttgggc	catgt	agaattt	840
ttttaggatc	ctgtttttgg	tgctaactct	gtcaacacta	gactgagaat	tgttgttac	900
aagtagctt	catgttgac	gacaagatcc	tctgtgtt	cattccagg	ctcatgattt	960
tatttttttt	ttgcatcttc	atccgc	cataatggc	tttatctact	tgcaattc	1020

SEQ ID NO: 10 moltype = DNA length = 841

FEATURE Location/Qualifiers
source 1..841
 mol_type = genomic DNA
 organism = Zea mays

SEQUENCE: 10

gtataacaacc	gaccttgtct	cccccgattt	ctcaactacg	tagtgtatgt	acgcattcggt	60
aggttagatgg	qaatttcggg	ccactgggtt	ggggggtttta	atttgcgcta	tcgtttcggt	120
ttgcctgtgg	ttcggaggt	agattgggt	cgcaggtagg	ttgtcgctg	gatctggag	180
aggcgaggag	ctaaattcgc	atagctgtta	ataactcacc	cgggtgtcta	tgaaaaggcg	240
taggcgtcgc	ctgtgtcg	tgctgttagt	tactactt	tttcttctaa	tataggggt	300
tcoccttcgt	caacttttt	ttaaaacgg	ttcttgggtt	tcggctgtgt	tagtgcgt	360
ggaccttgtt	gcccattgtt	gcatgtatg	atttgtgcgt	aatttagtag	atcatttagat	420
tacctaatac	tcggatcccg	gaatttttgc	ctagtccca	ttatttgc	cccttgcacg	480
catgtgttt	gtgtacttgc	aggaatctgt	ccatatgc	cggatctatg	ggtcggcc	540
tgtatgttgc	atgttgc	tttgcgttcat	gttcgactt	accttgcgc	cgagcaaaaa	600
gaaggataaa	tgcgtgttgc	caatctgggt	atacggc	tcgatgtgtc	tgaatgaaga	660
ttggggatgt	tcttctgtt	atttattttgt	caattttttat	tctgtatatt	cattttgtt	720
ccagtttgc	atgtgcattat	taacacttca	attcttaggtc	tattgtatgt	gtatagtagc	780
actctttcaa	tctttcgttgc	gtacagctga	tgccttgc	tgccttgc	ccttgcttta	840
g						841

SEQ ID NO: 11 moltype = DNA length = 1044

FEATURE Location/Qualifiers
source 1..1044
 mol_type = genomic DNA
 organism = Zea mays

SEQUENCE: 11

gtccgtgcgc	ggcgccccgg	atggacatct	atagccgc	ggtcatggat	agatagataa	60
ttcgcgtcc	gtgataaaggc	cggactgtca	cgttccgc	ggggagtaatt	atttacacac	120
tcttcagac	caacggccat	tcgtggacgc	tggatccca	taccaaatttgc	ctacaagctc	180
aatatgtgtt	gggtgggtt	ctggccattt	ttagaatatca	tctctatacg	tctttataca	240
gaacgaaaaa	aaaacgagcg	gccaaggatgg	tgttggatgt	tagatcgac	ggcaaggcg	300
tgctctccgc	ctttttaaag	gctgtccgc	tcgcacccttc	tcctctccgt	cgcattttcc	360
gtccggctt	tccgttaccc	ggccgttta	aaccctgtt	cccatccat	cttcgtttcc	420
gtccggccgc	ctgtcgatgg	gttctgggtt	gaccccttc	acttttcctc	tcgcaaaactc	480
aattctccgt	gataccgtcg	cttttttgtt	tttgttgcgtt	ttcctctgtt	tgatttttgg	540
gcgttttgc	ctttttat	aggccctggg	aatcatgggc	atggtttgc	tggctactgc	600
tagcacttgt	ttgcgtatgt	ttttagggcc	tgtgtatgt	ggcttatttttgc	tggctcgctt	660
ctacttaacta	ctagggcac	gttgcattac	aatcaggatgt	tatgtggcat	tgcctatatt	720
cacagttatgg	aaaaaaatgt	attttaatgg	ttgaaatcat	acgtggccgt	gtcctgtt	780
aatagggtct	tattatagga	cgtgcacgttgc	atgcacatgt	attaggatgt	ttgttcttt	840
gtaccatgtt	taaacacgcac	cagatccat	ttaatgtaa	ctacatcaga	ttgttgc	900
tgttctacta	caagttatgtt	cagcatggca	ggaggaaatgt	tgctctttaa	tctgttgc	960
aggcgcatttgc	cgaaatgtt	gttcttac	catcagg	tttacccat	tttacccat	1020
cttgcattttgc	tctgttttttgc	gcag				1044

SEQ ID NO: 12 moltype = DNA length = 648

FEATURE Location/Qualifiers
source 1..648
 mol_type = genomic DNA
 organism = Zea mays

SEQUENCE: 12

gtaaagcgacg	acaacgagca	gtggccggca	ggattggac	ccccccatacc	ttctctctct	60
gggtttcgt	ctctctcg	tgctcagaaa	gttccggagg	cgccgggtt	cgtctctct	120
cgtcgctcc	gtccaaaggc	ttttttttca	gaaccagtcc	ttttgtatc	cagactaaaa	180
tttatttgc	tttattttat	caataaaattt	catggttca	aaatgc	gaca	240
ttaaatagat	acatattttt	taacaaaaga	aaaatcatat	atataata	aaaacaatt	300
agtggcatcc	atacatatgg	atgc	ccaaac	aattatcggt	tgagggaaatc	360
tatcttaaag	gttggaaac	tggcaatcg	aggagcatga	gctggtaggg	cactcg	420
gggtggagcc	ggtttgcgt	cgcggaaatgg	accgaccat	catgactcgc	ttggcacgc	480
gtgaggtgag	tgtgatgtgt	gtcagcc	cgttgcgtt	ttgggttttgc	gtctataaaa	540
taccccgcc	tccatctt	ctctgggtt	tggtgttgc	tcctatct	tgcacgcaca	600
cgcacaaacgc	tccatcttgc	tcctcg	gttgcgtt	actactatgt	agtttagag	648

SEQ ID NO: 13 moltype = DNA length = 632

FEATURE Location/Qualifiers
source 1..632

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mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 13
gtgaggagcc ttctctctct ctctctctct ttctctgtct ctctctcttc ctaaccttc 60
ttcccttctgt ctctgtcccc cacgcgtctgc tcttctggaa ttttctattt gcgtctgccg 120
ccatttgttt cggctacttt gtgegcgcgc aagcgcacaaac cacgggggtc tctctcggt 180
tcgcctatct gccgaatcgc cactgcaga tcttctaccg ctttctgtgt gcgtcgacat 240
ctggactccg gagtcggac gtcggcggt ctgtttctgt egcttgcgtt cttttttcca 300
gtatgttcc gtttttctc gaattccatt ttttatctc tctttttcc ctcgtggacg 360
aagcaaaacga agcaaaacgc cctgcacatc gagactcaatc gactgtactg ttttttttc 420
cattgggtt ttcctaaga ttctttttt gtcgtccga cagcagcacc 480
cggtgcaccc atttcagcac ttcttcgcgc tctgtttcca taatattttt tcttttttt 540
ttccatctct tttttttgt gtgtgtata gcttttgcgtt gactgaaacg cagcacacac 600
ttacacaac caaacatttt ttttggcgc ag 632

SEQ ID NO: 14      moltype = DNA length = 1405
FEATURE           Location/Qualifiers
source            1..1405
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 14
gtatataactc ttctctctct atccgccttc tgattcatgc ggcccttgcg atgtgtgtga 60
tcttgttact tggccggctg tggttctttt tgcgtctctt gattcatgcg ggttaggatta 120
atctttgtt aatccgcacaa aaaggcccttg attattcgtat actatctatc tgcgtatccgc 180
aaaaaggcga tgaacttcc atgttctcg tagtgcgtgt tgcgtatcga ttctgtgtgc 240
tcatcaacta gtcgtggcgc gcaaggactc gatgcacgtt ccatttcgac ggtgcgtaca 300
acaaggcattt ctgcggatc aatatctcg ttttatttctc agaaggacgg tcgttgcgt 360
ttattnaaag gggaaaaaaag aatcgatgtt gtagatgtc gcttcgtatc ccagaatggc 420
ttaaaggacg atgggtacgg atggaaacctt ttctctctca tgcgtgcgc atagcggaaac 480
ggaaagctttt gtcagtccag ggaggggaaa aaatttcaaa gtcgttgcgc gccccttgc 540
tgcgtatcgc acgtggcggc acgtgcgtcaaa caattaaacg aatgcctacg agageggacc 600
ctgtatcttt tgcgtatgtt atgtgcgtatc gtcgttgcgtt ttaggtttt gaccggaaaa 660
tagctcgctt ttccacaggg gggaaataag gtgcgttgcgtt gaaatgcacag tcggatattgg 720
gttcagaacc aggcttttagt attacaatag agaaactggc acgttgcgtt gacgtgcgt 780
ttatcgaaat gtcgtcaata tcgcgttgcgtt cgtatatctt atatgtttat tgcgttgcgt 840
gacatcggtc ttcgtatgtt tcggacact gtttctccgc tccgtatcgc gattttattt 900
tttttacggc cggtcataatc catggtttcgat tataggatat tcatattca gagacgtcta 960
tcgttcttta ggcgttccacc gcaactctgc tgcgttgcgtt cattattttt gtttctctc 1020
atcggttccacc ggggggttccacc agtgcgtatc ttcgttctca gttttttttt tgcgtatcgtt 1080
caggctgtttt tcaatgttac tcgttgcgtt gtcgttgcgtt ggcgttgcgtt 1140
gaccggccgggtt atagtagggg ttcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1200
cgccggccggcc ggtatcgtt aaaaaaggct tcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1260
attcaaaaaa ccaaaacgcg ataaacaaaaa tgcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1320
cttcgtatcgc tgcgtatcgc ttcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1380
taatctgttccgcgt cgcgc 1405

