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(54) Title: PLANT REGULATORY ELEMENTS AND USES THEREOF

(57) Abstract: The invention provides DNA molecules and constructs, and their nucleotide sequences, useful for modulating gene expression in plants, and for specifying intracellular or extracellular localization of a gene product of interest. Transgenic plants, plant cells, plant parts, and seeds, comprising the DNA molecules operably linked to heterologous transcribable polynucleotides are also provided.

## **TITLE OF THE INVENTION**

### **PLANT REGULATORY ELEMENTS AND USES THEREOF**

#### **REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of United States provisional application Nos.  
5 61/295,160 filed January 14, 2010; 61/295,162 filed January 14, 2010; 61/339,057 filed  
February 26, 2010; 61/308,919 filed February 27, 2010; 61/308,921 filed February 27, 2010;  
and 61/331,924 filed May 6, 2010, all herein incorporated by reference in their entirety.

#### **INCORPORATION OF SEQUENCE LISTING**

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#### **FIELD OF THE INVENTION**

The invention relates to the field of plant molecular biology and plant genetic  
engineering and DNA molecules useful for modulating gene expression in plants, and for  
15 specifying intracellular or extracellular localization of a gene product.

#### **BACKGROUND**

Regulatory elements are genetic elements that regulate gene activity by modulating  
the transcription of an operably linked transcribable polynucleotide molecule. Such elements  
include promoters, leaders, introns, and 3' untranslated regions and are useful in the field of  
20 plant molecular biology and plant genetic engineering.

#### **SUMMARY OF THE INVENTION**

The present invention provides novel gene regulatory elements and transit peptide  
encoding sequences for use in plants. The present invention also provides DNA constructs  
comprising the regulatory elements and transit peptide encoding sequences. The present  
25 invention also provides transgenic plant cells, plants, and seeds comprising the regulatory  
elements and/or the transit peptide encoding sequences, operably linked to a transcribable  
polynucleotide molecule. The present invention also provides methods of making and using  
the regulatory elements, the DNA constructs comprising the regulatory elements, and the

transgenic plant cells, plants, and seeds comprising the regulatory elements operably linked to a transcribable polynucleotide molecule.

Thus, in one aspect, the present invention provides a DNA molecule comprising a DNA sequence selected from the group consisting of a) a sequence with at least 85 percent  
5 sequence identity to any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924; b) a sequence of any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924; and c) a fragment of any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924; wherein the fragment has gene regulatory activity or wherein the encoded peptide functions to localize an operably linked polypeptide within a cell; and wherein said sequence is operably linked to a heterologous transcribable  
10 polynucleotide molecule. In one embodiment, the sequence has at least 90 percent sequence identity to a DNA sequence selected from the group consisting of: SEQ ID NOs: 1-323 and SEQ ID NOS: 352-924. In another embodiment, the sequence has at least 95 percent sequence identity to a DNA sequence selected from the group consisting of: SEQ ID NOs: 1-323 and SEQ ID NOs: 352-924. In certain embodiments of the DNA molecule, the DNA  
15 sequence comprises a regulatory element. In some embodiments the regulatory element comprises a promoter. In particular embodiments, the heterologous transcribable polynucleotide molecule comprises a gene of agronomic interest, a gene capable of providing herbicide resistance in plants, or a gene capable of providing plant pest control in plants.

The invention also provides a plant cell comprising a DNA construct comprising a  
20 sequence of any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924, or a fragment or variant thereof, wherein said sequence is operably linked to a heterologous transcribable polynucleotide molecule. In certain embodiments, the transgenic plant cell is a monocotyledonous plant cell. In other embodiments, the transgenic plant cell is a dicotyledonous plant cell.

Also included is a transgenic plant, or part thereof, comprising a DNA molecule  
25 comprising a DNA sequence selected from the group consisting of: a) a sequence with at least 85 percent sequence identity to any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924; b) a sequence of any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924; and c) a fragment of any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924; wherein the fragment has gene regulatory  
30 activity or wherein the encoded peptide functions to localize an operably linked polypeptide within a cell; and wherein said sequence is operably linked to a heterologous transcribable polynucleotide molecule. In some embodiments a progeny plant of any generation of such a

transgenic plant, or a part thereof, is provided. Further, a transgenic seed, wherein the seed comprises such a DNA molecule is also provided.

In another aspect, the invention provides a DNA molecule comprising a DNA sequence encoding a chloroplast transit peptide, wherein the protein sequence of the encoded transit peptide is selected from the group consisting of: a) a transit peptide protein sequence of any of SEQ ID NOs: 324-350; and b) a transit peptide protein sequence with at least 95 percent sequence identity to any of SEQ ID NOs: 324-350; wherein the chloroplast transit peptide-encoding DNA molecule is operably linked to a heterologous transcribable polynucleotide molecule. In some embodiments, such a DNA molecule comprises a DNA sequence encoding a chloroplast transit peptide, wherein the DNA sequence is selected from the group consisting of SEQ ID NOs: 277-284, 289-293, 296, 301-304 and 307-316. The present invention thus provides a DNA construct encoding such a chloroplast transit peptide.

Also provided by the invention is a transgenic plant cell comprising a DNA sequence encoding a chloroplast transit peptide, wherein the sequence of the chloroplast transit peptide is selected from the group consisting of SEQ ID NOs: 324-350. In some embodiments, the transgenic plant cell is a monocotyledonous plant cell. In other embodiments, the transgenic plant cell is a dicotyledonous plant cell. A transgenic plant, or part thereof, comprising the DNA molecule is also contemplated by the invention, as well as a progeny transgenic plant of any generation, or part thereof, of the transgenic plant; and a transgenic seed, each comprising the DNA molecule.

### BRIEF DESCRIPTION OF THE FIGURES

- FIGS. 1a- 1c** depict alignment of size variants corresponding to SEQ ID NOs:24-25 for the Foxtail Millet Actin 8 promoter.
- FIGS. 2a- 2c** depict alignment of size variants corresponding to SEQ ID NOs:28-29 for the Foxtail Millet Alc1 promoter.
- FIGS. 3a- 3f** depict alignment of size variants corresponding to SEQ ID NOs:45-46 for the Foxtail Millet Cys promoter.
- FIGS. 4a- 4f** depict alignment of size variants corresponding to SEQ ID NOs:47-48 for the Foxtail Millet Dzs promoter.
- FIGS. 5a- 5c** depict alignment of size variants corresponding to SEQ ID NOs:58-59 for the Foxtail Millet Gst promoter.



**FIGS. 6a- 6C** depict alignment of size variants corresponding to SEQ ID NOs:60-61 for the Foxtail Millet Ifr promoter.

**FIGS. 7a- 7d** depict alignment of size variants corresponding to SEQ ID NOs:64-65 for the Foxtail Millet Nrt2 promoter.

5 **FIGS. 8a- 8e** depict alignment of size variants corresponding to SEQ ID NOs:74-75 for the Foxtail Millet Ppc promoter.

**FIGS. 9a- 9f** depict alignment of size variants corresponding to SEQ ID NOs:82-83 for the Foxtail Millet Prx3 promoter.

10 **FIGS. 10a- 10f** depict alignment of size variants corresponding to SEQ ID NOs:88-91 for the Foxtail millet Rcc3 promoter.

**FIGS. 11a- 11b** depict alignment of size variants corresponding to SEQ ID NOs:93-94 for the Foxtail Millet Ssp1 promoter.

**FIGS. 12a- 12d** depict alignment of size variants corresponding to SEQ ID NOs:96-97 for the Foxtail Millet Tip promoter.

15 **FIGS. 13a- 13d** depict alignment of size variants corresponding to SEQ ID NOs:98-99 for the Foxtail Millet TubA2-1 promoter.

**FIGS. 14a- 14c** depict alignment of size variants corresponding to SEQ ID NOs:102-103 for the Foxtail Millet Ubq1 promoter.

**FIG. 15** depicts transgene cassette configurations of the present invention.

20

## DETAILED DESCRIPTION OF THE INVENTION

The invention disclosed herein provides polynucleotide molecules having beneficial gene regulatory or other activity from foxtail millet, *Setaria italica*. The design, construction, and use of these polynucleotide molecules are one object of this invention. The nucleotide sequences of these polynucleotide molecules are provided among SEQ ID NO: 1 through  
25 SEQ ID NO:323 and SEQ ID NO:352 through SEQ ID NO:1060. These polynucleotide molecules are, for instance, capable of affecting the expression of an operably linked transcribable polynucleotide molecule in plant tissues, or of effecting localization of an encoded gene product, and therefore can selectively regulate gene expression, or activity of an encoded gene product, in transgenic plants. The present invention also provides methods  
30 of modifying, producing, and using the same. The invention also includes compositions, transformed host cells, transgenic plants, and seeds containing the promoters and/or other disclosed *S. italica* nucleotide sequences, and methods for preparing and using the same.

The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

## 5 DNA Molecules

As used herein, the term “DNA” or “DNA molecule” refers to a double-stranded DNA molecule of genomic or synthetic origin, *i.e.* a polymer of deoxyribonucleotide bases or a polynucleotide molecule, read from the 5’ (upstream) end to the 3’ (downstream) end. As used herein, the term “DNA sequence” refers to the nucleotide sequence of a DNA molecule.  
10 The nomenclature used herein is that required by Title 37 of the United States Code of Federal Regulations § 1.822 and set forth in the tables in WIPO Standard ST.25 (1998), Appendix 2, Tables 1 and 3.

As used herein, the term “isolated DNA molecule” refers to a DNA molecule at least partially separated from other molecules normally associated with it in its native or natural state. In one embodiment, the term “isolated” refers to a DNA molecule that is at least partially separated from the nucleic acids which normally flank the DNA molecule in its native or natural state. Thus, DNA molecules fused to regulatory or coding sequences with which they are not normally associated, for example as the result of recombinant techniques, are considered isolated herein. Such molecules are considered isolated even when integrated  
15 into the chromosome of a host cell or present in a nucleic acid solution with other DNA molecules.

Any number of methods well known to those skilled in the art can be used to isolate and manipulate a DNA molecule, or fragment thereof, disclosed in the present invention. For example, PCR (polymerase chain reaction) technology can be used to amplify a particular starting DNA molecule and/or to produce variants of the original molecule. DNA molecules, or fragment thereof, can also be obtained by other techniques such as by directly synthesizing the fragment by chemical means, as is commonly practiced by using an automated oligonucleotide synthesizer.  
25

As used herein, the term “sequence identity” refers to the extent to which two optimally aligned polynucleotide sequences or two optimally aligned polypeptide sequences are identical. An optimal sequence alignment is created by manually aligning two sequences, *e.g.* a reference sequence and another sequence, to maximize the number of nucleotide matches in the sequence alignment with appropriate internal nucleotide insertions, deletions,  
30

or gaps. As used herein, the term “reference sequence” refers to a sequence provided as the polynucleotide sequences of SEQ ID NOS: 1 through 323 and 352 through 924 or the polypeptide sequences of SEQ ID NOS: 324 through 350.

As used herein, the term “percent sequence identity” or “percent identity” or “% identity” is the identity fraction times 100. The “identity fraction” for a sequence optimally aligned with a reference sequence is the number of nucleotide matches in the optimal alignment, divided by the total number of nucleotides in the reference sequence, *e.g.* the total number of nucleotides in the full length of the entire reference sequence. Thus, one embodiment of the invention is a DNA molecule comprising a sequence that when optimally aligned to a reference sequence, provided herein as SEQ ID NOS: 1 through 323 and 352 through 924, has about 85 percent identity or higher, about 90 percent identity or higher, about 95 percent identity or higher, or at least 96 percent identity, 97 percent identity, 98 percent identity, or 99 percent identity to the reference sequence and has gene regulatory activity.

## 15 **Regulatory Elements**

A regulatory element is a DNA molecule having gene regulatory activity, *i.e.* one that has the ability to affect the transcription and/or translation of an operably linked transcribable polynucleotide molecule. The term “gene regulatory activity” thus refers to the ability to affect the expression pattern of an operably linked transcribable polynucleotide molecule by affecting the transcription and/or translation of that operably linked transcribable polynucleotide molecule. Gene regulatory activity may be positive and/or negative and the effect may be characterized by its temporal, spatial, developmental, tissue, environmental, physiological, pathological, cell cycle, and/or chemically responsive qualities as well as by quantitative or qualitative indications.

Regulatory elements such as promoters, leaders, introns, and transcription termination regions are DNA molecules that have gene regulatory activity and play an integral part in the overall expression of genes in living cells. The term “regulatory element” refers to a DNA molecule having gene regulatory activity, *i.e.* one that has the ability to affect the transcription and/or translation of an operably linked transcribable polynucleotide molecule. Isolated regulatory elements, such as promoters and leaders, that function in plants are therefore useful for modifying plant phenotypes through the methods of genetic engineering.