SEQ ID NO: 15      moltype = DNA length = 1361
FEATURE           Location/Qualifiers
source            1..1361
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 15
gttaaaggcggatc ttttagtttgcgtt atcgatcctt aagacaatac tgcgttgcgtt 60
gcacaaccat gccccgtcc atatccgcac cgtccgttgcgtt ctgaatccac cccgcatac 120
ctcatctggc accatcttc gtcgttgcgtt tgcgttgcgtt cgcgttgcgtt 180
ccgcggccaa accatcgccg atccgtccgc aacaggccatc cgcgttgcgtt 240
cagatccacgc ttccatctatc tgcgttgcgtt atccacagg gatccgttgcgtt 300
tcgttgcgtt cgcgttgcgtt ttgcgttgcgtt taggttgcgtt cgcgttgcgtt 360
ctcggttccacc gggggggaccgc cgcgttgcgtt tgcgttgcgtt gtcgttgcgtt 420
ttcttctttt tggccggccacc ggcgttgcgtt gtcgttgcgtt gtcgttgcgtt 480
tcgttgcgtt ttcgttgcgtt ttcgttgcgtt gtcgttgcgtt gtcgttgcgtt 540
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ccagggttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 660
gggttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 720
tttttttttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 780
tcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 840
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atgcacatctt atctatctt ttcgttgcgtt gtcgttgcgtt gtcgttgcgtt 960
gggttcttttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1020
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catcttccaa ttcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1140
ggccacacaa atgatagattt ttcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1200
cagtacaaatc ttcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1260
tcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1320
cttataagat ttcgttgcgtt gtcgttgcgtt gtcgttgcgtt gtcgttgcgtt 1361

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SEQ ID NO: 16	moltype = DNA length = 703
FEATURE	Location/Qualifiers
source	1..703
	mol_type = genomic DNA
	organism = Zea mays
SEQUENCE: 16	
gtgagaggattc gagagattcg tccgtaccag ggcttggatc tacgctccgg ccacccatcg 60	
gettcgggtc ggcgcacc aaccgagct aggagtcgc cattcatca aacgcctcg 120	
acggcgactg cgcatcggtt cagatccagg cgtcccttca ccggcagctc cgcctccat 180	
gcaatccgtc tcgttgttgc tgatccctcc ctggactcgg gtgtactgga agtccatcg 240	
gataaaagaa tttttttgtg tggatggac gcataatgc gttttgttc ggaattttt 300	
tgcggcttgg cgtgtgtca atacccgtt agttttcca ttttttttca gggttcaacc 360	
aaaccccttcg ctgacagacc ctctctgtt cagtttgcgtt accccaactga ctttttttc 420	
ttgttcatat tctatgttgc tgctgagtgg catgccgtc gatattttgg aaagcagatt 480	
tttatgttgg caagtgttgc tgcgatgttct ttgcgttgcata tttaagtttcc acctgagatc 540	
tgatattgtc ggtccaaat tgctgtatc ttgacttattt gaggacacgt tcttaggtaa 600	
atcattggaa gacatatttc acttcgcgtt ggacacgtac ttctccaaga tgatgccttc 660	
acctgttca aaccccttgcg atttttatata actcgcttgc cag 703	
SEQ ID NO: 17	moltype = DNA length = 1341
FEATURE	Location/Qualifiers
source	1..1341
	mol_type = genomic DNA
	organism = Zea mays
SEQUENCE: 17	
gtgaggccgc gaccaagaag gcgttaggcgg cggcgctggc cgtggggggg cggtggcacg 60	
atacgcttgc cggctgggc acgcgttgc ctgttgcgtt ctctcgcaagg aaaaagtttc 120	
tgtgttccaa tggaaatgg atacaacgtt tggattctaa tggaaatggg atcgaacgg 180	
tgtgatagat acaaacaatgtt ctgaagatct tttatagtagt acatagatag atcgactaa 240	
gcaaaaaaat cttagcccccc gtttttttcg ttggattgaa ttccattttt ataattataa 300	
tttagtcaaa actaaatgg tttatataattt tatataatcg atatattttgt atattatcc 360	
aaatcatacg agagagatag ttatatacta tttttatgtt atagcgaac aaatagatga 420	
gtgtgtata agttgtacat cggaaaaataa gcgtgtaaat ctatagaatc aattttccatc 480	
tccacccca ttaatttgcg ataggctt atgataactt tggaaatggg tggatgtca 540	
catttttttta aaaaatataa ctatattttt atgttgcgtt aaatttctcg aaataaaaga 600	
aaacaaacgca gacccatggaa ataatgttca tataacaatc tataacaac tcaaaagatg 660	
agaataaaaa aaagtaacgg cgtgtttgtt ttgcagggtt gactgttct gggttcatcc 720	
ggacctatgtt cggagccatc attatcattt gtttgcgtt ggggaacgtat gtcgtccgtc 780	
actgcgttgc ttaataataa tactaacaatc tggaaatggc tcacttgcgaa agaaatgtca 840	
agaccgcgtt ctcggggcc acggccatcgat ggttgcgttca aaccatcaaa ccaaacacgc 900	
tgtataattt ccggaaaccgc ccggcgatcgatc tggcgttgc tggatgtgg gttcgaaagg 960	
ggatcccgtg tgcgtgggtcc acacgttccatc gttgtgcggcg tgcgttcaact gccccggccc 1020	
ggccatgtgc gggatgggggg gggatggggc tgacgttgc taaatgtca ggcgtatggc 1080	
cgcggatcgatccttccatc tggatgttgc tggatgttgc tggatgttgc tggatgttgc 1140	
gatccggccgcg cggccatggc tggatgttgc tggatgttgc tggatgttgc tggatgttgc 1200	
ccggccgtatcgatc tggatgttgc tggatgttgc tggatgttgc tggatgttgc tggatgttgc 1260	
ccccccatctt tgcgttgc tttttttttt ctggatgttgc tggatgttgc tggatgttgc 1320	
tcggccgtc ggttgc tggatgttgc 1341	
SEQ ID NO: 18	moltype = DNA length = 1125
FEATURE	Location/Qualifiers
source	1..1125
	mol_type = genomic DNA
	organism = Zea mays
SEQUENCE: 18	
gtactaataa actgtatgtt catttattca tggcacggcc taccaatgc aataaaatcta 60	
tccacgtca tgagaagaaa acccgagatcg agagagatcg agagagatcg ggcttgcctt 120	
cccgccggc cggttagtgc caatttggca cgcgttccatc gaggcaagca gaggcctcg 180	
gtcgccgtgg gtttgcgttgc cggggacggc agatccatcg ctctgttgc tggatccagg 240	
cggtggatcgatc tgcgttgc tggatgttgc tggatgttgc tggatgttgc tggatgttgc 300	
tggcaaggccgc ctttttttttgcgttgc tggatgttgc tggatgttgc tggatgttgc 360	
tgcaaaaatca acgtgtccatc ccctgggtttt gtcgtccgcgc gcaccatcgca aaacaaatcg 420	
gaaaccgaaa gactgttgc aaggccaaatcg caagtgttgc gggatgttgc tggatgttgc 480	
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aatctgttgc ttttttttttgcgttgc tggatgttgc tggatgttgc tggatgttgc tggatgttgc 720	
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SEQ ID NO: 19	moltype = DNA length = 894

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FEATURE	Location/Qualifiers
source	1..894
	mol_type = genomic DNA
	organism = Zea mays
SEQUENCE: 19	
gtgaggcattc cgatcgattt ttctttctt ctttaactaca ctcccttgcg atatggggac 60	
gacactcggt agtggcgtga ggtgaggtaa atcgcgttag ttagtgtta gggtttgc 120	
gtttcaaaaa ggaccagggg ttgggcttc cgtgttgaac cgtcaatccg acgttagt 180	
agtgcggatt cgggggttga tcgatggaaa gagggggttg ccgcactttt ggtgtggta 240	
taggggtttt cgattttttt gtctgttgcg gctgttttc ttcgaggag tagattttca 300	
ttgtacttaa aatccctat gtgggttgcg aaacacgtat ttgggttgtt atatggttta 360	
aacgtgaaga ctatggtagt gtggaccat gattttggatc cttttctgt gcattatagt 420	
taaaatcggtt aggtgcacc tataatctatc ttttgcgtt taggggtattt ttatagacga 480	
gatcccccttcttgggctcta aaaatagcaa gaaaaagaca tttttggc aagttaatgt 540	
cctgtattat tctgaacgag atatgttac tttttataa gtttgcgtt ttgggtctgga 600	
atatggttgc gttcatcttc caattttgtt gtttgcgtt tttttttttt tagttctt 660	
gtggggcattt tggtggccaca gaaatgtatg attttaaagaa aggttttaggc agaagggtac 720	
cttaagtgtt gtcccaatc aagtaacaaat ttgttagcact ttgtttttt ctttttttt 780	
attatatgaa atttcggcca tttttttttt tttttttttt aatgttgcac 840	
actacttccc agtcctatgt atacttataa gttttttttt tttttttttt tcag 894	
SEQ ID NO: 20	moltype = DNA length = 33
FEATURE	Location/Qualifiers
misc_feature	1..33
	note = intron_TS1 fwd primer
source	1..33
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 20	
caagcgtatcg caggtgagcg cttacaccc tcc	33
SEQ ID NO: 21	moltype = DNA length = 35
FEATURE	Location/Qualifiers
misc_feature	1..35
	note = Intron_TS1 rev primer
source	1..35
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 21	
tcgggtacctt ggattgcaaa aaaaacagtgc atcag	35
SEQ ID NO: 22	moltype = DNA length = 33
FEATURE	Location/Qualifiers
misc_feature	1..33
	note = Intron_TS4_forward primer
source	1..33
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 22	
caagcgtatcg caggtgatcc ttgacgcgtt cga	33
SEQ ID NO: 23	moltype = DNA length = 32
FEATURE	Location/Qualifiers
misc_feature	1..32
	note = Intron_TS4_reverse primer
source	1..32
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 23	
tcgggtacctt aataatggcaaa ataaaattgg tt	32
SEQ ID NO: 24	moltype = DNA length = 33
FEATURE	Location/Qualifiers
misc_feature	1..33
	note = Intron_TS5 forward primer
source	1..33
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 24	
caagcgtatcg caggtacgccc cgctctctcg ctc	33
SEQ ID NO: 25	moltype = DNA length = 29
FEATURE	Location/Qualifiers
misc_feature	1..29
	note = Intron_TS5 reverse primer
source	1..29

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mol_type = other DNA
organism = synthetic construct
SEQUENCE: 25
tcgggtacct gcacgaacga atttatgag                                29

SEQ ID NO: 26          moltype = DNA  length = 33
FEATURE               Location/Qualifiers
misc_feature          1..33
                      note = Intron_TS6 forward primer
source                1..33
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 26
caagcgatcg caggtaaact cctacgcctc cct                                33

SEQ ID NO: 27          moltype = DNA  length = 29
FEATURE               Location/Qualifiers
misc_feature          1..29
                      note = Intron_TS6 reverse primer
source                1..29
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 27
tcgggtacct gtaaaagttt tccagttca                                29

SEQ ID NO: 28          moltype = DNA  length = 34
FEATURE               Location/Qualifiers
misc_feature          1..34
                      note = Intron_TS7 forward primer
source                1..34
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 28
caagcgatcg caggtaaggt tcccttcct cctc                                34

SEQ ID NO: 29          moltype = DNA  length = 30
FEATURE               Location/Qualifiers
misc_feature          1..30
                      note = Intron_TS7 reverse primer
source                1..30
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 29
tcgggtacct gcacaagata cacacaaaaca                                30

SEQ ID NO: 30          moltype = DNA  length = 35
FEATURE               Location/Qualifiers
misc_feature          1..35
                      note = Intron_TS8 forward primer
source                1..35
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 30
caagcgatcg caggtaaatc tcaaatttat catgt                                35

SEQ ID NO: 31          moltype = DNA  length = 33
FEATURE               Location/Qualifiers
misc_feature          1..33
                      note = Intron_TS8 reverse primer
source                1..33
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 31
tcgggtacct gaattgcaag tagataaaga cca                                33

SEQ ID NO: 32          moltype = DNA  length = 33
FEATURE               Location/Qualifiers
misc_feature          1..33
                      note = Intron_TS10 forward primer
source                1..33
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 32
caagcgatcg caggtataca accgacacccg tct                                33

SEQ ID NO: 33          moltype = DNA  length = 29

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FEATURE	Location/Qualifiers
misc_feature	1..29
	note = Intron_TS10 reverse primer
source	1..29
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 33	
tcgggtacct aaagcaagga agggatca	29
SEQ ID NO: 34	moltype = DNA length = 35
FEATURE	Location/Qualifiers
misc_feature	1..35
	note = Intron_TS11 forward primer
source	1..35
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 34	
caaggcgtacg caggtccgtc ccggggcgcc cgat	35
SEQ ID NO: 35	moltype = DNA length = 32
FEATURE	Location/Qualifiers
misc_feature	1..32
	note = Intron_TS11 reverse primer
source	1..32
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 35	
tcgggtacct gaaaaagaca gagcaataca ag	32
SEQ ID NO: 36	moltype = DNA length = 33
FEATURE	Location/Qualifiers
misc_feature	1..33
	note = Intron_TS12 primer
source	1..33
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 36	
caaggcgtacg caggtaagcg acgacaacga gca	33
SEQ ID NO: 37	moltype = DNA length = 28
FEATURE	Location/Qualifiers
misc_feature	1..28
	note = Intron_TS12 reverse primer
source	1..28
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 37	
tcgggtacct ctaactaact agtagtaa	28
SEQ ID NO: 38	moltype = DNA length = 33
FEATURE	Location/Qualifiers
misc_feature	1..33
	note = Intron_TS13 forward primer
source	1..33
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 38	
caaggcgtacg caggtgagga gccttctctc tct	33
SEQ ID NO: 39	moltype = DNA length = 35
FEATURE	Location/Qualifiers
misc_feature	1..35
	note = Intron_TS13 reverse primer
source	1..35
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 39	
tcgggtacct ggcgcaaaaaa aaaatgtttg gttgt	35
SEQ ID NO: 40	moltype = DNA length = 34
FEATURE	Location/Qualifiers
misc_feature	1..34
	note = Intron_TS14 forwrad primer
source	1..34
	mol_type = other DNA
	organism = synthetic construct

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SEQUENCE: 40
caaggcgtatcg caggtatata ctctctctct ctca                                34

SEQ ID NO: 41      moltype = DNA  length = 36
FEATURE          Location/Qualifiers
misc_feature     1..36
note = Intron_TS14 reverse primer
source           1..36
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 41
tcgggtacct gcgagcggaa tgagaacaga ttagt                                36

SEQ ID NO: 42      moltype = DNA  length = 43
FEATURE          Location/Qualifiers
misc_feature     1..43
note = Intron_TS15 forward primer
source           1..43
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 42
cctccgttcc aaggcgtatcg aggttaagcag gagagagagc tct                                43

SEQ ID NO: 43      moltype = DNA  length = 41
FEATURE          Location/Qualifiers
misc_feature     1..41
note = Intron_TS15 reverse primer
source           1..41
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 43
aggctaagtt aaagtgcgggt acctgaaata tcaaagagga a                                41

SEQ ID NO: 44      moltype = DNA  length = 34
FEATURE          Location/Qualifiers
misc_feature     1..34
note = Intron_TS16 forward primer
source           1..34
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 44
caaggcgtatcg caggtgagat ttgcgagat tcgt                                34

SEQ ID NO: 45      moltype = DNA  length = 34
FEATURE          Location/Qualifiers
misc_feature     1..34
note = Intron_TS16 reverse primer
source           1..34
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 45
tcgggtacct gccaagcggag tatataaaaaa tcac                                34

SEQ ID NO: 46      moltype = DNA  length = 42
FEATURE          Location/Qualifiers
misc_feature     1..42
note = Intron_TS17 forward primer
source           1..42
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 46
cctccgttcc aaggcgtatcg aggttgaggcc gcgaccaaga ag                                42

SEQ ID NO: 47      moltype = DNA  length = 43
FEATURE          Location/Qualifiers
misc_feature     1..43
note = Intron_TS17 reverse primer
source           1..43
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 47
aggctaagtt aaagtgcgggt acctgcgaga gcccacggcc cga                                43

SEQ ID NO: 48      moltype = DNA  length = 43
FEATURE          Location/Qualifiers
misc_feature     1..43

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source          note = Intron_TS24 forward primer
                1..43
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 48
cctccgcttc aagcgatcgc aggtactaat aaactgtat ttc           43

SEQ ID NO: 49      moltype = DNA  length = 44
FEATURE          Location/Qualifiers
misc_feature     1..44
note = Intron_TS24 reverse primer
source          1..44
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 49
aggctaaggtaaagtcgggt acctgcaaaa tcggattca cggt           44

SEQ ID NO: 50      moltype = DNA  length = 46
FEATURE          Location/Qualifiers
misc_feature     1..46
note = Intron_TS27 forward primer
source          1..46
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 50
cctccgcttc aagcgatcgc aggtgaggca tccgatcgat ttttct           46

SEQ ID NO: 51      moltype = DNA  length = 47
FEATURE          Location/Qualifiers
misc_feature     1..47
note = Intron_TS27 reverse primer
source          1..47
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 51
aggctaaggtaaagtcgggt acctgaaata tcaaagaggaa aaaatct           47

SEQ ID NO: 52      moltype = DNA  length = 100
FEATURE          Location/Qualifiers
source          1..100
mol_type = genomic DNA
organism = Zea mays
SEQUENCE: 52
gtccgttccc gtccccagatc cgtccatggc ttctgtccaga tctgacactgt cctgacacac 60
cctcacccgg atctgtccct cttcccccctc tccccctgcag           100

SEQ ID NO: 53      moltype = DNA  length = 147
FEATURE          Location/Qualifiers
source          1..147
mol_type = genomic DNA
organism = Zea mays
SEQUENCE: 53
gtctgtttcg tcctcgcttag gtttcatttc qcggctgtt tttgtccgttg gggctagatc 60
cgggtcgtag ttcaacagat ctgttcgtt ttgttacaga tctgcgttgc ctcgaatcga 120
gcatgacatgtt ttcatgtat tatgcag           147

SEQ ID NO: 54      moltype = DNA  length = 82
FEATURE          Location/Qualifiers
source          1..82
mol_type = genomic DNA
organism = Zea mays
SEQUENCE: 54
gtgcgtgcat ggcacacgtc tgcttctgcc tccctttccc ttttctccg aaagaactga 60
aacggAACGc atcttcgctc ag           82

SEQ ID NO: 55      moltype = DNA  length = 85
FEATURE          Location/Qualifiers
source          1..85
mol_type = genomic DNA
organism = Zea mays
SEQUENCE: 55
gtgcgtcaact gtccaggtgc ttgggttggc tcagaatatt gttggcggtg acactgtctt 60
ctctcgatcg atcgatcgat gacag           85

SEQ ID NO: 56      moltype = DNA  length = 107
FEATURE          Location/Qualifiers

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source          1..107
               mol_type = genomic DNA
               organism = Zea mays
SEQUENCE: 56
gtcggtttcc aatctgttga ccatggatcc acagatcgga gcagttctt catagtactc 60
agcgatgtt ttgggtccta aatttcctt ccccggtgt tgtttag                                107

SEQ ID NO: 57      moltype = DNA  length = 604
FEATURE           Location/Qualifiers
source            1..604
               mol_type = genomic DNA
               organism = Zea mays
SEQUENCE: 57
gtatgttcc ttttttatttc cttegtgtca tatttctggg tgcgagttt gtgcctagat 60
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tgctggatc ggaatatgtt tttcaatta cttatctgca acttgtggat gcgactt 600
tcag                                              604

SEQ ID NO: 58      moltype = DNA  length = 659
FEATURE           Location/Qualifiers
source            1..659
               mol_type = genomic DNA
               organism = Zea mays
SEQUENCE: 58
gtaaagtatcg attgctgatt gctcggttgc gcttgcgttgc ttatgttgc 60
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SEQ ID NO: 59      moltype = DNA  length = 9794
FEATURE           Location/Qualifiers
misc_feature     1..9794
note = Vector Sequence
source            1..9794
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 59
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                      organism = synthetic construct
SEQUENCE: 71
gatctgcttc gtttggtac agatctgcgt tcgctcgaat cgag           44

SEQ ID NO: 72        moltype = DNA  length = 43
FEATURE             Location/Qualifiers
misc_feature         1..43
                      note = i2_sense oligonucleotide 4
source               1..43
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 72
catgacgttt tcatgtgtt atgcagctgg cgccttggga tcc            43

SEQ ID NO: 73        moltype = DNA  length = 52
FEATURE             Location/Qualifiers
misc_feature         1..52
                      note = i2_antisense oligonucleotide 1
source               1..52
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 73
ggcacaaaaca gaccgcgaaa tgaaacctag cgaggacgaa gcagacotgg cg      52

SEQ ID NO: 74        moltype = DNA  length = 53
FEATURE             Location/Qualifiers
misc_feature         1..53
                      note = i2_antisense oligonucleotide 2
source               1..53
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 74