Regulatory elements may be characterized by their expression pattern, *i.e.* as constitutive and/or by their temporal, spatial, developmental, tissue, environmental,

physiological, pathological, cell cycle, and/or chemically responsive expression pattern, and any combination thereof, as well as by quantitative or qualitative indications. A promoter is useful as a regulatory element for modulating the expression of an operably linked transcribable polynucleotide molecule.

5 As used herein, a “gene expression pattern” is any pattern of transcription of an operably linked DNA molecule into a transcribed RNA molecule. Expression may be characterized by its temporal, spatial, developmental, tissue, environmental, physiological, pathological, cell cycle, and/or chemically responsive qualities as well as by quantitative or qualitative indications. The transcribed RNA molecule may be translated to produce a  
10 protein molecule or may provide an antisense or other regulatory RNA molecule, such as a dsRNA, a tRNA, an rRNA, a miRNA, and the like.

As used herein, the term “protein expression” is any pattern of translation of a transcribed RNA molecule into a protein molecule. Protein expression may be characterized by its temporal, spatial, developmental, or morphological qualities as well as by quantitative  
15 or qualitative indications.

As used herein, the term “promoter” refers generally to a DNA molecule that is involved in recognition and binding of RNA polymerase II and other proteins (trans-acting transcription factors) to initiate transcription. A promoter may be initially isolated from the 5’ untranslated region (5’ UTR) of a genomic copy of a gene. Alternately, promoters may be  
20 synthetically produced or manipulated DNA molecules. Promoters may also be chimeric, that is a promoter produced through the fusion of two or more heterologous DNA molecules. Promoters useful in practicing the present invention include SEQ ID NOS: 23 through 105 and SEQ ID NOS: 353 through 536 or fragments or variants thereof.

In one embodiment, fragments are provided of a promoter sequence disclosed herein.  
25 Promoter fragments may exhibit promoter activity, and may be useful alone or in combination with other promoters and promoter fragments, such as in constructing chimeric promoters. In specific embodiments, fragments of a promoter are provided comprising at least about 50, 95, 150, 250, 500, or about 750 contiguous nucleotides of a polynucleotide molecule having promoter activity disclosed herein.

30 A promoter or promoter fragment may also be analyzed for the presence of known promoter elements, *i.e.* DNA sequence characteristics, such as a TATA-box and other known transcription factor binding site motifs. Identification of such known promoter elements may be used by one of skill in the art to design variants of the promoter having a similar expression pattern to the original promoter.

As used herein, the term “enhancer” or “enhancer element” refers to a cis-acting transcriptional regulatory element, a.k.a. cis-element, which confers an aspect of the overall expression pattern, but is usually insufficient alone to drive transcription, of an operably linked polynucleotide sequence. Unlike promoters, enhancer elements do not usually include a transcription start site (TSS) or TATA box. A promoter may naturally comprise one or more enhancer elements that affect the transcription of an operably linked polynucleotide sequence. An isolated enhancer element may also be fused to a promoter to produce a chimeric promoter.cis-element, which confers an aspect of the overall modulation of gene expression. A promoter or promoter fragment may comprise one or more enhancer elements that effect the transcription of operably linked genes. Many promoter enhancer elements are believed to bind DNA-binding proteins and/or affect DNA topology, producing local conformations that selectively allow or restrict access of RNA polymerase to the DNA template or that facilitate selective opening of the double helix at the site of transcriptional initiation. An enhancer element may function to bind transcription factors that regulate transcription. Some enhancer elements bind more than one transcription factor, and transcription factors may interact with different affinities with more than one enhancer domain. Enhancer 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 DNA sequence similarity analysis using known cis-element motifs or enhancer elements as a target sequence or target motif with conventional DNA sequence comparison methods, such as BLAST. The fine structure of an enhancer domain can be further studied by mutagenesis (or substitution) of one or more nucleotides or by other conventional methods. Enhancer elements can be obtained by chemical synthesis or by isolation from regulatory elements that include such elements, and they can be synthesized with additional flanking nucleotides that contain useful restriction enzyme sites to facilitate subsequence manipulation. Thus, the design, construction, and use of enhancer elements according to the methods disclosed herein for modulating the expression of operably linked transcribable polynucleotide molecules are encompassed by the present invention.

As used herein, the term “leader” refers to a DNA molecule isolated from the untranslated 5’ region (5’ UTR) of a genomic copy of a gene and defined generally as a nucleotide segment between the transcription start site (TSS) and the protein coding sequence start site. Alternately, leaders may be synthetically produced or manipulated DNA elements.

A leader can be used as a 5' regulatory element for modulating expression of an operably linked transcribable polynucleotide molecule. Leader molecules may be used with a heterologous promoter or with their native promoter. Promoter molecules of the present invention may thus be operably linked to their native leader or may be operably linked to a heterologous leader. Leaders useful in practicing the present invention include SEQ ID NOS: 106 through 171 and SEQ ID NOS: 537 through 588 or fragments or variants thereof.

As used herein, the term "chimeric" refers to a single DNA molecule produced by fusing a first DNA molecule to a second DNA molecule, where neither first nor second DNA molecule would normally be found in that configuration, *i.e.* fused to the other. The chimeric DNA molecule is thus a new DNA molecule not otherwise normally found in nature. As used herein, the term "chimeric promoter" refers to a promoter produced through such manipulation of DNA molecules. A chimeric promoter may combine two or more DNA fragments; an example would be the fusion of a promoter to an enhancer element. Thus, the design, construction, and use of chimeric promoters according to the methods disclosed herein for modulating the expression of operably linked transcribable polynucleotide molecules are encompassed by the present invention.

As used herein, the term "variant" refers to a second DNA molecule that is in composition similar, but not identical to, a first DNA molecule and yet the second DNA molecule still maintains the general functionality, *i.e.* same or similar expression pattern, of the first DNA molecule. A variant may be a shorter or truncated version of the first DNA molecule and/or an altered version of the sequence of the first DNA molecule, such as one with different restriction enzyme sites and/or internal deletions, substitutions, and/or insertions. A "variant" can also encompass a regulatory element having a nucleotide sequence comprising a substitution, deletion and/or insertion of one or more nucleotides of a reference sequence, wherein the derivative regulatory element has more or less or equivalent transcriptional or translational activity than the corresponding parent regulatory molecule. The regulatory element "variants" will also encompass variants arising from mutations that naturally occur in bacterial and plant cell transformation. In the present invention, a polynucleotide sequence provided as SEQ ID NOS: 1 through 325 and 352 through 924 may be used to create variants that are in composition similar, but not identical to, the polynucleotide sequence of the original regulatory element, while still maintaining the general functionality, *i.e.* same or similar expression pattern, of the original regulatory element. Production of such variants of the present invention is well within the ordinary skill of the art in light of the disclosure and is encompassed within the scope of the present

invention. Chimeric regulatory element “variants” comprise the same constituent elements as a reference sequence but the constituent elements comprising the chimeric regulatory element may be operatively linked by various methods known in the art such as, restriction enzyme digestion and ligation, ligation independent cloning, modular assembly of PCR products during amplification, or direct chemical synthesis of the regulatory element as well as other methods known in the art. The resulting chimeric regulatory element “variant” can be comprised of the same, or variants of the same, constituent elements of the reference sequence but differ in the sequence or sequences that comprise the linking sequence or sequences which allow the constituent parts to be operatively linked. In the present invention, a polynucleotide sequence provided as SEQ ID NOS: 1 through 323 and 352 through 924 provide a reference sequence wherein the constituent elements that comprise the reference sequence may be joined by methods known in the art and may comprise substitutions, deletions and/or insertions of one or more nucleotides or mutations that naturally occur in bacterial and plant cell transformation.

## 15 **Constructs**

As used herein, the term “construct” means any recombinant polynucleotide molecule such as a plasmid, cosmid, virus, autonomously replicating polynucleotide molecule, phage, or linear or circular single-stranded or double-stranded DNA or RNA polynucleotide molecule, derived from any source, capable of genomic integration or autonomous replication, comprising a polynucleotide molecule where one or more polynucleotide molecule has been linked in a functionally operative manner, *i.e.* operably linked. As used herein, the term “vector” means any recombinant polynucleotide construct that may be used for the purpose of transformation, *i.e.* the introduction of heterologous DNA into a host cell.

As used herein, the term “operably linked” refers to a first molecule joined to a second molecule, wherein the molecules are so arranged that the first molecule affects the function of the second molecule. The two molecules may or may not be part of a single contiguous molecule and may or may not be adjacent. For example, a promoter is operably linked to a transcribable polynucleotide molecule if the promoter modulates transcription of the transcribable polynucleotide molecule of interest in a cell.

The constructs of the present invention are generally double Ti plasmid border DNA constructs that have the right border (RB or AGRtu.RB) and left border (LB or AGRtu.LB) regions of the Ti plasmid isolated from *Agrobacterium tumefaciens* comprising a T-DNA, that along with transfer molecules provided by the *A. tumefaciens* cells, permit the integration

of the T-DNA into the genome of a plant cell (*see*, for example, US Patent 6,603,061). The constructs may also contain the plasmid backbone DNA segments that provide replication function and antibiotic selection in bacterial cells, for example, an *Escherichia coli* origin of replication such as *ori322*, a broad host range origin of replication such as *oriV* or *oriRi*, and  
5 a coding region for a selectable marker such as Spec/Strp that encodes for Tn7 aminoglycoside adenylyltransferase (*aadA*) conferring resistance to spectinomycin or streptomycin, or a gentamicin (Gm, Gent) selectable marker gene. For plant transformation, the host bacterial strain is often *A. tumefaciens* ABI, C58, or LBA4404; however, other strains known to those skilled in the art of plant transformation can function in the present  
10 invention.

Methods are known in the art for assembling and introducing constructs into a cell in such a manner that the transcribable polynucleotide molecule is transcribed into a functional mRNA molecule that is translated and expressed as a protein product. For the practice of the present invention, conventional compositions and methods for preparing and using constructs  
15 and host cells are well known to one skilled in the art, *see*, for example, *Molecular Cloning: A Laboratory Manual, 3<sup>rd</sup> edition Volumes 1, 2, and 3* (2000) J.F. Sambrook, D.W. Russell, and N. Irwin, Cold Spring Harbor Laboratory Press. Methods for making recombinant vectors particularly suited to plant transformation include, without limitation, those described in U.S. Patent No. 4,971,908; 4,940,835; 4,769,061; and 4,757,011 in their entirety. These  
20 types of vectors have also been reviewed in the scientific literature (*see*, for example, Rodriguez, *et al.*, *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston, (1988) and Glick, *et al.*, *Methods in Plant Molecular Biology and Biotechnology*, CRC Press, Boca Raton, FL. (1993)). Typical vectors useful for expression of nucleic acids in higher plants are well known in the art and include vectors derived from  
25 the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* (Rogers, *et al.*, *Methods in Enzymology* 153: 253-277 (1987)). Other recombinant vectors useful for plant transformation, including the pCaMVCN transfer control vector, have also been described in the scientific literature (*see*, for example, Fromm, *et al.*, *Proc. Natl. Acad. Sci. USA* 82: 5824-5828 (1985)).

30 Various regulatory elements may be included in a construct. Any such regulatory elements may be provided in combination with other regulatory elements. Such combinations can be designed or modified to produce desirable regulatory features. Constructs of the present invention would typically comprise at least one regulatory element



operably linked to a transcribable polynucleotide molecule operably linked to a 3' transcription termination molecule.

Constructs of the present invention may include any promoter or leader known in the art. For example, a promoter of the present invention may be operably linked to a  
5 heterologous non-translated 5' leader such as one derived from a heat shock protein gene (*see*, for example, U.S. Patent No. 5,659,122 and 5,362,865). Alternatively, a leader of the present invention may be operably linked to a heterologous promoter such as the Cauliflower Mosaic Virus 35S transcript promoter (*see*, U.S. Patent No. 5,352,605).

As used herein, the term "intron" refers to a DNA molecule that may be isolated or  
10 identified from the genomic copy of a gene and may be defined generally as a region spliced out during mRNA processing prior to translation. Alternately, an intron may be a synthetically produced or manipulated DNA element. An intron may contain elements enhancer elements that effect the transcription of operably linked genes. An intron may be used as a regulatory element for modulating expression of an operably linked transcribable  
15 polynucleotide molecule. A DNA construct may comprise an intron, and the intron may or may not be heterologous with respect to the transcribable polynucleotide molecule sequence. Examples of introns in the art include the rice actin intron (U.S. Patent No. 5,641,876) and the corn HSP70 intron (U.S. Patent No. 5,859,347). Introns useful in practicing the present invention include SEQ ID NOS: 172 through 267, SEQ ID NOS: 317 through 323 and SEQ  
20 ID NOS: 589 through 778.