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ctgtacaaa acgaagcaga tctgttgaac cacgaccgg atcttagcccc aac	53
SEQ ID NO: 75 moltype = DNA length = 45	
FEATURE Location/Qualifiers	
misc_feature 1..45	
note = i2_antisense oligonucleotide 3	
source 1..45	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 75 cataatcaca tgaaaacgtc atgctcgatt cgagcgaacg cagat	45
SEQ ID NO: 76 moltype = DNA length = 41	
FEATURE Location/Qualifiers	
misc_feature 1..41	
note = i2_antisense flanking primer	
source 1..41	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 76 gtggtaagcg aatgcaggaa tggatcccaa ggcccagct g	41
SEQ ID NO: 77 moltype = DNA length = 37	
FEATURE Location/Qualifiers	
misc_feature 1..37	
note = i3_sense flanking primer	
source 1..37	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 77 ccatcgtac tcgatatccc ggactggcgc caggtgc	37
SEQ ID NO: 78 moltype = DNA length = 45	
FEATURE Location/Qualifiers	
misc_feature 1..45	
note = i3_sense oligonucleotide 2	
source 1..45	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 78 gtgcatgcgc acgctctgct tctgcctccc ttccctttt cctcc	45
SEQ ID NO: 79 moltype = DNA length = 50	
FEATURE Location/Qualifiers	
misc_feature 1..50	
note = i3_sense oligonucleotide 3	
source 1..50	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 79 gaaagaactg aaacggaacg catttcgct cagctggcgc ctggggatcc	50
SEQ ID NO: 80 moltype = DNA length = 39	
FEATURE Location/Qualifiers	
misc_feature 1..39	
note = i3_antisense oligonucleotide 1	
source 1..39	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 80 tgcgcatgca cgcacctggc gccagtccgg gatatcgag	39
SEQ ID NO: 81 moltype = DNA length = 54	
FEATURE Location/Qualifiers	
misc_feature 1..54	
note = i3_antisense oligo2	
source 1..54	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 81 cgttccgtt cagtttttc ggaggaaaaag gggaaagggg gcagaaggcag agcg	54
SEQ ID NO: 82 moltype = DNA length = 43	
FEATURE Location/Qualifiers	
misc_feature 1..43	
note = i3_antisense flanking primer	

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source	1..43	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 82		
gtcgagggtga tgtggatccc aaggcgccag ctgagcaga atg		43
SEQ ID NO: 83	moltype = DNA length = 50	
FEATURE	Location/Qualifiers	
misc_feature	1..50	
	note = i4_sense flanking primer	
source	1..50	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 83		
ccatcagata tcccgactg gcgccaggtg cgtcaactgtc caggtgcttg		50
SEQ ID NO: 84	moltype = DNA length = 50	
FEATURE	Location/Qualifiers	
misc_feature	1..50	
	note = i4_s2_sense oligo 3	
source	1..50	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 84		
gcttggatca gaatattgtt ggcggtgaca ctgtcttc tcgatcgatc		50
SEQ ID NO: 85	moltype = DNA length = 45	
FEATURE	Location/Qualifiers	
misc_feature	1..45	
	note = i4_sense oligonucleotide 3	
source	1..45	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 85		
gatcgatgac agctggcgcc ttgggatcca catcaatcac catgc		45
SEQ ID NO: 86	moltype = DNA length = 49	
FEATURE	Location/Qualifiers	
misc_feature	1..49	
	note = i4_antisense oligonucleotide 1	
source	1..49	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 86		
caaatattctg atccaagCCA agcacctgga cagtgacgca cctggcgCC		49
SEQ ID NO: 87	moltype = DNA length = 50	
FEATURE	Location/Qualifiers	
misc_feature	1..50	
	note = i4_antisense oligonucleotide 2	
source	1..50	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 87		
cggcagctgt catcgatcga tcgatcgaga gaagacagtg tcaccgccaa		50
SEQ ID NO: 88	moltype = DNA length = 43	
FEATURE	Location/Qualifiers	
misc_feature	1..43	
	note = i4_antisense flanking primer	
source	1..43	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 88		
gcttggAACGA tggaatgcat ggtgattgtat gtggatcccc agg		43
SEQ ID NO: 89	moltype = DNA length = 48	
FEATURE	Location/Qualifiers	
misc_feature	1..48	
	note = i5_flanking sense primer	
source	1..48	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 89		
ccatcagata tcccgactg gcgccaggtc ggTTCCAAT ctgttgac		48

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SEQ ID NO: 90	moltype = DNA length = 47
FEATURE	Location/Qualifiers
misc_feature	1..47
	note = i5_sense oligonucleotide 2
source	1..47
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 90	
catggatcca cagatcgagg cagttcttc atagtactca gcgatct	47
SEQ ID NO: 91	moltype = DNA length = 50
FEATURE	Location/Qualifiers
misc_feature	1..50
	note = i5_sense oligonucleotide 3
source	1..50
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 91	
gtttgggtcc taaatttctt ttccccggct gttgttagc tggcgcccttg	50
SEQ ID NO: 92	moltype = DNA length = 50
FEATURE	Location/Qualifiers
misc_feature	1..50
	note = i5_antisense oligonucleotide 1
source	1..50
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 92	
ctccgatctg tggatccatg gtcaacagat tggaaaccga cctggcgcca	50
SEQ ID NO: 93	moltype = DNA length = 45
FEATURE	Location/Qualifiers
misc_feature	1..45
	note = i5_antisense oligonucleotide 2
source	1..45
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 93	
gaaaattttagg acccaaacag atcgctgagt actatgaaag aactg	45
SEQ ID NO: 94	moltype = DNA length = 44
FEATURE	Location/Qualifiers
misc_feature	1..44
	note = i5_antisense flanking primer
source	1..44
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 94	
cgtatgtat ctcaaggcgc cagctaaaca acagccgggg aaag	44
SEQ ID NO: 95	moltype = DNA length = 535
FEATURE	Location/Qualifiers
source	1..535
	mol_type = genomic DNA
	organism = Zea mays
SEQUENCE: 95	
gtccgccttg ttctctctct gtctcttgat ctgactaatac ttggtttatg attcggttag 60 taattttggg gaaagcttcg tccacagttt ttttcgatg aacagtgcg cagttggcgct 120 gtatctgtat gtatctctgc aatcggtgtg aacttatttc ttatctccca ttactccca 180 tgaaaaggct agtaatcttt ctcgatgtaa catcgccag cactgtctatt accggtgttgt 240 ccatccgaca gtctggctga acacatcata cgatctatgg agcaaaaaatc tatctccct 300 gttctttaat gaaggacgtc atttcatta gtatgtatca ggaatgttgc aacttgcag 360 gaggcggttc ttctttgaa tttaactaac tcggttgatg gccctgttcc tcggacgtaa 420 ggcccttgct gtcacacaca tgcatttcg aattttaccc tgtttagcaa gggcgaaaag 480 tttgcattt gatgatgtat cttgactatg cgatgtctt cctggacccg tgcag 535	
SEQ ID NO: 96	moltype = DNA length = 344
FEATURE	Location/Qualifiers
source	1..344
	mol_type = genomic DNA
	organism = Zea mays
SEQUENCE: 96	
gtacagtaca cacacatatg tataatatgtat tgatgtatcc cttcgatcga aggcatgcct 60 tggtcgaata actgagtagt catttttata cgttattttg acaagtcagt agttcatcca 120 tttgcattccat ttttcagct aggaagttt gttacactgg ctttggctta ataactgagt 180 agtcatttttta ttacgttgg tgcacaagtc agtagctcat ccatctgtcc cattttttc 240	

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acttaggaag ttgggttaca ctggacttg tctaataact gagtagtcat tttattacgt 300
tgttcgaca agtcattagc tcatccatct gtcccattt tcag 344

SEQ ID NO: 97      moltype = DNA  length = 1014
FEATURE           Location/Qualifiers
source            1..1014
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 97
gtatgcttc tctctgttc atctccgtgc taaacctctg tctctgggt gggttttgc 60
tgggatttt agctaactcg ctggccggg tagaaaaagac cgtgcacct gatgagctca 120
agcgcgtccc tttagccgcgt cctgtcccc cgccattctc tgccgttgc ctgtgttccc 180
gtgactcgcc ggggtgcgtca tcegcgtaaat cttgtctggg ctctgcgtac atgttctgg 240
ctagtgggtt ttatagatc ctctgcgtca aaaccgtgc tgcgtgcgc acagaactct 300
ccctgtctt ctctgggggt ttgggttacg ttgggttagt aagttggat ttgcacatgg 360
ataaaagtgtt tctaaggctc gtgggttgcgt tgagatcttgc tgcgttattgc gtgcgtgtc 420
cacttctttt gcaatccgag gaatgaattt gtcgttact cggtttggg gattattagc 480
gcgaaaaaaa actctttttt ttgttctt tactacgaaa aagcatcttgc tggattttgc 540
tatcttttt tactacgaaa aacttttgcgt tcttaggaattt tgaatttgtg atgtccattc 600
ttgcagtgcg ctgtgttta ttggggagcc aaatccattt atttttgc tctagggtct 660
gaatggaatc agtactattt agacaaaatc aatccaatca agttgattt tttttttaaa 720
aatattatca cagaactaag tgcttgcgtc gaatcgtac tggcttttgc ttgggtggagg 780
atcaataactt gcttttttgc ttgggtggca actgttttgc tataagatc catgtgttcc 840
ttgttggatg aatcatatat agtatacgct catactacaa atctgtttt caaatttagg 900
ttgttggc atgtatcaatt ttttttgc ttttttgc ttttttgc ttttttgc ttttttgc 960
ttgttcttca acaactgggt ctgtgtatc ttgaccgtatc agtcgtatc gcag 1014

SEQ ID NO: 98      moltype = DNA  length = 1010
FEATURE           Location/Qualifiers
source            1..1010
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 98
gtacggccgt cgtccccc ccccccctt ctctacccctc tctagatcgg cggtccggc 60
catgggttagg gcccggtagt tctacttctg ttcatgtttt tggtagatcc gtgtttgtgt 120
tagatccgtg ctgcgtatcggt ctgcgtatcggt atgcgtccgtc tacgtcgac acgttctgt 180
tggtacttgc ccagggtttc tctttggggat atctgggttgc ggctctagcc gttccggcaga 240
cgggatcgat ttcatgtttt tttttgttgc gttgtatccgg gttttgggttgc cccttttctt 300
ttatccat atatccgttgc cactgttttgc tccgggtatccgttgc ttttttttgc 360
ttgggttgcgt tgcgtgttgc ttgggtggcgt gtcgttgcgt atccggatgt aattctgtttt 420
caaactactt ggtgttttgc ttaattttgc atctgtatgt gtgtccata catattcata 480
gttacgaattt gaagatgtatc gatggaaata tgcgtatcgttgc ataggatatac atgttgcgt 540
gggtttactt gatgtatccgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 600
ttgggtggccgt tgcgttgcgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 660
tgcgttgcgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 720
agatggatgg aatatccgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 780
atatacatgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 840
tataataaac aagtatgttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 900
tgcgttgcgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 960
actgttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 1010

SEQ ID NO: 99      moltype = length =
SEQUENCE: 99
000

SEQ ID NO: 100      moltype = length =
SEQUENCE: 100
000

SEQ ID NO: 101      moltype = DNA  length = 867
FEATURE           Location/Qualifiers
source            1..867
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 101
gtatgggtga cgtgggtggca ctgcgtatcg tgggttgc tctgttttgc ttgggtgcgg 60
gtatgggtgc cggcgatgggt gcaggccgtc cactccaaacg accccccggc gcaggttgc 120
gccaccacac agttcaggaa gctgttttgc atagttgaga ttgggtatcc atgtgcgtt 180
agcattccatc atggatagatc tttaggggtt gatgttttgc ccatgtccgc ggttattttgc 240
catatccaaa cgtgggggtt tgggttgcgttgc ttttttttgc ttttttttgc ttttttttgc 300
acttgcgttgc tggatgttgc gctatccaaa ttgttgcgttgc cacccttca ttttgcgtt 360
acttaattttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 420
catatgcgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 480
tgcgttgcgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 540
ttgttgcgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 600
aaatggatgttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc ttttttttgc 660

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ttgtctaaa aaggcatgg agagtctcg tacctcttt catattctg tacatcgtag 720
atagcagott tgatatctt tggatttt gtatttggt gtgaatccag atgagtgcac 780
ggtaacttctt cttaattcc cactaaact ttatgtgaaa gttaaatgt aacttgctg 840
ttgagttcc ataaatttct ctctcgtag 867
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SEQ ID NO: 102 moltype = DNA length = 847
FEATURE Location/Qualifiers
source 1..847
mol_type = genomic DNA
organism = Zea mays
SEQUENCE: 102
gtgcgacccc ctcgtattct cttecttcaa aagcatctc atcaccatct tcgattgtt 60
tctgatctgt cgctgaggag tgctgcaatt tgcaatttgc gggcgtagt actcgaatcg 120
gggtgaaga ctgaagtggc tttaggttagg gttttttttt ctgcagatct gttgttaga 180
ctactacatcc ctgcacgatt taagctgcca ccatcctgtg ttttaggtgt 240
atcgtgatt ttcgtttta atacttgcg qcagaaataa caggatgtc cgatcgatt 300
tggcatctcg tattttgtga tggcacgc aattcttgac agatttgcgat gtcgtataaa 360
gtcatctgcc gttccgtgcg cgggtttggat ctgggttgcg tttgttagaa ctgcgcatac 420
tttgggttgcg tttttttttt ctgcacgat cttttttacta ttttgtgaat 480
caagctctga ttctgttaact tttaactatg ttgttttcat ttcttcggcc tgatccaaaa 540
ttttccagtg gaacgtgtc ttctttgtg ctgtacaact gcaatatttc gtactcaag 600
ctctgattctt gtaaaaatttta cccatgttgc tcccatatttc cagatctgat gcaggaaatga 660
cgcttttagt ctatgttgcg ctgtgcact gtaatacgcg tttctcttc 720
ctatgtatggatgtcg ctgcgtttt gattcaataa ttattacac taatggcacc 780
tcgcacatcat gttcctttga ttcgtttatg atctgaacca cattaaacac cttcttattt 840
cacgcac 847
```

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SEQ ID NO: 103 moltype = DNA length = 1448
FEATURE Location/Qualifiers
source 1..1448
mol_type = genomic DNA
organism = Zea mays
SEQUENCE: 103
gtgaggccgc cccggggggg ttccctcagat ctggggccga tgctgggtcc gcctagatcc 60
acgcgtttctt cggctgtcc ggcgaggatt atgtttttttt agtgcgcgtc gtttgttaat 120
aggatatgcg caccgttctc taagagtggg tgtagatctt gccccggggta gaaggcccg 180
agttttgtca cccgttgcgtt tattttgtgg gtttgcgcgt gatctggcata tctgggggtg 240
aagttccat tatttagctg cggatgcctg ggtgagctcg aatgcatttc tatttttacc 300
ctcttcgggg accgcgtacat ctgttgcgtt agcacaataat catcatttttgcg 360
agcgcgttcc aggttgcgtt attttttgcg aatggaaagac aaatctatcat gtctgtgtgg 420
gggtgggtggg tgggggctcg gtacccctgg ggactaaca ttgggtgtatc ccttcccccc 480
ttcaaaacctt tcaaaactatg tacttttta tgatataattt ttgttcaacta ctggcaccgt 540
cccaactgtg gttttttttt aagggtgggtt catgtgtatg gtcactgtgtg ttttgtgtatg 600
attcgcattca cggggcccaa gttaggtgtgg caaacaattt ctttttccca cctttttggac 660
gtgtgtatgg tttttttttt ggtttttttt gttttttttt gttttttttt tttttttttt 720
ctttattgaa gtttgcgtt aatgcatttc cgtgttgcgtt ccttgcgtgtg gtgtgtcaatg 780
aaaagggtgtt gttttttttt gatctgcgtt ggcgccttc ttcttagacta 840
aaactcggtt atggaaatgtt cgtgtgtgtt cttttttttt ttgttgcgtt aaacacgtt 900
caagttttttt agagcaccat ttgttgcgtt gatctgcgtt ttgttgcgtt ttgttgcgtt 960
ttctctgcctt tttttttttt acgtgttgcgtt ttgttgcgtt ttgttgcgtt ttgttgcgtt 1020
atcagcatggt atggcccttag tttttttttt atacttgcgtt gatctgtggg gttttttttt 1080
taaggccgtt accaatctttt gacccgtat gatctgtgtt tttttttttt ttgttgcgtt 1140
gacacagggaa gatgtgtgtt ttgttgcgtt ttgttgcgtt ttgttgcgtt ttgttgcgtt 1200
acctgtgtgg ttgacaaatgtt gatctgtgtt gttttttttt gttttttttt gttttttttt 1260
tttctgtatcc tttttttttt aacttgcgtt gatctgtgtt tttttttttt ttgttgcgtt 1320
taccattgtt tttttttttt gatctgtgtt gatctgtgtt tttttttttt ttgttgcgtt 1380
tttacatctt atgtatccat accatcatgtt tgatctgtt aaaaaaaaaactt ttgttgcgtt 1440
atggacac 1448
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SEQ ID NO: 104 moltype = DNA length = 169
FEATURE Location/Qualifiers
source 1..169
mol_type = genomic DNA
organism = Zea mays
SEQUENCE: 104
gtccgtgcctttt cttttcaattt tcacccgcattt aagggttccattt cttttcatcg 60
tctgggtgtt tttttttttt ttgttgcgtt ttgttgcgtt ttgttgcgtt ttgttgcgtt 120
ttgttgcgtt ttgttgcgtt ttgttgcgtt ttgttgcgtt ttgttgcgtt ttgttgcgtt 169
```

```
SEQ ID NO: 105 moltype = DNA length = 1500
FEATURE Location/Qualifiers
source 1..1500
mol_type = genomic DNA
organism = Zea mays
SEQUENCE: 105
atctttttgtt gttttttttt gttttttttt gttttttttt gttttttttt gttttttttt 60
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aattttctct	cggctatgtg	aaccatcaaa	tatggtaact	aacaatatata	aaaatataag	120
ataaatttgtt	gotatggta	atcagtattt	tgtggcattt	tccatcgat	atgaaacattt	180
ctatgttagg	ctgcattcca	ttataaagt	atgtctgtt	tttacatagg	ccgataaata	240
tattnntcat	atctgtatcc	taaaaggttt	taggcttagt	aggtctaaa	cagagtata	300
gaagtgtat	ttcagtgc	tgggttatga	ggatggcgat	gttagtctt	atgggcaga	360
ggtaaccaag	aaagacactt	ttcgttactt	aggatcaat	cttcaaaagg	agggagacat	420
cgaggaggat	gtcagtca	gaattaaagt	cggatggttt	aagtggcgac	aagctgcggg	480
tgtcttatgc	gaccacggg	tgccacgaa	actaaaaggc	aaatttcata	ggacacgaa	540
ccggccgcgt	atgttgc	gagcagaat	ttggcccact	aaaagacgac	atgtccaa	600
actaagtgt	gcagagatgc	gtatgttgc	ctggatgtt	ggccacacaa	ggagagatc	660
agtccggaaat	gtatgtat	gagagagat	aggagtggcg	ccaatttggg	agaagcttat	720
gcaacatgc	tttgatgtt	ttggacat	ccaaacga	cctgaagg	caccagtgc	780
tatcgaata	attaggcgtc	ccgaaaatgt	gaagagagg	agaggtcgac	caacttgc	840
gtggacagag	gtgttga	gagacttgc	ggagtggaa	aatgacaag	agctcgcgc	900
agatagtag	gggttga	gtgcatttca	cgtgcacag	ccctgattt	tagttcgct	960
tttcctctt	aatcttgc	ccttttgc	tgtccattt	agatcttgc	ggctttgt	1020
ggttttatct	ctttatgt	tttcccgtt	tcgttgc	cggttctt	ttgccttgc	1080
ttcccttttc	tggttttt	gggttgc	ctgagg	catacgggtt	ttcatctca	1140
gcctacccca	acgtgttgc	gacaaaagg	tttgcgtt	gttgcgtt	ttgtatctgt	1200
atctaaag	gtgagagaga	agggttata	agaaaaacc	tcgtgcgtt	ccactgaa	1260
ccggggccaa	tttgcac	agacgtgc	ccaccgcact	acaagacca	ggccta	1320
gcccatcagg	aggcgcac	gcaatgccc	caaactaaa	ccctaccc	gcaagtata	1380
atatccccc	aacctcgtt	cttgcatttca	ttatcacggc	ggcgttgc	gagcgt	1440
cgaggagta	gcagcgc	gccccgc	ccatctcga	gttgc	ccacc	1500

SEQ ID NO: 106	moltype = DNA	length = 1500				
FEATURE	Location/Qualifiers					
source	1..1500					
	mol_type = genomic DNA					
	organism = Zea mays					
SEQUENCE: 106						
ataagcgcta	ctacttgaga	aatgat	tttac	aaaatgt	ttttatgc	60
atcaaactag	cacccaaat	taaacaa	atgttgc	tgtatctt	ttaagcgcta	120
tttgagaac	tctgtttt	caatgaa	atatttctc	acataaaattt	aattcattt	180
ttcttagaaa	aaaaaaaggaa	aatcttgc	aaaacagat	tcccaaaacta	gccataa	240
tgcacggagt	tttcttgc	cgagat	tcaat	tgcataa	gat	300
atgc	aaattt	atagcacc	ttttat	tttttttt	tttttttt	360
taacat	ttttat	tttttttgc	aaagat	tttttttt	ttatcgaa	420
tttgatgtt	tttgc	tttttttt	tttttttt	tttttttt	ttatcgcccc	480
attagaatgt	tttttttt	tttttttt	tttttttt	tttttttt	ttatcgat	540
tttctat	tttttttt	tttttttt	tttttttt	tttttttt	ttatcgat	600
ca	tttttttt	tttttttt	tttttttt	tttttttt	ttatcgat	660
ccat	tttttttt	tttttttt	tttttttt	tttttttt	ttatcgat	720
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tttttttt	tttttttt	tttttttt	tttttttt	tttttttt	ttatcgat	1140
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SEQ ID NO: 107	moltype = DNA	length = 1500				
FEATURE	Location/Qualifiers					
source	1..1500					
	mol_type = genomic DNA					
	organism = Zea mays					
SEQUENCE: 107						
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tagtacagga	aaaaggaa	gagtcgat	cgttcg	actgtctgt	tttgc	180
gttaggtacc	tatgttgggg	gagaagagat	cgttcgat	agtgttgc	tttgc	240
ttgaacgtat	acggatata	gtatgttgc	cattgc	aaagg	gccaagaca	300
ccatccatcc	attgttgc	gtagcaaa	aacagat	tctgttgc	agtctt	360
tgagccatac	tttctc	cggttgc	gacgtgggg	aatc	taatc	420
gcaaccaat	cgggggc	gtgagg	agaaatttac	tatgttgc	ctctca	480