As used herein, the term "3' transcription termination molecule" or "3' UTR" refers to a DNA molecule that is used during transcription to produce the 3' untranslated region (3' UTR) of an mRNA molecule. The 3' untranslated region of an mRNA molecule may be generated by specific cleavage and 3' polyadenylation, a.k.a. polyA tail. A 3' UTR may be  
25 operably linked to and located downstream of a transcribable polynucleotide molecule and may include polynucleotides that provide a polyadenylation signal and other regulatory signals capable of affecting transcription, mRNA processing, or gene expression. PolyA tails are thought to function in mRNA stability and in initiation of translation. Examples of 3' transcription termination molecules in the art are the nopaline synthase 3' region (*see*, Fraley, *et al.*, *Proc. Natl. Acad. Sci. USA*, 80: 4803-4807 (1983)); wheat hsp17 3' region; pea rubisco small subunit 3' region; cotton E6 3' region (U.S. Patent 6,096,950); 3' regions disclosed in WO0011200A2; and the coixin 3' UTR (U.S. Patent No. 6,635,806). Sequences of 3' UTR  
30 regions useful in practicing the present invention are provided as SEQ ID NOS: 268 through 276 and SEQ ID NOS: 779 through 924.

3' UTRs are a basic prerequisite for the recombinant expression of specific genes. In animal systems, a machinery of 3' UTRs has been well defined (*e.g.* Zhao *et al.*, *Microbiol Mol Biol Rev* 63:405–445 (1999); Proudfoot, *Nature* 322:562-565 (1986); Kim *et al.*, *Biotechnology Progress* 19:1620-1622 (2003); Yonaha and Proudfoot, *EMBO J.* 19:3770-3777 (2000); Cramer *et al.*, *FEBS Letters* 498:179-182 (2001); Kuerstem and Goodwin, *Nature Reviews Genetics* 4:626-637 (2003)). Effective termination of RNA transcription is required to prevent unwanted transcription of trait- unrelated (downstream) sequences, which may interfere with trait performance (see below for more details). Arrangement of multiple gene expression cassettes in local proximity to one another (*e.g.* within one T- DNA) may cause suppression of gene expression of one or more genes in said construct in comparison to independent insertions (Padidam and Cao, *BioTechniques* 31:328-334 (2001)). This may interfere with achieving adequate levels of expression, for instance in cases where strong gene expression from all cassettes is desired.

In plants, clearly defined polyadenylation signal sequences are not known. Hasegawa *et al.*, *Plant J.* 33:1063-1072, (2003)) were not able to identify conserved polyadenylation signal sequences in both *in vitro* and *in vivo* systems in *Nicotiana sylvestris* and to determine the actual length of the primary (non-polyadenylated) transcript. A weak 3' UTR has the potential to generate read-through, which may affect the expression of the genes located in the neighboring expression cassettes (Padidam and Cao, *BioTechniques* 31:328-334 (2001)). Appropriate control of transcription termination can prevent read-through into sequences (*e.g.* other expression cassettes) localized downstream and can further allow efficient recycling of RNA polymerase, to improve gene expression. Efficient termination of transcription (release of RNA Polymerase II from the DNA) is pre-requisite for re-initiation of transcription and thereby directly affects the overall transcript level. Subsequent to transcription termination, the mature mRNA is released from the site of synthesis and template to the cytoplasm. Eukaryotic mRNAs are accumulated as poly(A) forms *in vivo*, so that it is difficult to detect transcriptional termination sites by conventional methods. However, prediction of functional and efficient 3' UTRs by bioinformatics methods is difficult in that there are no conserved sequences which would allow easy prediction of an effective 3' UTR.

From a practical standpoint, it is preferred that a 3' UTR used in a transgene cassette possesses the following characteristics. The 3' UTR must be able to efficiently and effectively terminate transcription of the transgene and prevent read-through of the transcript into any neighboring DNA sequence which can be comprised of another transgene cassette as in the case of multiple cassettes residing in one T-DNA, or the neighboring chromosomal

DNA into which the T-DNA has inserted. The 3' UTR should not cause a reduction in the transcriptional activity imparted by the promoter, leader and introns that are used to drive expression of the transgene. In plant biotechnology, the 3' UTR is often used for priming of amplification reactions of reverse transcribed RNA extracted from the transformed plant and used to (1) assess the transcriptional activity or expression of the transgene cassette once integrated into the plant chromosome; (2) assess the copy number of insertions within the plant DNA; and (3) assess zygosity of the resulting seed after breeding. The 3' UTR is also used in amplification reactions of DNA extracted from the transformed plant to characterize the intactness of the inserted cassette.

3' UTRs useful in providing expression of a transgene in plants are identified based upon the expression of expressed sequence tags (ESTs) in cDNA libraries made from messenger RNA isolated from seed, flower and other tissues derived from Foxtail millet (*Setaria italica* (L.) Beauv). Libraries of cDNA are made from tissues isolated from *S. italica* using methods known to those skilled in the art from flower tissue, seed, leaf and root. The resulting cDNAs are sequenced using various sequencing methods known in the art. The resulting ESTs are assembled into clusters using bioinformatics software such as *clc\_ref\_assemble\_complete* version 2.01.37139 (CLC bio USA, Cambridge, Massachusetts 02142). Transcript abundance of each cluster is determined by counting the number of cDNA reads for each cluster. The identified 3' UTRs may be comprised of sequence derived from cDNA sequence as well as sequence derived from genomic DNA. The cDNA sequence is used to design primers, which are then used with GenomeWalker™ (Clontech Laboratories, Inc, Mountain View, CA) libraries constructed following the manufacturer's protocol to clone the 3' region of the corresponding genomic DNA sequence to provide a longer termination sequence. Analysis of relative transcript abundance either by direct counts or normalized counts of observed sequence reads for each tissue library can be used to infer properties about patterns of expression. For example, some 3' UTRs may be found in transcripts seen in higher abundance in root tissue as opposed to leaf. This is suggestive that the transcript is highly expressed in root and that the properties of root expression may be attributable to the transcriptional regulation of the promoter, the lead, the introns or the 3' UTR. Empirical testing of 3' UTRs identified by the properties of expression within specific organs, tissues or cell types can result in the identification of 3' UTRs that enhance expression in those specific organs, tissues or cell types.

Constructs and vectors may also include a transit peptide coding sequence that expresses a linked peptide that is useful for targeting of a protein product, particularly to a

chloroplast, leucoplast, or other plastid organelle; mitochondria; peroxisome; vacuole; or an extracellular location. For descriptions of the use of chloroplast transit peptides, see U.S. Patent No. 5,188,642 and U.S. Patent No. 5,728,925. Many chloroplast-localized proteins are expressed from nuclear genes as precursors and are targeted to the chloroplast by a chloroplast transit peptide (CTP). Examples of such isolated chloroplast proteins include, but are not limited to, those associated with the small subunit (SSU) of ribulose-1,5,-bisphosphate carboxylase, ferredoxin, ferredoxin oxidoreductase, the light-harvesting complex protein I and protein II, thioredoxin F, enolpyruvyl shikimate phosphate synthase (EPSPS), and transit peptides described in U.S. Patent No. 7,193,133. It has been demonstrated *in vivo* and *in vitro* that non-chloroplast proteins may be targeted to the chloroplast by use of protein fusions with a heterologous CTP and that the CTP is sufficient to target a protein to the chloroplast. Incorporation of a suitable chloroplast transit peptide such as the *Arabidopsis thaliana* EPSPS CTP (CTP2) (See, Klee *et al.*, *Mol. Gen. Genet.* 210:437-442 (1987)) or the *Petunia hybrida* EPSPS CTP (CTP4) (See, della-Cioppa *et al.*, *Proc. Natl. Acad. Sci. USA* 83:6873-6877 (1986)) has been shown to target heterologous EPSPS protein sequences to chloroplasts in transgenic plants (See, U.S. Patent Nos. 5,627,061; 5,633,435; and 5,312,910 and EP 0218571; EP 189707; EP 508909; and EP 924299). Sequences encoding transit peptides useful for the present invention are provided as SEQ ID NOS: 277 through 316. Protein sequences of transit peptides useful for the present invention are provided as SEQ ID NOS: 324 through 350.

### **Transcribable polynucleotide molecules**

As used herein, the term “transcribable polynucleotide molecule” refers to any DNA molecule capable of being transcribed into a RNA molecule, including, but not limited to, those having protein coding sequences and those having sequences useful for gene suppression. A “transgene” refers to a transcribable polynucleotide molecule heterologous to a host cell and/or a transcribable polynucleotide molecule artificially incorporated into a host cell’s genome.

A promoter of the present invention may be operably linked to a transcribable polynucleotide molecule that is heterologous with respect to the promoter molecule. As used herein, the term “heterologous” refers to the combination of two or more polynucleotide molecules when such a combination would not normally be found in nature. For example, the two molecules may be derived from different species and/or the two molecules may be derived from different genes, *e.g.* different genes from the same species or the same genes

from different species. A promoter is thus heterologous with respect to an operably linked transcribable polynucleotide molecule if such a combination is not normally found in nature, *i.e.* that transcribable polynucleotide molecule is not naturally occurring operably linked in combination with that promoter molecule.

5           The transcribable polynucleotide molecule may generally be any DNA molecule for which expression of an RNA transcript is desired. Such expression of an RNA transcript may result in translation of the resulting mRNA molecule and thus protein expression. Alternatively, a transcribable polynucleotide molecule may be designed to ultimately cause decreased expression of a specific gene or protein. This may be accomplished by using a  
10 transcribable polynucleotide molecule that is oriented in the antisense direction. One of ordinary skill in the art is familiar with using such antisense technology. Briefly, as the antisense transcribable polynucleotide molecule is transcribed, the RNA product hybridizes to and sequesters a complimentary RNA molecule inside the cell. This duplex RNA molecule cannot be translated into a protein by the cell's translational machinery and is  
15 degraded in the cell. Any gene may be negatively regulated in this manner.

Thus, one embodiment of the invention is a regulatory element of the present invention, such as those provided as SEQ ID NOS: 1 through 276, SEQ ID NOS: 317 through 323 and 352 through 924, operably linked to a transcribable polynucleotide molecule so as to modulate transcription of the transcribable polynucleotide molecule at a desired level or in a  
20 desired pattern upon introduction of said construct into a plant cell. In one embodiment, the transcribable polynucleotide molecule comprises a protein-coding region of a gene, and the promoter affects the transcription of an RNA molecule that is translated and expressed as a protein product. In another embodiment, the transcribable polynucleotide molecule comprises an antisense region of a gene, and the promoter affects the transcription of an  
25 antisense RNA molecule or other similar inhibitory RNA molecule in order to inhibit expression of a specific RNA molecule of interest in a target host cell.

### **Genes of Agronomic Interest**

Transcribable polynucleotide molecules may be genes of agronomic interest. As used herein, the term "gene of agronomic interest" refers to a transcribable polynucleotide  
30 molecule that when expressed in a particular plant tissue, cell, or cell type provides a desirable characteristic associated with plant morphology, physiology, growth, development, yield, product, nutritional profile, disease or pest resistance, and/or environmental or chemical tolerance. Genes of agronomic interest include, but are not limited to, those

encoding a yield protein, a stress resistance protein, a developmental control protein, a tissue differentiation protein, a meristem protein, an environmentally responsive protein, a senescence protein, a hormone responsive protein, an abscission protein, a source protein, a sink protein, a flower control protein, a seed protein, an herbicide resistance protein, a disease resistance protein, a fatty acid biosynthetic enzyme, a tocopherol biosynthetic enzyme, an amino acid biosynthetic enzyme, a pesticidal protein, or any other agent such as an antisense or RNAi molecule targeting a particular gene for suppression. The product of a gene of agronomic interest may act within the plant in order to cause an effect upon the plant physiology or metabolism or may be act as a pesticidal agent in the diet of a pest that feeds on the plant.