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gtctcgttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	720
aatggacgt	aaatcatcg	aaatcg	ccagcgt	ccatcg	tgtgttgc	780

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gggttcgtg	tcgaatttc	acctggcact	gagagccgta	atagcacttc	ctgtccctgc	960
agcgtcaat	aatcccggtt	caaggatgg	gtaaatttgt	cgatagatc	gcaaatctta	1020
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gtccccagcc	gogcatatgt	tatgcgtgt	cgagtagtgc	gcagggtttc	tctcgccage	1140
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cacaagattc	caccatcacc	acccatgtt	ttagcggtta	taccccgctt	ccgttacccc	1260
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atggtgccct	cgaaacgccc	tccagactcc	agagagaacc	aggcatggc	tcagagagag	1380
agagaaagcg	gcgacaaga	accaaaaacga	acccaaacgg	aaaaggcgtt	tcttgcaaa	1440
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SEQ ID NO: 108 moltype = DNA length = 1500
 FEATURE Location/Qualifiers
 source 1..1500
 mol_type = genomic DNA
 organism = Zea mays

SEQUENCE: 108

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ggcgagtgt	gcacactgt	gccaaacctaa	ggaccaaaacg	tttagcccta	accattgcac	180
catgtgtcgc	ttatgttat	atagagtata	taatatgtat	tagtaacaat	ttgaaaattta	240
aaattaaat	catgtttgaa	aaaaatctc	atttaataata	aaaattatcat	atatgatata	300
tagaattcat	aacaatgtac	gagtaactaa	ctagttctat	acttaagcat	aaatagaaaag	360
cgttagcaatg	tgatgcacact	ttgtctagcg	gatattttga	tactgttag	aagtattaaa	420
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aagttaatgt	agtccatgtt	tcgtccatgt	aaaaataataa	tttgcataata	atagatttaat	540
tagacttaat	agatccatct	cgtcggttag	tctttatctta	tataattact	tttgcgttta	600
gactatattt	attttttagt	attgacattt	aaacatccga	tatgtccag	acttgatgtt	660
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atcaaatgt	attcacacaa	tcacacatct	gggcctaact	ttcatcggt	ctgtccacga	780
cggcgacccgt	gaggcgagggt	caattccctg	gccccagcat	agcttggagc	ttgcacgcta	840
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tcAACCCG	aaataaaaaa	tgacccatctt	catgggcac	gcaagccgac	aactaccac	960
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gcacgcggac	ggggcccgac	gggcgagaag	atcccgact	tgccccc	gaagatacag	1080
gatcaaggat	ttttaccgc	agtttctat	tccacgactt	tatccacacc	agcagattcg	1140
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gcctccctct	ttttgtact	agcacataga	gtttcgccgc	aatcgatcgc	cgactgactc	1440
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SEQ ID NO: 109 moltype = DNA length = 1500
 FEATURE Location/Qualifiers
 source 1..1500
 mol_type = genomic DNA
 organism = Zea mays

SEQUENCE: 109

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tacatccctc	gggtcgagg	gacccatca	tgaggccct	ccccccctaa	gtctagcttc	180
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ccttggaccc	cttcaaggct	ctgaaggctt	cttggatctt	atggcttta	atgtggaaac	360
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aaccccccgg	caagaatgt	catgggatgt	tggtccat	gccacaaatcg	tctctactta	480
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taagcattat	aaaataataag	caattacacc	gtatctgtat	gcatggactc	gagtagataa	720
ctatgtat	atataatcc	cacttgcgc	agatatgc	attacaatgt	catgttcaag	780
cgcattttgt	gtcccgatgt	agtccctgtc	tctaacaaga	tgaagtaatc	gagcatgttc	840
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cccccgtat	tccactggcc	tcggatgggg	tcaggacggc	cgccggcgcc	1440	
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SEQ ID NO: 110      moltype = DNA  length = 1500
FEATURE           Location/Qualifiers
source            1..1500
                  mol_type = genomic DNA
                  organism = Zea mays

SEQUENCE: 110
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aactctagcc cattttagact ttgtgagggg tcatttgcgt tttcttgcgtt gctttgttt 240
gttgggtttt ctttcttctt ctttcattt cttctcaaga aacttgcgtt caaaggcaaga 300
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SEQ ID NO: 111      moltype = DNA  length = 1500
FEATURE           Location/Qualifiers
source            1..1500
                  mol_type = genomic DNA
                  organism = Zea mays

SEQUENCE: 111
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SEQ ID NO: 112      moltype = DNA  length = 1500
FEATURE           Location/Qualifiers
source            1..1500
                  mol_type = genomic DNA
                  organism = Zea mays

SEQUENCE: 112
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SEQ ID NO: 113	moltype = DNA	length = 1500				
FEATURE	Location/Qualifiers					
source	1..1500					
	mol_type = genomic DNA					
	organism = Zea mays					
SEQUENCE: 113						
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gaaggcggtt	ggacacgaaat	caaaaagg	tagtacggg	accccgcgat	tttagcgat	1260
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agtgtccacg	gcccggccca	cccagtata	gctaactaca	cgacacccac	ctgttccct	1380
cggtctcc	accccggtcc	tctctgtcc	cgtcgcttcc	gcccctgc	gccccgact	1440
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SEQ ID NO: 114	moltype = DNA	length = 1500				
FEATURE	Location/Qualifiers					
source	1..1500					
	mol_type = genomic DNA					
	organism = Zea mays					
SEQUENCE: 114						
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ccagcaagg	ccaaagacat	ctccggctc	cacattggca	tgtacatgg	gctgtcgat	240
tctctcgct	agacacgtt	cactctgtt	caccccccatt	tgtacatct	gttcccttc	300
ttacgactat	aaaaggaaat	accaggcc	tcttagagg	ggttggcc	ggggggacga	360
ggacgagaca	ggcgcttc	tggggccgt	cggttccctt	accccgctt	acgtgttta	420
ccccctact	gcaagcgac	ccgacccccc	cggggacac	acacgaaggc	cgccggatct	480
ccacccctt	cacggccgtc	tccggccacc	tcgccttc	cccttgc	ctcaccacac	540
cgctcgaccc	atctgggtt	gggcacgc	cacactcact	cgtcggctcg	ggggcccccc	600
ggtctcgaaa	cgccgacaaa	accgttagaa	ataaataact	tccgagagg	aactgttgc	660
tcttagaaat	aaacataact	tcctacgtt	ttttttataaa	gttgcgttgg	tttagttt	720
acatgttgc	ccgtgtgtt	ggtcaagcg	gccaactact	tccttagagg	ggccgttgg	780
agtttagcat	ggcagctaac	ttcctgtcc	ctcctctaga	aaatgttactt	ttaattgtt	840
atcccgccgtt	gtggtcaat	gagccgttac	cctccctacgg	cttcctctag	aaagtttagat	900
ttcagctttt	gaccagccaa	acgaaaatgt	cgtgtcaag	attacaggaa	caatccaaag	960
attacacca	tcatattaa	atggaggat	caagaaaagg	agaatctaaa	tccttaagagc	1020

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tagtttata	acctcgaaaa	tttgacagtt	ctctaccgat	gttcgcacac	gcagcagccc	1080
tttgtccat	ttttttcccg	cgtccctctgt	gcccccaggta	ggggatgtgc	cctttataaaa	1140
ctttcacctc	caactcactc	gtatctccctt	tcgagagatt	ctgatatttt	ccatcaacaa	1200
gaaacagatt	tgtaaaattt	tcatcaggcc	acatccatata	gaccgcgtg	gaccacaaat	1260
ttataaaacac	agtggtaattt	ataacaagaa	aacaacattt	tgatggccaa	aatctaattt	1320
gttcatcttc	cttccgttc	gccaatttca	aatcgaaatc	gttccctaat	tcgaaatcga	1380
atgggggtcg	tcttgcgtgt	cgtgcgtgg	tgccgcgagc	acttgactgc	acccgacccc	1440
cctcccttca	gtccccacac	actgcaccc	ggccgcgggt	cctccttaggg	tttcgcgcgg	1500

SEQ ID NO: 115	moltype = DNA	length = 1500				
FEATURE	Location/Qualifiers					
source	1..1500					
	mol_type = genomic DNA					
	organism = Zea mays					
SEQUENCE: 115						
actggacta	gattcgatctc	gtcttttaat	cttcggctga	caaatttagtt	ttataatccg	60
actacatttta	atacccgaaa	cgagggttca	aacattcgat	gggacagggg	ctaaatttta	120
gaggggtgtt	acccaaacacc	cccgtagtcc	aaaactcgag	gttaatgggt	ctatgactta	180
attttttggg	acacaaaaaaac	ataaaaattt	ggaaaacaaa	tatattctt	agactcatag	240
gaacccttat	agattttccc	aaattttttt	tgatttttaa	aattcaatct	tttgaaccga	300
aaaaatttca	aattttacac	agatottgtat	tctgtgcagt	gttgggtatg	ggaaaagcg	360
aaaaaccatc	gttatgtttt	tgacaaatat	gaaaatggga	caaaaacaac	atgtgtgtt	420
tttcgacgtt	ttccgtttttt	cttgcgtttt	tcacaatagc	tgtttttat	ccacatata	480
tatctcattt	tagataatac	atgaacaaat	cataattgtat	tatattatct	ctcaacaaat	540
taacccgtaa	tgaatttattt	ttctttgata	gtcatatgtat	cattacaata	tttcgcttcc	600
atatgtatgg	atgtgtatgg	ttaatcgatt	gcaacactac	tttttattttt	atactctatg	660
tgacacattt	ttccgtttttt	atttacat	tatcccgatc	tgtttagat	atcgattttt	720
tccgtcccg	ttttatctta	tttgcgtatag	tttcaatttta	atctttttt	cgaaaataaag	780
tatgaaaata	aaaataagag	agattgttac	gttcgatccg	gttttgaacc	ctagctatac	840
tttgcgcgtt	ttgcacttgg	ccggccattt	cataggcggg	cacagtccg	actcagcagt	900
gacagagatgc	gcgtgcgacaa	cacacgttca	aatttcaaaa	ctgaaaacggg	cggtataaaa	960
cagaacccgc	tgctcccgagg	agccctacgc	agataaattt	acccacatca	atggggccca	1020
aatattttata	accatctttt	ggtcccacat	gttcgtgtca	caacatctc	taccccgagg	1080
aaagatagcc	gtctcgccaa	gaccccgago	ccggccggctc	cgccggaccc	gccgcggact	1140
cacacccacc	gttgcggcc	gctgagccgt	tcqaaggccaa	aacgggttgc	aaccacccag	1200
gctgcgcgtt	cggttaccat	cacgcgttta	gccccggaa	agacggcgcc	taggttctcc	1260
ggcccgccgg	gogccatcac	ggggccggcc	cgccgcgttc	tcccaacgtt	cctataaaaag	1320
ccggccggccgg	gctgagccgg	attatcgctt	cagctccggc	tcttcacaaa	cgccggcgca	1380
aactctcgcc	cgagcccgac	agatcttcaa	ttccccattt	cgcccaacga	tcgaccttca	1440
cgccagttcc	ggtcttcttc	gaaggcgtcg	cgccgggtt	tttgagagga	gaggaggaag	1500

SEQ ID NO: 116	moltype = DNA	length = 1500				
FEATURE	Location/Qualifiers					
source	1..1500					
	mol_type = genomic DNA					
	organism = Zea mays					
SEQUENCE: 116						
atgagctatt	atccataaaaa	ttatctaata	ttcatttattt	ttccataaaat	tgtatcattt	60
tgcaggatcc	gogagcttgg	acgagccgg	tcggctcgcc	tcgctgaaaa	aacgacgtcg	120
aaacaggaggc	tcaagctcgcc	tcgtttgagg	ctcgccggcc	gtccggagct	cgagccggct	180
cgccgagctc	gagcttattt	tccaaaccctt	gccccctggcc	agtggatgtt	gttcccccgt	240
gagcgttccaa	cggtccccc	cccccttcac	gtcttttcgt	gtggagatctc	tcaccccttc	300
tccgcttaatc	agctataata	attacaaaat	taatttttttt	atttactttt	cagataacaa	360
tatgttattat	aacactacaa	aaaattgtat	aatcattttt	aattccaaaa	accgtatgtt	420
aaaaggtaaa	taaccccgagg	ttaaggggac	ttgaatagga	aatgtttttt	atatgaggaa	480
aaataaaggga	catgtttttt	aggatagata	tatthaataa	taaaatagaaa	ttatgaatgt	540
aggggatttt	gaggggggat	gttttttttt	agcataaaga	taagatgtt	ccaccttct	600
tgagccatcg	tccgtctcgct	ggcaggatgt	gatatatggc	catatgggtt	tttgcgttttt	660
tcagttgacc	ccgataaaaa	tttaataat	tttaaaaaat	caactttttt	tgaaaaaaca	720
tttgcgtatca	taatgtatgg	gacccttattt	cgacatcat	ctttgttttt	tttgcgtttt	780
ctcgctatcg	cttttttttt	gacagatgtt	gcacgttggg	gggtgttttt	ttcttttttt	840
agcacaggct	agcatgttta	ggctgcctta	tcttgcgtat	cttgcgttcc	aaatataacc	900
atggacaattt	taaatagacac	aacgacatgc	atgtcgatgc	tgtttttttt	caggaaggcc	960
caccctgtgg	ccaggcttca	atccggatgt	gttggatgtt	tctccgtccg	ctcgcccttgc	1020
cagcgcgggg	gagccgat	ggccggccgt	ccgttcaac	gacggggaaa	agcttagctt	1080
cccgacacac	ttaggttgc	aaacaaacgc	agtttctaa	cacaactagt	acccaaacacc	1140
ccgtcgctaa	tgcgtatcc	aagaagatca	ttagtttttt	caatgttgc	ggcaaatgac	1200
acgtcgttgc	aagcttactc	acttgcattca	gatctttcca	tgcctatgtt	tataacccta	1260
aataaacggg	ataagctaaa	agatattttt	ctcttggggcc	caccttggcc	cccgccggccca	1320
ccgcaccacgc	aaggccttgc	attttcacac	ctaacccttc	caagtttact	gcatttacga	1380
cccaacccccc	tttcttcttc	cttcggcc	cctccgttca	ttccacactc	cctctccgg	1440
acgcacccat	cccgctat	aaggcgtcg	cccgccggcc	aggcgttcc	cggtgcaggg	1500

SEQ ID NO: 117	moltype = DNA	length = 1500
FEATURE	Location/Qualifiers	
source	1..1500	

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mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 117
gtctttgagc tgagggttcc ctggagaag gtgattgttaa tggatgtacca tttggattt 60
atgaagggtc cttatactcc aacatgttgc accatttcgt gtgttccctt cttctgttcc 120
ttgttagtgc gtcagaact tgaacctccg tgatggggag caccagotc gtggccaaag 180
gtgtcacaac ttgatcacct tgcgaagccaa ttgtcggtgc cgtggaaatg agttctccgg 240
agggtggccat caatgttgc cacttgttgc cgaatgtgt gaattaaagaa caaggcaaca 300
cagtcgttag ggattaaaga cttctgttgc ccgaacatc gtttcttgc ggattcaatg 360
atcatcgac aaggccatg aaggacatgc cttcatcata tcataaaataa ataaaaatgt 420
aaagagataa atacattgc gattatctt taatacatcata atactgtac tccgtaaaac 480
atgtataat atcaataaaa ttcaatgttat attgatacatc tcggcttgc cgaagggtaa 540
gatgcgagcg agtgattaca attcagctgtg aacagtgggg tggttattgtt catctattta 600
taggcacccgg acgcataccca gggaaatata cattcagccac cttcaacatt catctagaga 660
caaccttagat taacaagggtc tatctgttgc ttcttcttgc tgcttgactt gaacagaaac 720
taaatgttag ctgttgcatt tgactatgtt gattctacatc taatgttgc ttgaaatgtt 780
tcggcagaaa aaaagttagac ttatgttacca tttaccggaa gatgttttg ttgaaaactt 840
ttgtcgaaaaa aagaagacacc caacacttca cgtccggccca ataaatgttgc acgtcgatc 900
catgtactat ggcgttcaaa aaagatcaa agaatacatcata tacatctttt ataaaaccca 960
acgtatattt ttttagttaaa aaataaaaat gcaaaaaaaaaa atggttccc acagccctag 1020
caaaacacaa tgcgcacttc attccacccc ccgttgcattt gatgtttggg gacaaaaata 1080
caggggaccca catgtcgatc aaaatctgcatc catgcgttgc gtcatggcac ggaatgttgc 1140
tttactacta ggcaaccaggc acgttgcatttgc ctgactgtgg ggcacccgtt accttgggtc 1200
cacaaggctac tggatgttgc ttcttgcattt ttccgtttaaag ccataatct tcaatgttgc 1260
ccaggaaattt gggccggcc ttcttgcattt ttccgtttaaag ccataatct tcaatgttgc 1320
acgggtccaa gattatgttgc ggcacccatgtt gcaatgttgc aagctgttgc cggacccgtt 1380
acgcgaggtc tggatgttgc cggccggccatc tggatgttgc atggggaaagg ggattaaata 1440
tcgtcgacccggc aacccttttgc gtccttgc cccgttttttgc ttgggggtttt 1500

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SEQ ID NO: 118      moltype = DNA length = 1329
FEATURE          Location/Qualifiers
source           1..1329
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 118
gttaactcgca tcatccatcc tcccgcttcc actctccctt caccccttgc gcttgcgtt 60
tatacgaaca tacgatttat tacgggttat atgggggtt cgttccatc atctggcgat 120
ctattatcgat agctccgatc cctcgatcta gtaattgtgg gatgttgcgtt taaggggtc 180
tgagatgggtt tgggtgggtt tgggtcggtt tgacgttcc aacagcccttgc ttctttaggg 240
ttggatgttcc tggatgttgc ttcttgcattt aaataatgttgc ctgatgttgc atggatgttgc 300
ctttagatgttgc gaaaccttgcat cttatgttgc ttttgcatttgc ttttgcatttgc 360
ccaaacaggcttgc ttttgcatttgc gcttgcgtt ctgggttgcgtt agatcgatc ttttgcatttgc 420
ggctcgatc ttttgcatttgc ccgttgcgtt ccgttgcgtt atggatgttgc atggatgttgc 480
tgcgttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 540
gtgtatcgatc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 600
caacccgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 660
ctggccgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 720
atataatgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 780
tgcgttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 840
cctatctgtt ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 900
tttcactgtt ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 960
gttctatataca catgttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 1020
tgcgttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 1080
gttgaacatgtt ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 1140
ttgtcgccgtt ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 1200
tggcatgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 1260
tataatgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 1320
tcttgcgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 1380

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SEQ ID NO: 119      moltype = DNA length = 1076
FEATURE          Location/Qualifiers
source           1..1076
mol_type = genomic DNA
organism = Zea mays

SEQUENCE: 119
gacggagatc aacgtccctt ttttgcatttgc ttttgcatttgc ttttgcatttgc 60
aaaacactttt cacttataat ttttgcatttgc ttttgcatttgc ttttgcatttgc 120
aaacatattt gtacaatgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 180
tagtgcgttgc aacatccatc acgttgcgttgc ttttgcatttgc ttttgcatttgc 240
aaataatgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 300
atccatgttgc aacatccatc acgttgcgttgc ttttgcatttgc ttttgcatttgc 360
tggatgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 420
ctcgatgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 480
aatttgcgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 540
actgttgcgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 600
attatttgcgttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc ttttgcatttgc 660

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ttataaaaaa tccactaaaaa acaggtaaaa taagcttca attttacact acgaaaaagt	720
cagctttaa aaaaaactgc tttaaatccag tccttttagt taattttat cttttagaa	780
acaaaaagcca aaactaaaaac caaaccaaac ctacctttaa aaccgatcta ataggaacgc	840
ggtgttttgg acaactagat attaattta gaggttagac cgccacgaaa gcgtcactgc	900
acacggcatt cccctccccct agcgttatcg tcgcaccata aataaccate ctcttcctcgc	960
cttccccac atctcatctt cgctgtgtt ctggggcata cgccggacaca gccccgatcc	1020
gaatcgctgt ctttgcgagc ctgcggatc ccccactccc ctccccctcgc ttcaag	1076
 SEQ ID NO: 120 moltype = DNA length = 42	
FEATURE Location/Qualifiers	
misc_feature 1..42	
note = TS2 fwd primer	
source 1..42	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 120	
cctcccgcttc aagcgatcgc aggttaactgc gatcatccat cc	42
 SEQ ID NO: 121 moltype = DNA length = 46	
FEATURE Location/Qualifiers	
misc_feature 1..46	
note = TS2 rev primer	
source 1..46	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 121	
aggctaagg aaagtccccgtt acctgcaagg aaaccaaaaa aaggtt	46
 SEQ ID NO: 122 moltype = DNA length = 42	
FEATURE Location/Qualifiers	
misc_feature 1..42	
note = pTS2 fwd primer	
source 1..42	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 122	
tccaaaggctt ggcgcgcgcac ggagatcaac gtccctattt ac	42