In one embodiment of the invention, a promoter of the present invention is incorporated into a construct such that the promoter is operably linked to a transcribable polynucleotide molecule that is a gene of agronomic interest. The expression of the gene of agronomic interest is desirable in order to confer an agronomically beneficial trait. A beneficial agronomic trait may be, for example, but not limited to, herbicide tolerance, insect control, modified yield, fungal disease resistance, virus resistance, nematode resistance, bacterial disease resistance, plant growth and development, starch production, modified oils production, high oil production, modified fatty acid content, high protein production, fruit ripening, enhanced animal and human nutrition, biopolymers, environmental stress resistance, pharmaceutical peptides and secretable peptides, improved processing traits, improved digestibility, enzyme production, flavor, nitrogen fixation, hybrid seed production, fiber production, and biofuel production. Examples of genes of agronomic interest known in the art include those for herbicide resistance (U.S. Patent No. 6,803,501; 6,448,476; 6,248,876; 6,225,114; 6,107,549; 5,866,775; 5,804,425; 5,633,435; and 5,463,175), increased yield (U.S. Patent Nos. USRE38,446; 6,716,474; 6,663,906; 6,476,295; 6,441,277; 6,423,828; 6,399,330; 6,372,211; 6,235,971; 6,222,098; and 5,716,837), insect control (U.S. Patent Nos. 6,809,078; 6,713,063; 6,686,452; 6,657,046; 6,645,497; 6,642,030; 6,639,054; 6,620,988; 6,593,293; 6,555,655; 6,538,109; 6,537,756; 6,521,442; 6,501,009; 6,468,523; 6,326,351; 6,313,378; 6,284,949; 6,281,016; 6,248,536; 6,242,241; 6,221,649; 6,177,615; 6,156,573; 6,153,814; 6,110,464; 6,093,695; 6,063,756; 6,063,597; 6,023,013; 5,959,091; 5,942,664; 5,942,658; 5,880,275; 5,763,245; and 5,763,241), fungal disease resistance (U.S. Patent Nos. 6,653,280; 6,573,361; 6,506,962; 6,316,407; 6,215,048; 5,516,671; 5,773,696; 6,121,436; 6,316,407; and 6,506,962), virus resistance (U.S. Patent Nos. 6,617,496; 6,608,241; 6,015,940; 6,013,864; 5,850,023; and 5,304,730), nematode resistance (U.S.

Patent No. 6,228,992), bacterial disease resistance (U.S. Patent No. 5,516,671), plant growth and development (U.S. Patent Nos. 6,723,897 and 6,518,488), starch production (U.S. Patent Nos. 6,538,181; 6,538,179; 6,538,178; 5,750,876; 6,476,295), modified oils production (U.S. Patent Nos. 6,444,876; 6,426,447; and 6,380,462), high oil production (U.S. Patent Nos. 6,495,739; 5,608,149; 6,483,008; and 6,476,295), modified fatty acid content (U.S. Patent Nos. 6,828,475; 6,822,141; 6,770,465; 6,706,950; 6,660,849; 6,596,538; 6,589,767; 6,537,750; 6,489,461; and 6,459,018), high protein production (U.S. Patent No. 6,380,466), fruit ripening (U.S. Patent No. 5,512,466), enhanced animal and human nutrition (U.S. Patent Nos. 6,723,837; 6,653,530; 6,5412,59; 5,985,605; and 6,171,640), biopolymers (U.S. Patent Nos. USRE37,543; 6,228,623; and 5,958,745, and 6,946,588), environmental stress resistance (U.S. Patent No. 6,072,103), pharmaceutical peptides and secretable peptides (U.S. Patent Nos. 6,812,379; 6,774,283; 6,140,075; and 6,080,560), improved processing traits (U.S. Patent No. 6,476,295), improved digestibility (U.S. Patent No. 6,531,648) low raffinose (U.S. Patent No. 6,166,292), industrial enzyme production (U.S. Patent No. 5,543,576), improved flavor (U.S. Patent No. 6,011,199), nitrogen fixation (U.S. Patent No. 5,229,114), hybrid seed production (U.S. Patent No. 5,689,041), fiber production (U.S. Patent Nos. 6,576,818; 6,271,443; 5,981,834; and 5,869,720) and biofuel production (U.S. Patent No. 5,998,700).

Alternatively, a gene of agronomic interest can affect the above mentioned plant characteristic or phenotype by encoding a RNA molecule that causes the targeted modulation of gene expression of an endogenous gene, for example via antisense (see *e.g.* US Patent 5,107,065); inhibitory RNA (“RNAi”, including modulation of gene expression via miRNA-, siRNA-, trans-acting siRNA-, and phased sRNA-mediated mechanisms, *e.g.* as described in published applications US 2006/0200878 and US 2008/0066206, and in US patent application 11/974,469); or cosuppression-mediated mechanisms. The RNA could also be a catalytic RNA molecule (*e.g.* a ribozyme or a riboswitch; see *e.g.* US 2006/0200878) engineered to cleave a desired endogenous mRNA product. Thus, any transcribable polynucleotide molecule that encodes a transcribed RNA molecule that affects an agronomically important phenotype or morphology change of interest may be useful for the practice of the present invention. Methods are known in the art for constructing and introducing constructs into a cell in such a manner that the transcribable polynucleotide molecule is transcribed into a molecule that is capable of causing gene suppression. For example, posttranscriptional gene suppression using a construct with an anti-sense oriented transcribable polynucleotide molecule to regulate gene expression in plant cells is disclosed

in U.S. Patent Nos. 5,107,065 and 5,759,829, and posttranscriptional gene suppression using a construct with a sense-oriented transcribable polynucleotide molecule to regulate gene expression in plants is disclosed in U.S. Patent Nos. 5,283,184 and 5,231,020. Expression of a transcribable polynucleotide in a plant cell can also be used to suppress plant pests feeding on the plant cell, for example, compositions isolated from coleopteran pests (U.S. Patent Publication No. US20070124836) and compositions isolated from nematode pests (U.S. Patent Publication No. US20070250947). Plant pests include, but are not limited to arthropod pests, nematode pests, and fungal or microbial pests. Exemplary transcribable polynucleotide molecules for incorporation into constructs of the present invention include, for example, DNA molecules or genes from a species other than the target species or genes that originate with or are present in the same species, but are incorporated into recipient cells by genetic engineering methods rather than classical reproduction or breeding techniques. The type of polynucleotide molecule can include, but is not limited to, a polynucleotide molecule that is already present in the plant cell, a polynucleotide molecule from another plant, a polynucleotide molecule from a different organism, or a polynucleotide molecule generated externally, such as a polynucleotide molecule containing an antisense message of a gene, or a polynucleotide molecule encoding an artificial, synthetic, or otherwise modified version of a transgene.

### Selectable Markers

As used herein the term “marker” refers to any transcribable polynucleotide molecule whose expression, or lack thereof, can be screened for or scored in some way. Marker genes for use in the practice of the present invention include, but are not limited to transcribable polynucleotide molecules encoding  $\beta$ -glucuronidase (GUS described in U.S. Patent No. 5,599,670), green fluorescent protein and variants thereof (GFP described in U.S. Patent No. 5,491,084 and 6,146,826), proteins that confer antibiotic resistance, or proteins that confer herbicide tolerance. Useful antibiotic resistance markers, including those encoding proteins conferring resistance to kanamycin (*nptII*), hygromycin B (*aph IV*), streptomycin or spectinomycin (*aad*, *spec/strep*) and gentamycin (*aac3* and *aacC4*) are known in the art. Herbicides for which transgenic plant tolerance has been demonstrated and the method of the present invention can be applied, include, but are not limited to: amino-methyl-phosphonic acid, glyphosate, glufosinate, sulfonyleureas, imidazolinones, bromoxynil, delapone, dicamba, cyclohezanedione, protoporphyrinogen oxidase inhibitors, and isoxasflutole herbicides. Transcribable polynucleotide molecules encoding proteins involved in herbicide tolerance are



known in the art, and include, but are not limited to, a transcribable polynucleotide molecule encoding 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS for glyphosate tolerance described in U.S. Patent No. 5,627,061; 5,633,435; 6,040,497; and 5,094,945); a transcribable polynucleotide molecule encoding a glyphosate oxidoreductase and a glyphosate-N-acetyl transferase (GOX described in U.S. Patent No. 5,463,175; GAT described in U.S. Patent publication No. 20030083480, and dicamba monooxygenase U.S. Patent publication No. 20030135879); a transcribable polynucleotide molecule encoding bromoxynil nitrilase (*Bxn* for Bromoxynil tolerance described in U.S. Patent No. 4,810,648); a transcribable polynucleotide molecule encoding phytoene desaturase (*crtI*) described in Misawa, *et al.*, *Plant Journal* 4:833-840 (1993) and Misawa, *et al.*, *Plant Journal* 6:481-489 (1994) for norflurazon tolerance; a transcribable polynucleotide molecule encoding acetohydroxyacid synthase (AHAS, *aka* ALS) described in Sathasiivan, *et al.*, *Nucl. Acids Res.* 18:2188-2193 (1990) for tolerance to sulfonylurea herbicides; and the *bar* gene described in DeBlock, *et al.*, *EMBO Journal* 6:2513-2519 (1987) for glufosinate and bialaphos tolerance. The promoter molecules of the present invention can express linked transcribable polynucleotide molecules that encode for phosphinothricin acetyltransferase, glyphosate resistant EPSPS, aminoglycoside phosphotransferase, hydroxyphenyl pyruvate dehydrogenase, hygromycin phosphotransferase, neomycin phosphotransferase, dalapon dehalogenase, bromoxynil resistant nitrilase, anthranilate synthase, aryloxyalkanoate dioxygenases, acetyl CoA carboxylase, glyphosate oxidoreductase, and glyphosate-N-acetyl transferase.

Included within the term “selectable markers” are also genes which encode a secretable marker whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers that encode a secretable antigen that can be identified by antibody interaction, or even secretable enzymes which can be detected catalytically. Selectable secreted marker proteins fall into a number of classes, including small, diffusible proteins which are detectable, (*e.g.* by ELISA), small active enzymes which are detectable in extracellular solution (*e.g.* alpha-amylase, beta-lactamase, phosphinothricin transferase), or proteins which are inserted or trapped in the cell wall (such as proteins which include a leader sequence such as that found in the expression unit of extension or tobacco pathogenesis related proteins also known as tobacco PR-S). Other possible selectable marker genes will be apparent to those of skill in the art and are encompassed by the present invention.

## Cell Transformation

The invention is also directed to a method of producing transformed cells and plants which comprise a promoter operably linked to a transcribable polynucleotide molecule.

5 The term “transformation” refers to the introduction of nucleic acid into a recipient host. As used herein, the term “host” refers to bacteria, fungi, or plant, including any cells, tissue, organs, or progeny of the bacteria, fungi, or plant. Plant tissues and cells of particular interest include protoplasts, calli, roots, tubers, seeds, stems, leaves, seedlings, embryos, and pollen.

10 As used herein, the term “transformed” refers to a cell, tissue, organ, or organism into which a foreign polynucleotide molecule, such as a construct, has been introduced. The introduced polynucleotide molecule may be integrated into the genomic DNA of the recipient cell, tissue, organ, or organism such that the introduced polynucleotide molecule is inherited by subsequent progeny. A “transgenic” or “transformed” cell or organism also includes  
15 progeny of the cell or organism and progeny produced from a breeding program employing such a transgenic organism as a parent in a cross and exhibiting an altered phenotype resulting from the presence of a foreign polynucleotide molecule. The term “transgenic” refers to a bacteria, fungi, or plant containing one or more heterologous polynucleic acid molecules.

20 There are many methods for introducing polynucleic acid molecules into plant cells. The method generally comprises the steps of selecting a suitable host cell, transforming the host cell with a recombinant vector, and obtaining the transformed host cell. Suitable methods include bacterial infection (*e.g. Agrobacterium*), binary bacterial artificial chromosome vectors, direct delivery of DNA (*e.g. via PEG-mediated transformation, desiccation/inhibition-mediated DNA uptake, electroporation, agitation with silicon carbide*  
25 *fibers, and acceleration of DNA coated particles, etc.* (reviewed in Potrykus, *et al., Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42: 205 (1991)).

30 Technology for introduction of a DNA molecule into cells is well known to those of skill in the art. Methods and materials for transforming plant cells by introducing a plant DNA construct into a plant genome in the practice of this invention can include any of the well-known and demonstrated methods including, but not limited to:

- (1) chemical methods (Graham and Van der Eb, *Virology* 54:536-539 (1973) and Zatloukal, *et al., Ann. N.Y. Acad. Sci.* 660: 136-153 (1992));
- (2) physical methods such as microinjection (Capecchi, *Cell* 22:479-488 (1980)), electroporation (Wong and Neumann, *Biochim. Biophys. Res. Commun.*, 107:584-

- 587 (1982); Fromm, *et al*, *Proc. Natl. Acad. Sci. USA* 82:5824-5828 (1985); U.S. Patent No. 5,384,253) particle acceleration (Johnston and Tang, *Methods Cell Biol.* 43(A):353-365 (1994); Fynan, *et al.*, *Proc. Natl. Acad. Sci. USA* 90:11478-11482 (1993)); and microprojectile bombardment (as illustrated in U.S. Patent No. 5,015,580; 5,550,318; 5,538,880; 6,160,208; 6,399,861; and 6,403,865);
- (3) viral vectors (Clapp, *Clin. Perinatol.* 20:155-168 (1993); Lu, *et al.*, *J. Exp. Med.* 178:2089-2096 (1993); Eglitis and Anderson, *Biotechniques* 6:608-614 (1988));
- (4) receptor-mediated mechanisms (Curiel *et al.*, *Hum. Gen. Ther.* 3:147-154 (1992) and Wagner, *et al.*, *Proc. Natl. Acad. Sci. USA* 89:6099-6103 (1992); (5) bacterial mediated mechanisms such as *Agrobacterium*-mediated transformation (as illustrated in U.S. Patent No. 5,824,877; 5,591,616; 5,981,840; and 6,384,301); direct introduction into pollen by injecting a plant's reproductive organs (Zhou, *et al.*, *Methods in Enzymology* 101:433, (1983); Hess, *Intern Rev. Cytol.* 107:367 (1987); Luo, *et al.*, *Plant Mol Biol. Reporter* 6:165 (1988); Pena, *et al.*, *Nature* 325:274 (1987));
- (7) protoplast transformation (as illustrated in U.S. Patent No. 5,508,184); and
- (8) injection into immature embryos (Neuhaus, *et al.*, *Theor. Appl. Genet.* 75:30 (1987)).

Any of the above described methods may be utilized to transform a host cell with one or more promoters and/or constructs of the present. Host cells may be any cell or organism such as a plant cell, algae cell, algae, fungal cell, fungi, bacterial cell, or insect cell. Preferred hosts and transformed cells include cells from: plants, *Aspergillus*, yeasts, insects, bacteria and algae.

Methods for transforming dicotyledonous plants, primarily by use of *Agrobacterium tumefaciens* and obtaining transgenic plants have been published for cotton (U.S. Patent No. 5,004,863; 5,159,135; and 5,518,908); soybean (U.S. Patent No. 5,569,834 and 5,416,011; *see also*, McCabe, *et al.*, *Biotechnology* 6:923 (1988) and Christou *et al.*, *Plant Physiol.* 87:671-674 (1988)); *Brassica* (U.S. Patent No. 5,463,174); peanut (Cheng *et al.*, *Plant Cell Rep.* 15:653-657 (1996) and McKently *et al.*, *Plant Cell Rep.* 14:699-703 (1995)); papaya; and pea (Grant *et al.*, *Plant Cell Rep.* 15:254-258 (1995)).

Transformations of monocotyledon plants using electroporation, particle bombardment, and *Agrobacterium* have also been reported. Transformation and plant regeneration have been achieved in asparagus (Bytebier, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 84:5354 (1987); barley (Wan and Lemaux, *Plant Physiol.* 104:37 (1994)); maize (Rhodes, *et*

5 *al.*, *Science* 240:204 (1988), Gordon-Kamm, *et al.*, *Plant Cell* 2:603-618 (1990), Fromm, *et al.*, *Bio/Technology*, 8:833 (1990), Koziel *et al.*, *Bio/Technology* 11:194 (1993), and Armstrong, *et al.*, *Crop Science* 35:550-557 (1995)); oat (Somers, *et al.*, *Bio/Technology* 10:1589 (1992)); orchard grass (Horn, *et al.*, *Plant Cell Rep.* 7:469 (1988)); rye (De la Pena, *et al.*, *Nature*, 325:274 (1987)); sugarcane (Bower and Birch, *Plant Journal* 2:409 (1992)); tall fescue (Wang, *et al.*, *Bio/Technology* 10:691 (1992)); and wheat (Vasil, *et al.*, *Bio/Technology* 10:667 (1992) and U.S. Patent No. 5,631,152).

10 The regeneration, development, and cultivation of plants from transformed plant protoplast or explants is well known in the art (*see*, for example, Weissbach and Weissbach, *Methods for Plant Molecular Biology*, (Eds.), Academic Press, Inc., San Diego, CA (1988) and Horsch *et al.*, *Science* 227:1229-1231 (1985)). Transformed cells are generally cultured in the presence of a selective media, which selects for the successfully transformed cells and induces the regeneration of plant shoots and roots into intact plants (Fraley, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 80: 4803 (1983)). Transformed plants are typically obtained within two to  
15 four months.

The regenerated transgenic plants are self-pollinated to provide homozygous transgenic plants. Alternatively, pollen obtained from the regenerated transgenic plants may be crossed with non-transgenic plants, preferably inbred lines of agronomically important species. Descriptions of breeding methods that are commonly used for different traits and crops can be found in one of several reference books, *see*, for example, Allard, *Principles of*  
20 *Plant Breeding*, John Wiley & Sons, NY, U. of CA, Davis, CA, 50-98 (1960); Simmonds, *Principles of crop improvement*, Longman, Inc., NY, 369-399 (1979); Sneep and Hendriksen, *Plant breeding perspectives*, Wageningen (ed), Center for Agricultural Publishing and Documentation (1979); Fehr, *Soybeans: Improvement, Production and Uses*, 2nd Edition, Monograph, 16:249 (1987); Fehr, *Principles of variety development, Theory and Technique*,  
25 (Vol. 1) and *Crop Species Soybean* (Vol 2), Iowa State Univ., Macmillan Pub. Co., NY, 360-376 (1987). Conversely, pollen from non-transgenic plants may be used to pollinate the regenerated transgenic plants.

30 The transformed plants may be analyzed for the presence of the genes of interest and the expression level and/or profile conferred by the regulatory elements of the present invention. Those of skill in the art are aware of the numerous methods available for the analysis of transformed plants. For example, methods for plant analysis include, but are not limited to Southern blots or northern blots, PCR-based approaches, biochemical analyses, phenotypic screening methods, field evaluations, and immunodiagnostic assays. The

expression of a transcribable polynucleotide molecule can be measured using TaqMan® (Applied Biosystems, Foster City, CA) reagents and methods as described by the manufacturer and PCR cycle times determined using the TaqMan® Testing Matrix. Alternatively, the Invader® (Third Wave Technologies, Madison, WI) reagents and methods as described by the manufacturer can be used transgene expression.

The seeds of the plants of this invention can be harvested from fertile transgenic plants and be used to grow progeny generations of transformed plants of this invention including hybrid plant lines comprising the construct of this invention and expressing a gene of agronomic interest.

The present invention also provides for parts of the plants of the present invention. Plant parts, without limitation, include leaves, stems, roots, tubers, seeds, endosperm, ovule, and pollen. The invention also includes and provides transformed plant cells which comprise a nucleic acid molecule of the present invention.

The transgenic plant may pass along the transgenic polynucleotide molecule to its progeny. Progeny includes any regenerable plant part or seed comprising the transgene derived from an ancestor plant. The transgenic plant is preferably homozygous for the transformed polynucleotide molecule and transmits that sequence to all offspring as a result of sexual reproduction. Progeny may be grown from seeds produced by the transgenic plant. These additional plants may then be self-pollinated to generate a true breeding line of plants. The progeny from these plants are evaluated, among other things, for gene expression. The gene expression may be detected by several common methods such as western blotting, northern blotting, immuno-precipitation, and ELISA.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified. It should be appreciated by those of skill in the art that the techniques disclosed in the following examples represent techniques discovered by the inventors to function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention, therefore all matter set forth or shown in the accompanying drawings is to be interpreted as illustrative and not in a limiting sense.

## EXAMPLES

Regulatory elements useful to drive expression of an operably linked transcribable polynucleotide in transgenic plants were isolated, and the expression pattern of these regulatory elements operably linked to a transcribable polynucleotide molecule was analyzed  
5 in transgenic soy plants.

### **Example 1: Identification and Cloning of Novel Regulatory Elements:**

#### **Promoters, Leaders and Introns.**

Regulatory elements were identified and isolated from genomic DNA of the monocot  
10 species, Foxtail millet (*Setaria italica* (L.) Beauv). Proprietary genomic and EST and public genomic and EST sequence was used to design primers, which were then used with GenomeWalker™ (Clontech Laboratories, Inc, Mountain View, CA) libraries constructed following the manufacturer's protocol to clone the 5' region of the corresponding genomic DNA sequence. In the case of promoters leaders and introns, this cloned region comprised  
15 the 5'transcriptional regulatory, 5' UTR and if present, intron sequence upstream of the protein-coding region for each gene from *S. italica*. Using this sequence, regulatory elements were bioinformatically identified within the 5' region for each gene. Bioinformatic analysis was used to identify the transcriptional start site (TSS) and any bi-directionality, introns, or upstream coding sequence present in the sequence. Using the results of this analysis,  
20 regulatory elements were defined within the 5' sequence upstream of the coding sequence of the gene. Primers were then designed to amplify the regulatory elements. The corresponding DNA molecule for each regulatory element was amplified using standard polymerase chain reaction conditions with primers containing unique restriction enzyme sites and genomic DNA isolated from *S. italica*. The resulting DNA fragments were ligated into a base plant  
25 expression vector using standard restriction enzyme digestion of compatible restriction sites and DNA ligation methods.

Sequences of regulatory elements, transit peptide encoding elements and transit peptide protein sequences identified from *S. italica* are listed in Table 1 below. Sequences of the regulatory elements identified from *S. italica* are provided herein as SEQ ID NOS: 1  
30 through 276, SEQ ID NOS: 317 through 323 and 352 through 924. Transcriptional regulatory element groups (EXP) comprised of a promoter, leader and intron operably linked or a promoter, leader, intron and leader operably linked are provided herein as SEQ ID NOS: 1 through 22. Promoter sequences are provided herein as SEQ ID NOS: 23 through 105 and

SEQ ID NOS: 353 through 536. Leader sequences are provided herein as SEQ ID NOS: 106 through 171 and SEQ ID NOS: 537 through 588. Intron sequences are provided as SEQ ID NOS: 172 through 267, SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778. Sequences comprising 3' UTRs are provided herein as SEQ ID NOS: 268 through 276 and SEQ ID NOS: 779 through 924. Sequences encoding transit peptides are provided herein as SEQ ID NOS: 277 through 316. The genomic DNA encoding some of the transit peptides also are comprised by introns presented as SEQ ID NOS: 317 through 323. Transit peptide protein sequences are provided herein as SEQ ID NOS: 324 through 350. An enhancer element is provided as SEQ ID NO: 352.

10 **Table 1. Regulatory elements and corresponding promoters, leaders and introns; and transit (localization) sequences.**

Annotation	SEQ ID NO:	Description
EXP-FMV.35S-SETit.Ccoamt	1	Enhanced Caffeyoyl CoA methyltransferase
EXP-FMV.35S-SETit.Gst:a	2	Enhanced Glutathione S-transferase
EXP-FMV.35S-SETit.Gst:b	3	Enhanced Glutathione S-transferase
EXP-FMV.35S-SETit.Ifr	4	Enhanced Isoflavone Reducatase
EXP-FMV.35S-SETit.Pox	5	Enhanced Peroxidase
EXP-FMV.35S-SETit.Rcc3:a	6	Enhanced Lipid Transfer Protein-RCc3
EXP-FMV.35S-SETit.Rcc3:b	7	Enhanced Lipid Transfer Protein-RCc3
EXP-FMV.35S-SETit.Tip	8	Enhanced Tonoplast intrinsic protein
EXP-SETit.Act8:1:1	9	Actin 8
EXP-SETit.Act8:1:2	10	Actin 8
EXP-SETit.Act8:c	11	Actin 8-1
EXP-SETit.CLUS120796-1	12	Cluster 120796-1
EXP-SETit.CLUS19108	13	Cluster 19108
EXP-SETit.Ppdk:1:1	14	Pyruvate orthophosphate dikinase
EXP-SETit.TubA2:1:1	15	Tubulin A2
EXP-SETit.TubA2:1:3	16	Tubulin A2
EXP-SETit.TubA2-1:1:2	17	Tubulin A2-1
EXP-SETit.TubA2-2:1:1	18	Tubulin A2-2
EXP-SETit.TubA3:1:3	19	Tubulin A3
EXP-SETit.Ubq1:1:1	20	Ubiquitin 1
EXP-SETit.Ubq1:1:3	21	Ubiquitin 1
EXP-SETit.Ubq5	22	Ubiquitin 5
P-SETit.25509-1:1:3	23	Cluster 25509
P-SETit.Act8-1:1:5	24	Actin 8
P-SETit.Act8-1:1:6	25	Actin 8