 SEQ ID NO: 123 moltype = DNA length = 42	
FEATURE Location/Qualifiers	
misc_feature 1..42	
note = pTS2_TS2 rev primer	
source 1..42	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 123	
acaggacgga ccatggctgc aaggaaacca aaaaaaggta ag	42
 SEQ ID NO: 124 moltype = DNA length = 42	
FEATURE Location/Qualifiers	
misc_feature 1..42	
note = pTS2 reverse primer	
source 1..42	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 124	
acaggacgga ccatggccctt gaagcgaggg gagggggagtgg gg	42
 SEQ ID NO: 125 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
misc_feature 1..33	
note = pTS1 fwd primer	
source 1..33	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 125	
cttggcgccgc ctgccacgca aactaaaagg caa	33
 SEQ ID NO: 126 moltype = DNA length = 42	
FEATURE Location/Qualifiers	
misc_feature 1..42	
note = pTS1 rev primer	
source 1..42	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 126	

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cctgcgatcg cgggtggcaag ctcgagatgg ggagccgcta ct	42
SEQ ID NO: 127	moltype = DNA length = 39
FEATURE	Location/Qualifiers
misc_feature	1..39
	note = pTS27 fwd primer
source	1..39
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 127	
tcgaagttg gcgccccccg gctataccgc tcccgccct	39
SEQ ID NO: 128	moltype = DNA length = 42
FEATURE	Location/Qualifiers
misc_feature	1..42
	note = pTS27 rev primer
source	1..42
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 128	
ggatgcctca cctgcgatcg caggacgaag cggcgatcgg gc	42
SEQ ID NO: 129	moltype = DNA length = 21
FEATURE	Location/Qualifiers
misc_feature	1..21
	note = GUS-F primer
source	1..21
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 129	
tttacgtggc aaaggattcg a	21
SEQ ID NO: 130	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = GUS-R primer
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 130	
gccccaatcc agtccattaa	20
SEQ ID NO: 131	moltype = DNA length = 21
FEATURE	Location/Qualifiers
misc_feature	1..21
	note = Gr5-F primer
source	1..21
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 131	
ggcagtttg ttgatgctca t	21
SEQ ID NO: 132	moltype = DNA length = 26
FEATURE	Location/Qualifiers
misc_feature	1..26
	note = Gr5-R primer
source	1..26
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 132	
tgtgttatat ctttgctttg aaccat	26
SEQ ID NO: 133	moltype = DNA length = 23
FEATURE	Location/Qualifiers
misc_feature	1..23
	note = GUS probe
source	1..23
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 133	
aacgtgtga tggcacgca cca	23
SEQ ID NO: 134	moltype = DNA length = 17
FEATURE	Location/Qualifiers
misc_feature	1..17
	note = Gr5 probe

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source          1..17
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 134
ttgaagtac aaagcca                                17
SEQ ID NO: 135          moltype = DNA  length = 50976
FEATURE
misc_feature      1..50976
source           note = vector sequence
               1..50976
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 135
gttaccatcg caatatatcc tgtcaaacac tgatagttt aactgaaggc gggaaacgac 60
aatctgtatca ttagccggaga attaaggqag tcacgttatg acccccgcgg atgacccggg 120
acaaggccgtt ttacgtttgg aactgacaga accgcaacgt tgaaggagcc actcagcaag 180
ctggatcgat ttaatacata ctcatactatg ggcaatttgat ggcgttgtt aacgtcttc 240
aactggaa ggcgttaccgg acgttgttgg tgcgttgtt aacgttgtt acaaggttgt 300
acaaaaaaaac aggttttaac ttcgaaacgcg gtggaccgaa gtttgcgtgc ctgcgttgca 360
gcgttaccgg gtcgtgtcccc tctcttagaga taatgagcat tgcgttgtt agttataaaa 420
aatttaccca tttttttttt gtcacactt gtttgcgtt aactttatcta tctttatata 480
tatatttaaa cttttacttata cgaataataat aatctatagt aactacaataa tatcgttgtt 540
tttagagaata atataataatgaa acgttgttgcg tttttttttt ggcataatggat gtatggac 600
aacaggactc tacaggatcc tttttttttt gtttgcgttgtt tttttttttt tttttttttt 660
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ttaagaaaac tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 840
taaaatataaag tgactaaaaaa tttttttttt tttttttttt tttttttttt tttttttttt 900
acatggttttt ttgtttcgat agataatggc accctttttt tttttttttt tttttttttt 960
ggcacccaaaac tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1020
tctctgtgtcc tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1080
ctgttccggat tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1140
cttcttccccc tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1200
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tcgttgcgtt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1320
cctccggatcc aaggatccgc tttttttttt tttttttttt tttttttttt tttttttttt 1380
cgggtttccg tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1440
tccgttgcgtt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1500
gacacgtttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1560
ggccgttccggc aacacggatc gttttttttt tttttttttt tttttttttt tttttttttt 1620
tttgcctttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1680
gtttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1740
tagaattttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 1800
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organism = Zea mays

SEQUENCE: 143

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note = Vector sequence
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organism = synthetic construct

SEQUENCE: 144

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1. A recombinant DNA construct comprising an intron operably linked to a promoter, a heterologous polynucleotide, and a terminator wherein the intron comprises a nucleotide sequence that has at least 95% sequence identity to SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138.

2. (canceled)

3. (canceled)

4. The recombinant DNA construct of claim 1, wherein the promoter comprises a nucleotide sequence that has at least 95% identity to SEQ ID NO: 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 119, 136 or 139.

5. The recombinant DNA construct of claim 4, wherein the terminator comprises a nucleotide sequence that has at least 95% identity to SEQ ID NO: 140, 141, 142 or 143.

6. The recombinant DNA construct of claim 1, wherein the intron comprises the nucleotide sequence of SEQ ID NO: 4, 8, 13, 19, 52, 53, 56, 57, 58, 101, 102, 103, 104, 118, 137 or 138.

7. The recombinant DNA construct of claim 4, wherein the promoter comprises the nucleotide sequence of SEQ ID NO: 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 119, 136 or 139.

8. The recombinant DNA construct of claim 5, wherein the terminator comprises the nucleotide sequence of SEQ ID NO: 140, 141, 142 or 143.

9. (canceled)

10. The recombinant DNA construct of claim 1, wherein the intron enhances expression of the heterologous polynucleotide in a plant.

11. (canceled)

12. (canceled)

13. (canceled)

14. (canceled)

15. (canceled)

16. A plant comprising the recombinant DNA construct of claim 10.

17. A seed comprising the recombinant DNA construct of claim 10.

18. (canceled)

19. (canceled)

20. (canceled)

21. (canceled)

22. (canceled)

23. A method for identifying an intron useful for enhancing transgene expression in a monocotyledenous plant comprising the steps of:

- (a) scanning a plurality of monocot introns for the presence of a sequence motif identical to SEQ ID NO: 99;
- (b) selecting an intron from step (a) comprising a nucleotide sequence that contains at least one copy of a sequence motif identical to SEQ ID NO: 99; and
- (c) measuring the enhancing effect of the intron of step (b) on the expression of an operably linked heterologous polynucleotide.

24. (canceled)

25. A method for modulating transgene expression in a plant comprising the steps of:

- (a) introducing into a regenerable plant cell the recombinant DNA construct of claim 1;
- (b) regenerating a transgenic plant from the regenerable plant cell after step (a) wherein the transgenic plant comprises the recombinant DNA construct; and
- (c) obtaining a progeny plant derived from the transgenic plant of step (b), wherein the progeny plant comprises the recombinant DNA construct and exhibits enhanced transgene expression when compared to a plant comprising in its genome the recombinant DNA construct without the corresponding intron sequence.

26. The method of claim 25 wherein said plant is a monocot.

* * * * *