P-SETit.Act8-1-1:1:2	26	Actin 8-1
P-SETit.Aip-1:1:1	27	Auxin-induced protein
P-SETit.Alc1-1:1:1	28	Alpha-coixin
P-SETit.Alc1-1:1:2	29	Alpha-coixin
P-SETit.Alc2-1:1:2	30	Alpha-coixin
P-SETit.Ali1-1:1:3	31	Aluminum-induced protein
P-SETit.Cab1-1:1:1	32	Chlorophyll a/b binding protein-1
P-SETit.Cab3-1:1:3	33	Chlorophyll a/b binding protein-3
P-SETit.Cbl7-1:1:1	34	Calcineurin B-like protein
P-SETit.Ccoamt-1:1:2	35	Cafeoyl CoA methyltransferase
P-SETit.Cda-1:1:1	36	Cell death associated protein
P-SETit.CLUS1164825-1-1:1:1	37	Cluster 1164825-1
P-SETit.CLUS1165324-1:1:1	38	Cluster 1165324-1
P-SETit.CLUS120796-1-1:1:1	39	Cluster 120796-1
P-SETit.CLUS19108-1:1:2	40	Cluster 19108
P-SETit.CLUS882664-1-1:1:2	41	Cluster 882664-1
P-SETit.CP29-1:1:4	42	Chloroplast protein-29
P-SETit.Cyp-1-1:1:1	43	Cysteine protease-1
P-SETit.Cyp78a-1:1:2	44	Cysteine protease-78a
P-SETit.Cys-1:1:2	45	Cysteine synthase
P-SETit.Cys-1:1:3	46	Cysteine synthase
P-SETit.Dzs-1:1:4	47	Delta zein storage protein
P-SETit.Dzs-1:1:5	48	Delta zein storage protein
P-SETit.Eie-1:1:1	49	Elongation Factor
P-SETit.EST CLUS675389-2-1:1:2	50	Cluster 675389-2
P-SETit.Fba-1:1:1	51	Fructose-bisphosphate aldolase
P-SETit.FM54-1:1:2	52	Cluster 1102871_1
P-SETit.FM63-1:1:2	53	Cluster 1019870_1
P-SETit.Fst-1:1:1	54	Flavonol 4-sulfotransferase
P-SETit.Gapdh2-1:1:3	55	Glyceraldehyde-3-phosphate dehydrogenase
P-SETit.Grcw2-1:1:1	56	Glycine-rich cell wall structural protein 2
P-SETit.Grf-1:1:2	57	Putative growth-regulating factor
P-SETit.Gst-1:1:1	58	Glutathione S-transferase
P-SETit.Gst-1:1:2	59	Glutathione S-transferase
P-SETit.Ifr-1:1:2	60	Isoflavone Reductase
P-SETit.Ifr-1:1:3	61	Isoflavone Reductase
P-SETit.LaDo-1:1:2	62	leucoanthocianidin dioxygenase
P-SETit.Mt1-1:1:2	63	Metallothionein-like protein 1
P-SETit.Nrt2-1:1:2	64	Nitrate transporter-like protein



P-SETit.Nrt2-1:1:3	65	Nitrate transporter-like protein
P-SETit.OMT2.1-1:1:2	66	O-methyltransferase-2.1
P-SETit.OMT2.2-1:1:2	67	O-methyltransferase-2.2
P-SETit.OMT2.3-1:1:1	68	O-methyltransferase-2.3
P-SETit.Omt3-1:1:3	69	O-methyltransferase-3
P-SETit.Omt4_2-1:1:2	70	O-methyltransferase-4.2
P-SETit.Pip2-1:1:3	71	Aquaporin
P-SETit.Pip2-3-1:1:1	72	Aquaporin
P-SETit.Pox-1:1:1	73	Peroxidase 1
P-SETit.Ppc-1:1:3	74	Phosphoenolpyruvate carboxylase
P-SETit.Ppc-1:1:4	75	Phosphoenolpyruvate carboxylase
P-SETit.Ppdk-1:1:1	76	Pyruvate orthophosphate dikinase
P-SETit.Pro1-1:1:2	77	Prolamin
P-SETit.Pro2-1:1:3	78	Prolamin
P-SETit.Prx-1:1:1	79	Peroxidase
P-SETit.Prx17-1:1:2	80	Peroxidase-17
P-SETit.Prx2-1:1:3	81	Peroxidase-2
P-SETit.Prx3-1:1:3	82	Peroxidase-3
P-SETit.Prx3-1:1:4	83	Peroxidase-3
P-SETit.Prx47-1:1:2	84	Peroxidase-47
P-SETit.Prx72-1:1:2	85	Peroxidase-72
P-SETit.PSI-4a-1:1:1	86	Photosystem 1 4a
P-SETit.Rbcs-1:1:1	87	Small subunit RUBISCO
P-SETit.Rcc3-1:1:1	88	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:10	89	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:11	90	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:16	91	Lipid Transfer Protein-RCc3
P-SETit.Srp-1:1:2	92	Stress responsive protein
P-SETit.Ssp1-1:1:1	93	Seed storage protein
P-SETit.Ssp1-1:1:2	94	Seed storage protein
P-SETit.Tga6-1:1:2	95	Teosinte glume architecture
P-SETit.Tip-1:1:1	96	Tonoplast intrinsic protein
P-SETit.Tip-1:1:4	97	Tonoplast intrinsic protein
P-SETit.TubA2-1-1:1:2	98	Tubulin A2-1
P-SETit.TubA2-1-1:1:3	99	Tubulin A2-1
P-SETit.TubA2-2-1:1:3	100	Tubulin A2-2
P-SETit.TubA3-1:1:3	101	Tubulin A3
P-SETit.Ubq1-1:1:1	102	Ubiquitin 1
P-SETit.Ubq1-1:1:3	103	Ubiquitin 1

P-SETit.Ubq5-1:1:2	104	Ubiquitin 5
P-SETit.Ucc1-1:1:2	105	Uclacyanin
L-SETit.Act8-1:1:2	106	Actin 8
L-SETit.Act8-1:1:3	107	Actin 8
L-SETit.Act8-1:1:4	108	Actin 8-1
L-SETit.Aip-1:1:1	109	Auxin-induced protein
L-SETit.Alc1-1:1:1	110	Alpha-coixin
L-SETit.Alc2-1:1:1	111	Alpha-coixin
L-SETit.Ali1-1:1:1	112	Aluminum-induced protein
L-SETit.Cab1-1:1:1	113	Chlorophyll a/b binding protein-1
L-SETit.Cab3-1:1:1	114	Chlorophyll a/b binding protein-3
L-SETit.Cbl7-1:1:1	115	Calcineurin B-like protein
L-SETit.Ccoamt-1:1:2	116	Cafeoyl CoA methyltransferase
L-SETit.Cda-1:1:1	117	Cell death associated protein
L-SETit.CLUS1164825-1-1:1:1	118	Cluster 1164825-1
L-SETit.CLUS120796-1-1:1:1	119	Cluster 120796-1
L-SETit.CLUS120796-1-1:1:2	120	Cluster 120796-1
L-SETit.CLUS19108-1:1:1	121	Cluster 19108
L-SETit.CLUS19108-1:1:2	122	Cluster 19108
L-SETit.CLUS882664-1-1:1:1	123	Cluster 882664-1
L-SETit.CP29-1:1:1	124	Chloroplast protein-29
L-SETit.Cyp-1:1:1	125	Cysteine protease-1
L-SETit.Cyp78a-1:1:1	126	Cysteine protease-78a
L-SETit.Cys-1:1:1	127	Cysteine synthase
L-SETit.Dzs-1:1:1	128	Delta zein storage protein
L-SETit.Eie-1:1:1	129	Elongation Factor
L-SETit.EST CLUS675389-2-1:1:1	130	Cluster 675389-2
L-SETit.Fba-1:1:1	131	Fructose-bisphosphate aldolase
L-SETit.Fst-1:1:1	132	Flavonol 4-sulfotransferase
L-SETit.Gapdh2-1:1:1	133	Glyceraldehyde-3-phosphate dehydrogenase
L-SETit.Grcw2-1:1:1	134	Glycine-rich cell wall structural protein 2
L-SETit.Gst-1:1:1	135	Glutathione S-transferase
L-SETit.Ifr-1:1:1	136	Isoflavone Reductase
L-SETit.LaDo-1:1:1	137	leucoanthocyanidin dioxygenase
L-SETit.Mt1-1:1:1	138	Metallothionein-like protein 1
L-SETit.Nrt2-1:1:2	139	Nitrate transporter-like protein
L-SETit.OMT2.1-1:1:1	140	O-methyltransferase-2.1
L-SETit.OMT2.2-1:1:1	141	O-methyltransferase-2.2
L-SETit.OMT2.2-1:1:2	142	O-methyltransferase-2.3

L-SETit.Omt3-1:1:1	143	O-methyltransferase-3
L-SETit.Omt4_2-1:1:1	144	O-methyltransferase-4.2
L-SETit.Pip2-1:1:1	145	Aquaporin
L-SETit.Pip2-3-1:1:1	146	Aquaporin
L-SETit.Pox-1:1:1	147	Peroxidase 1
L-SETit.Ppc-1:1:1	148	Phosphoenolpyruvate carboxylase
L-SETit.Ppdk-1:1:2	149	Pyruvate orthophosphate dikinase
L-SETit.Ppdk-1:1:4	150	Pyruvate orthophosphate dikinase
L-SETit.Pro1-1:1:1	151	Prolamin
L-SETit.Pro2-1:1:2	152	Prolamin
L-SETit.Prx-1:1:1	153	Peroxidase
L-SETit.Prx17-1:1:1	154	Peroxidase-17
L-SETit.Prx2-1:1:2	155	Peroxidase-2
L-SETit.Prx3-1:1:1	156	Peroxidase-3
L-SETit.Prx47-1:1:1	157	Peroxidase-47
L-SETit.Prx72-1:1:1	158	Peroxidase-72
L-SETit.PSI-4a-1:1:1	159	Photosystem 1 4a
L-SETit.Rbcs-1:1:1	160	Small subunit RUBISCO
L-SETit.Rcc3-1:1:1	161	Lipid Transfer Protein-RCc3
L-SETit.Rcc3-1:1:2	162	Lipid Transfer Protein-RCc3
L-SETit.Srp-1:1:1	163	Stress responsive protein
L-SETit.Ssp1-1:1:1	164	Seed storage protein
L-SETit.Tip-1:1:1	165	Tonoplast intrinsic protein
L-SETit.TubA2-1-1:1:1	166	Tubulin A2-1
L-SETit.TubA2-2-1:1:1	167	Tubulin A2-2
L-SETit.TubA3-1:1:1	168	Tubulin A3
L-SETit.Ubq1-1:1:1	169	Ubiquitin 1
L-SETit.Ubq5-1:1:1	170	Ubiquitin 5
L-SETit.Ucc1-1:1:1	171	Uclacyanin
I-SETit.Act8-1:1:2	172	Actin 8
I-SETit.CLUS120796-1-1:1:1	173	Cluster 120796-1
I-SETit.CLUS19108-1:1:1	174	Cluster 19108
I-SETit.Ppdk-1:1:1	175	Pyruvate orthophosphate dikinase
I-SETit.TubA2_1-1:1:2	176	Tubulin A2-1
I-SETit.Ubq1-1:1:1	177	Ubiquitin 1
I-SETit.Ubq5-1:1:2	178	Ubiquitin 5
I-SETit.14-3-3A-2-1:1:1	179	14-3-3-like protein A
I-SETit.14-3-3A-3-1:1:2	180	14-3-3-like protein A
I-SETit.14-3-3A-4-1:1:2	181	14-3-3-like protein A

I-SETit.14-3-3A-5-1:1:2	182	14-3-3-like protein A
I-SETit.14-3-3B-2-1:1:1	183	14-3-3-like protein B
I-SETit.14-3-3B-3-1:1:2	184	14-3-3-like protein B
I-SETit.14-3-3B-4-1:1:2	185	14-3-3-like protein B
I-SETit.14-3-3B-5-1:1:2	186	14-3-3-like protein B
I-SETit.14-3-3C-1-1:1:1	187	14-3-3-like protein C
I-SETit.14-3-3C-2-1:1:1	188	14-3-3-like protein C
I-SETit.14-3-3C-3-1:1:2	189	14-3-3-like protein C
I-SETit.14-3-3C-4-1:1:2	190	14-3-3-like protein C
I-SETit.14-3-3C-5-1:1:2	191	14-3-3-like protein C
I-SETit.14-3-3D-1-1:1:2	192	14-3-3-like protein D
I-SETit.14-3-3D-2-1:1:1	193	14-3-3-like protein D
I-SETit.14-3-3D-3-1:1:2	194	14-3-3-like protein D
I-SETit.14-3-3D-4-1:1:3	195	14-3-3-like protein D
I-SETit.14-3-3D-5-1:1:2	196	14-3-3-like protein D
I-SETit.14-3-3E-2-1:1:1	197	14-3-3-like protein E
I-SETit.14-3-3E-3-1:1:2	198	14-3-3-like protein E
I-SETit.14-3-3E-4-1:1:2	199	14-3-3-like protein E
I-SETit.14-3-3E-5-1:1:2	200	14-3-3-like protein E
I-SETit.40S-7S-1_1-1:1:2	201	40S ribosomal protein S7
I-SETit.40S-7S-1_2-1:1:2	202	40S ribosomal protein S7
I-SETit.40S-7S-1_3-1:1:2	203	40S ribosomal protein S7
I-SETit.40S-7S-1_4-1:1:2	204	40S ribosomal protein S7
I-SETit.40S-7S-2_2-1:1:1	205	40S ribosomal protein S7
I-SETit.40S-7S-2_3-1:1:1	206	40S ribosomal protein S7
I-SETit.40S-7S-2_4-1:1:1	207	40S ribosomal protein S7
I-SETit.40S-7S-3_1-1:1:2	208	40S ribosomal protein S7
I-SETit.40S-7S-3_2-1:1:2	209	40S ribosomal protein S7
I-SETit.40S-7S-3_3-1:1:2	210	40S ribosomal protein S7
I-SETit.60S_L10A1-1-1:1:2	211	60S ribosomal protein L10A1
I-SETit.60S_L10A1-2-1:1:2	212	60S ribosomal protein L10A1
I-SETit.60S_L10A1-3-1:1:2	213	60S ribosomal protein L10A1
I-SETit.60S_L10A1-4-1:1:1	214	60S ribosomal protein L10A1
I-SETit.60S_L10A1-5-1:1:2	215	60S ribosomal protein L10A1
I-SETit.ASA2-3-1:1:2	216	Anthranilate Synthase alpha 2 subunit
I-SETit.ClpD-1-1:1:1	217	ATP-dependent Clp protease ATP-binding subunit
I-SETit.DnaJ_1-1:1:2	218	Heat shock protein
I-SETit.DnaJ3-2-1:1:2	219	Heat shock protein
I-SETit.eEF1g_1-1:1:2	220	Elongation Factor 1 gamma

I-SETit.eEF1g_4-1:1:3	221	Elongation Factor 1 gamma
I-SETit.eIF5A1-1-1:1:1	222	Elongation Factor 5 alpha
I-SETit.eIF5A1-2-1:1:2	223	Elongation Factor 5 alpha
I-SETit.eIF5A1-3-1:1:2	224	Elongation Factor 5 alpha
I-SETit.eIF5A1-4-1:1:2	225	Elongation Factor 5 alpha
I-SETit.eIF5A1-5-1:1:2	226	Elongation Factor 5 alpha
I-SETit.eIF5A2-1-1:1:2	227	Elongation Factor 5 alpha
I-SETit.eIF5A2-2-1:1:3	228	Elongation Factor 5 alpha
I-SETit.eIF5A2-3-1:1:2	229	Elongation Factor 5 alpha
I-SETit.eIF5A2-4-1:1:2	230	Elongation Factor 5 alpha
I-SETit.eIF5A2-5-1:1:2	231	Elongation Factor 5 alpha
I-SETit.eIF5A3-1-1:1:1	232	Elongation Factor 5 alpha
I-SETit.eIF5A3-2-1:1:2	233	Elongation Factor 5 alpha
I-SETit.eIF5A3-3-1:1:2	234	Elongation Factor 5 alpha
I-SETit.eIF5A3-4-1:1:2	235	Elongation Factor 5 alpha
I-SETit.eIF5A3-5-1:1:2	236	Elongation Factor 5 alpha
I-SETit.GAD_1-1:1:2	237	UDP-glucuronic acid decarboxylase
I-SETit.GAD_2-1:1:2	238	UDP-glucuronic acid decarboxylase
I-SETit.GAD_3-1:1:2	239	UDP-glucuronic acid decarboxylase
I-SETit.GAD_4-1:1:2	240	UDP-glucuronic acid decarboxylase
I-SETit.Grf1-3-1:1:1	241	Putative growth-regulating factor
I-SETit.GRP-1-1:1:1	242	Glycine-rich RNA binding protein
I-SETit.LSm8-1-1:1:2	243	U6 snRNA-associated Sm-like protein LSm8
I-SETit.LSm8-2-1:1:1	244	U6 snRNA-associated Sm-like protein LSm8
I-SETit.LSm8-3-1:1:1	245	U6 snRNA-associated Sm-like protein LSm8
I-SETit.LSm8-4-1:1:2	246	U6 snRNA-associated Sm-like protein LSm8
I-SETit.PGK3_1-1:1:2	247	Phosphoglycerate kinase
I-SETit.PGK3_2-1:1:1	248	Phosphoglycerate kinase
I-SETit.PIP1_1_2-1:1:1	249	Aguaporin
I-SETit.PIP1-1_1-1:1:1	250	Aguaporin
I-SETit.PIP1-1_3-1:1:2	251	Aguaporin
I-SETit.PIP1-4_3-1:1:2	252	Aguaporin
I-SETit.PIP2-2_2-1:1:2	253	Aguaporin
I-SETit.PIP2-2_3-1:1:2	254	Aguaporin
I-SETit.PIP2-5_2-1:1:2	255	Aguaporin
I-SETit.PIP2-5_3-1:1:2	256	Aguaporin
I-SETit.Prx17-2-1:1:1	257	Peroxidase 17
I-SETit.Prx3-1-1:1:2	258	Peroxidase 3
I-SETit.SBD-1-1:1:2	259	Shwachman-Bodian-Diamond syndrome protein

I-SETit.SBD-2-1:1:1	260	Shwachman-Bodian-Diamond syndrome protein
I-SETit.SBD-3-1:1:2	261	Shwachman-Bodian-Diamond syndrome protein
I-SETit.TubA2_1-1:1:1	262	Tubulin A2
I-SETit.TubA2_2-1:1:1	263	Tubulin A2
I-SETit.TubA2_3-1:1:1	264	Tubulin A2
I-SETit.TubA3_1-1:1:1	265	Tubulin A3
I-SETit.TubA3_2-1:1:1	266	Tubulin A3
I-SETit.Wx1-1-1:1:2	267	Putative granule bound starch synthase
T-SETit.Act1-1:1:1	268	Actin 1
T-SETit.Act8-1:1:1	269	Actin 8
T-SETit.Ams1-1:1:1	270	S-adenosylmethionine synthetase 1
T-SETit.Ctpt-1:1:2	271	Triose phosphate/phosphate translocator, chloroplast precursor
T-SETit.Fba-1:1:1	272	Fructose-bisphosphate aldolase
T-SETit.Fnr-1:1:1	273	Ferredoxin-NADP+ reductase
T-SETit.Mes2-1:1:1	274	Methionine synthase 2
T-SETit.Ntr-1:1:1	275	Nitrite transporter
T-SETit.Sus2-1:1:1	276	Sucrose synthase 2
GOI-TS-APX	277	Ascorbate Peroxidase
GOI-TS-APX:1:2	278	Ascorbate Peroxidase
GOI-TS-APX2:1:1	279	Ascorbate Peroxidase
GOI-TS-CNT:1:2	280	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
GOI-TS-DHDPS:1:2	281	Dihydrodipicolinate synthase precursor, chloroplastic
GOI-TS-Fe-SD:1:1	282	Iron-superoxidedismutases, chloroplastic
GOI-TS-PPR:1:1	283	Pentatricopeptide repeat-containing protein, putative
TS-SETit.APG6-1:1:1	284	Casein lytic proteinase B3 heat shock protein-like
TS-SETit.APX.2.ex1-1:1:1	285	Ascorbate Peroxidase
TS-SETit.APX.ex1-1:1:1	286	Ascorbate Peroxidase
TS-SETit.APX.ex2-1:1:2	287	Ascorbate Peroxidase
TS-SETit.APX2.ex2-1:1:1	288	Ascorbate Peroxidase
TS-SETit.APX3-1:1:1	289	Ascorbate Peroxidase
TS-SETit.ASA2-1:1:1	290	Anthranilate Synthase alpha 2 subunit
TS-SETit.CC10-1:1:1	291	Chloroplast Chaperonin 10 Kd subunit
TS-SETit.CHoR1-1:1:1	292	Calcium homeostasis regulator
TS-SETit.ClpD-1:1:1	293	ATP-dependent Clp protease ATP-binding subunit

TS-SETit.CNT.ex1-1:1:1	294	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
TS-SETit.CNT.ex2-1:1:2	295	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
TS-SETit.CR88-1:1:1	296	Heat-shock protein putative
TS-SETit.DHDPS.Ex1-1:1:1	297	Dihydrodipicolinate synthase precursor, chloroplastic
TS-SETit.DHDPS.Ex2-1:1:1	298	Dihydrodipicolinate synthase precursor, chloroplastic
TS-SETit.Fe-SD.ex1-1:1:1	299	Iron-superoxidedismutases, chloroplastic
TS-SETit.Fe-SD.ex2-1:1:1	300	Iron-superoxidedismutases, chloroplastic
TS-SETit.G-typA-1:1:1	301	GTP-binding protein typA
TS-SETit.HDh-1:1:1	302	Haloacid dehalogenase-like hydrolase
TS-SETit.IMP-1:1:1	303	Inositol-1-monophosphatase, putative, chloroplastic
TS-SETit.MDH-1:1:1	304	Putative NAD-malate dehydrogenase
TS-SETit.PPR.ex1-1:1:1	305	Pentatricopeptide repeat-containing protein, putative
TS-SETit.PPR.ex2-1:1:2	306	Pentatricopeptide repeat-containing protein, putative
TS-SETit.PSPR-3-1:1:1	307	Plastid-specific 30S ribosomal protein 3
TS-SETit.RbcS_1-1:1:1	308	Small subunit RUBISCO
TS-SETit.RbcS_2-1:1:1	309	Small subunit RUBISCO
TS-SETit.RbcS_3-1:1:1	310	Small subunit RUBISCO
TS-SETit.RbcS_4-1:1:1	311	Small subunit RUBISCO
TS-SETit.ShkG-1:1:1	312	5-enolpyruvylshikimate-3-phosphate synthase precursor
TS-SETit.SRP43-1:1:1	313	Signal recognition particle 43 kDa protein, chloroplastic
TS-SETit.TDh-1:1:1	314	Threonine dehydratase biosynthetic, chloroplast precursor
TS-SETit.ThR-1:1:1	315	Thioredoxin
TS-SETit.Wx1-1:1:1	316	Putative granule bound starch synthase
I-SETit.APX.2-1:1:1	317	Ascorbate Peroxidase
I-SETit.APX-1:1:1	318	Ascorbate Peroxidase
I-SETit.APX-1:1:2	319	Ascorbate Peroxidase
I-SETit.CNT.1-1:1:1	320	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
I-SETit.DHDPS_1-1:1:1	321	Dihydrodipicolinate synthase precursor, chloroplastic
I-SETit.Fe-SD-1:1:1	322	Iron-superoxidedismutases, chloroplastic
I-SETit.PPR-1:1:2	323	Pentatricopeptide repeat-containing protein, putative
TS-SETit.APG6.pep	324	Casein lytic proteinase B3 heat shock protein-

		like
TS-SETit.APX.2.pep	325	Ascorbate Peroxidase
TS-SETit.APX.pep	326	Ascorbate Peroxidase
TS-SETit.APX3.pep	327	Ascorbate Peroxidase
TS-SETit.ASA2.pep	328	Anthranilate Synthase alpha 2 subunit
TS-SETit.CC10.pep	329	Chloroplast Chaperonin 10 Kd subunit
TS-SETit.CHoR1.pep	330	Calcium homeostasis regulator
TS-SETit.ClpD.pep	331	ATP-dependent Clp protease ATP-binding subunit
TS-SETit.CNT.pep	332	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
TS-SETit.CR88.pep	333	Heat-shock protein putative
TS-SETit.DHDPS.pep	334	Dihydrodipicolinate synthase precursor, chloroplastic
TS-SETit.Fe-SD.pep	335	Iron-superoxidedismutases, chloroplastic
TS-SETit.G-typA.pep	336	GTP-binding protein typA
TS-SETit.HDh.pep	337	Haloacid dehalogenase-like hydrolase
TS-SETit.IMP.pep	338	Inositol-1-monophosphatase, putative, chloroplastic
TS-SETit.MDH.pep	339	Putative NAD-malate dehydrogenase
TS-SETit.PPR.pep	340	Pentatricopeptide repeat-containing protein, putative
TS-SETit.PSPR-3.pep	341	Plastid-specific 30S ribosomal protein 3
TS-SETit.RbcS_1.pep	342	Small subunit RUBISCO
TS-SETit.RbcS_2.pep	343	Small subunit RUBISCO
TS-SETit.RbcS_3.pep	344	Small subunit RUBISCO
TS-SETit.RbcS_4.pep	345	Small subunit RUBISCO
TS-SETit.ShkG.pep	346	5-enolpyruvylshikimate-3-phosphate synthase precursor
TS-SETit.SRP43.pep	347	Signal recognition particle 43 kDa protein, chloroplastic
TS-SETit.TDh.pep	348	Threonine dehydratase biosynthetic, chloroplast precursor
TS-SETit.ThR.pep	349	Thioredoxin
TS-SETit.Wx1.pep	350	Putative granule bound starch synthase
E-FMV.35S-1:1:2	351	FMV 35S enhancer
E-SETit.Mth-1:1:2	352	Metallothionein
P-SETit.25509-1:1:1	353	Cluster 25509
P-SETit.25509-1:1:2	354	Cluster 25509
P-SETit.Act8-1:1:1	355	Actin 8
P-SETit.Act8-1:1:2	356	Actin 8
P-SETit.Act8-1:1:3	357	Actin 8



P-SETit.Act8-1:1:4	358	Actin 8
P-SETit.Act8-1:1:7	359	Actin 8
P-SETit.Act8-1-1:1:1	360	Actin 8
P-SETit.Aip-1:1:2	361	Auxin-induced protein
P-SETit.AKR-1:1:1	362	Aspartate kinase reductase
P-SETit.AKR-1:1:2	363	Aspartate kinase reductase
P-SETit.Alc2-1:1:1	364	Alpha-coixin
P-SETit.Ali1-1:1:1	365	Aluminum-induced protein
P-SETit.Ali1-1:1:2	366	Aluminum-induced protein
P-SETit.Ali1-1:1:4	367	Aluminum-induced protein
P-SETit.Cab3-1:1:1	368	Chlorophyll a/b binding protein 3
P-SETit.Cab3-1:1:2	369	Chlorophyll a/b binding protein 3
P-SETit.cab6-1:1:1	370	Chlorophyll a/b binding protein 6
P-SETit.Cab6-1:1:1	371	Chlorophyll a/b binding protein 6
P-SETit.Cab6-1:1:2	372	Chlorophyll a/b binding protein 6
P-SETit.Cafeoyl CoA methyltransferase-1:1:1	373	Cafeoyl CoA methyltransferase
P-SETit.Caffeoyl-CoA O-methyltransferase-1:1:1	374	Cafeoyl CoA methyltransferase
P-SETit.Caffeoyl-CoA O-methyltransferase-a-1:1:1	375	Cafeoyl CoA methyltransferase
P-SETit.Ccoamt-1:1:1	376	Cafeoyl CoA methyltransferase
P-SETit.Chi-1:1:1	377	Chitinase
P-SETit.chitinase-1:1:1	378	Chitinase
P-SETit.Chitinase-1:1:1	379	Chitinase
P-SETit.Chitinase-1:1:2	380	Chitinase
P-SETit.Chitinase-1:1:3	381	Chitinase
P-SETit.Chl A/b III-1:1:1	382	Chlorophyll a/b binding protein 3
P-SETit.Chl a/b pre-1:1:1	383	Chlorophyll a/b binding pre-protein
P-SETit.Class III citinase-1:1:1	384	Class III citinase
P-SETit.Class III citinase-1:1:2	385	Class III citinase
P-SETit.Class III citinase-1:1:3	386	Class III citinase
P-SETit.CLUS19108-1:1:1	387	Cluster 19108
P-SETit.CLUS882664-1-1:1:1	388	Cluster 882664
P-SETit.CLUS882664-1-1:1:2	389	Cluster 882664
P-SETit.Cp26-1:1:1	390	Chloroplast protein-26
P-SETit.Cp26-1:1:2	391	Chloroplast protein-26
P-SETit.CP29-1:1:1	392	Chloroplast protein-29
P-SETit.CP29-1:1:2	393	Chloroplast protein-29
P-SETit.CP29-1:1:3	394	Chloroplast protein-29

P-SETit.CP29-1:1:5	395	Chloroplast protein-29
P-SETit.Cyp78a-1:1:1	396	Cysteine protease-78a
P-SETit.Cys-1:1:1	397	Cystein protease
P-SETit.Cys-1:1:4	398	Cystein protease
P-SETit.Cys-1:1:5	399	Cystein protease
P-SETit.DRP-1:1:1	400	Dehydration responsive protein
P-SETit.DRP-1:1:2	401	Dehydration responsive protein
P-SETit.DRP-B-1:1:1	402	Dehydration responsive protein
P-SETit.Dzs-1:1:1	403	Delta zein storage protein
P-SETit.Dzs-1:1:2	404	Delta zein storage protein
P-SETit.Dzs-1:1:3	405	Delta zein storage protein
P-SETit.ESTCLUS675389-2-1:1:1	406	Cluster 675389-2
P-SETit.ESTCLUS675389-2-1:1:2	407	Cluster 675389-2
P-SETit.FBP-ald-1:1:1	408	Fructose 1,6-Bisphosphate Aldolase
P-SETit.FM54-1:1:1	409	Cluster 1102871_1
P-SETit.FM63-1:1:1	410	Cluster 1019870_1
P-SETit.foxtailRcc3-1:1:1	411	Lipid Transfer Protein-RCc3
P-SETit.FStr1-1:1:1	412	Flavonol 4-sulfotransferase
P-SETit.FStr1-1:1:2	413	Flavonol 4-sulfotransferase
P-SETit.FStr2-1:1:1	414	Flavonol 4-sulfotransferase
P-SETit.Gahdp-1:1:1	415	Glyceraldehyde-3-phosphate dehydrogenase
P-SETit.Gahdp-1:1:2	416	Glyceraldehyde-3-phosphate dehydrogenase
P-SETit.gapdh-1:1:1	417	Glyceraldehyde-3-phosphate dehydrogenase
P-SETit.Gapdh2-1:1:1	418	Glyceraldehyde-3-phosphate dehydrogenase
P-SETit.Gapdh2-1:1:2	419	Glyceraldehyde-3-phosphate dehydrogenase
P-SETit.Glutathione S-Transferase-1:1:1	420	Glutathione S-Transferase
P-SETit.Glutathione S-transferase-1:1:1	421	Glutathione S-Transferase
P-SETit.Glutathione S-transferase-1:1:2	422	Glutathione S-Transferase
P-SETit.GlutathioneS-transferase-1:1:3	423	Glutathione S-Transferase
P-SETit.Grew2-1:1:2	424	Glycine-rich cell wall structural protein 2
P-SETit.Gr-1:1:1	425	Putative growth-regulating factor
P-SETit.Ifr-1:1:1	426	Isoflavone Reducataase
P-SETit.Ifr2-1:1:1	427	Isoflavone Reducataase
P-SETit.Isoflavone Red Like-1:1:1	428	Isoflavone Reductase like
P-SETit.Isoflavone Reducataase	429	Isoflavone Reductase like

like-1:1:2		
P-SETit.Isoflavone Reductase 2-1:1:1	430	Isoflavone Reductase
P-SETit.Isoflavone Reductase Like-1:1:1	431	Isoflavone Reductase like
P-SETit.Isoflavone Reductase-1:1:1	432	Isoflavone Reductase
P-SETit.Isoflavone reductase-1:1:1	433	Isoflavone Reductase
P-SETit.Isoflavone reductase-1:1:2	434	Isoflavone Reductase
P-SETit.IsoflavoneReductase1-1:1:1	435	Isoflavone Reductase
P-SETit.IsoflavoneReductase1-1:1:2	436	Isoflavone Reductase
P-SETit.LaDo-1:1:1	437	leucoanthocianidin dioxygenase
P-SETit.Mt1-1:1:1	438	Metallothionein-like protein 1
P-SETit.Mt1-1:1:3	439	Metallothionein-like protein 1
P-SETit.Mth-1:1:1	440	Metallothionein
P-SETit.Mth-1:1:2	441	Metallothionein
P-SETit.Mth-1:1:4	442	Metallothionein
P-SETit.MTLP-1:1:1	443	Metallothionein-like protein
P-SETit.MTLP-1:1:2	444	Metallothionein-like protein
P-SETit.MTLP-1:1:3	445	Metallothionein-like protein
P-SETit.Nadp-Me-1:1:1	446	NADP malate enzyme
P-SETit.Nadp-me-1:1:1	447	NADP malate enzyme
P-SETit.Nitrite Transporter-1:1:1	448	Nitrite transporter
P-SETit.Nitrite transporter-1:1:1	449	Nitrite transporter
P-SETit.Nitrite Transporter-1:1:2	450	Nitrite transporter
P-SETit.Nitrite transporter-1:1:2	451	Nitrite transporter
P-SETit.Nitrite Transporter-1:1:3	452	Nitrite transporter
P-SETit.Nitritetransporter-1:1:3	453	Nitrite transporter
P-SETit.Nr1-1:1:1	454	Nitrate reductase
P-SETit.Nr1-1:1:2	455	Nitrate reductase
P-SETit.Nr1-1:1:3	456	Nitrate reductase
P-SETit.Nr1-1:1:4	457	Nitrate reductase
P-SETit.Nr1-1:1:5	458	Nitrate reductase
P-SETit.NRED-1:1:1	459	Nitrate reductase
P-SETit.Nrt2-1:1:1	460	Nitrate transporter-like protein

P-SETit.OMT2.1-1:1:1	461	O-methyltransferase
P-SETit.OMT2.2-1:1:1	462	O-methyltransferase
P-SETit.Omt3-1:1:1	463	O-methyltransferase
P-SETit.Omt3-1:1:2	464	O-methyltransferase
P-SETit.Omt4_2-1:1:1	465	O-methyltransferase
P-SETit.OMt4-1:1:1	466	O-methyltransferase
P-SETit.Peroxidase-1:1:1	467	Peroxidase
P-SETit.Peroxidase-1:1:2	468	Peroxidase
P-SETit.Pip2-1:1:1	469	Aquaporin
P-SETit.pip2-1:1:1	470	Aquaporin
P-SETit.Pip2-1:1:2	471	Aquaporin
P-SETit.pip2-1:1:2	472	Aquaporin
P-SETit.Pip2-3-1:1:2	473	Aquaporin
P-SETit.Pip2-3-1:1:3	474	Aquaporin
P-SETit.Pip2-3-1:1:4	475	Aquaporin
P-SETit.PIP2-5-1:1:1	476	Aquaporin
P-SETit.POX-1:1:1	477	Peroxidase
P-SETit.Ppc-1:1:1	478	Phosphoenolpyruvate carboxylase
P-SETit.Ppc-1:1:2	479	Phosphoenolpyruvate carboxylase
P-SETit.Ppc-1:1:5	480	Phosphoenolpyruvate carboxylase
P-SETit.Ppc-1:1:6	481	Phosphoenolpyruvate carboxylase
P-SETit.Ppdk-1:1:2	482	Pyruvate orthophosphate dikinase
P-SETit.Pro1-1:1:1	483	Prolamine 1
P-SETit.Pro2-1:1:1	484	Prolamine 2
P-SETit.Pro2-1:1:2	485	Prolamine 2
P-SETit.Prx17-1:1:1	486	Peroxidase 17
P-SETit.Prx2-1:1:1	487	Peroxidase 2
P-SETit.Prx2-1:1:2	488	Peroxidase 2
P-SETit.Prx2-1:1:4	489	Peroxidase 2
P-SETit.Prx3-1:1:1	490	Peroxidase 3
P-SETit.Prx3-1:1:2	491	Peroxidase 3
P-SETit.Prx3-1:1:5	492	Peroxidase 3
P-SETit.Prx4-1:1:1	493	Peroxidase 4
P-SETit.Prx47-1:1:1	494	Peroxidase 47
P-SETit.Prx47-1:1:3	495	Peroxidase 47
P-SETit.Prx72-1:1:1	496	Peroxidase 72
P-SETit.PSIRC-1:1:1	497	Photosystem 1 reaction center
P-SETit.Rbcs-1:1:2	498	Small subunit RUBISCO
P-SETit.Rcc3-1:1:12	499	Lipid Transfer Protein-RCc3

P-SETit.Rcc3-1:1:13	500	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:14	501	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:15	502	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:2	503	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:3	504	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:4	505	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:5	506	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:6	507	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:7	508	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:8	509	Lipid Transfer Protein-RCc3
P-SETit.Rcc3-1:1:9	510	Lipid Transfer Protein-RCc3
P-SETit.RUBP-1:1:1	511	RuBP-carboxylase/oxygenase
P-SETit.RUBP-pre-1:1:1	512	RuBP-carboxylase/oxygenase precursor
P-SETit.Srp-1:1:1	513	Stress responsive protein
P-SETit.Srp-1:1:3	514	Stress responsive protein
P-SETit.Tga6-1:1:1	515	Teosinte glume architecture
P-SETit.TGA6-1:1:1	516	Teosinte glume architecture
P-SETit.TGA6-1:1:2	517	Teosinte glume architecture
P-SETit.TIP-1:1:1	518	Tonoplast intrinsic protein
P-SETit.Tip-1:1:2	519	Tonoplast intrinsic protein
P-SETit.Tip-1:1:3	520	Tonoplast intrinsic protein
P-SETit.Tip-1:1:5	521	Tonoplast intrinsic protein
P-SETit.Tip-1:1:6	522	Tonoplast intrinsic protein
P-SETit.Tub3A-1:1:1	523	Tubulin A3
P-SETit.TubA-1:1:1	524	Tubulin A
P-SETit.TubA-1:1:2	525	Tubulin A
P-SETit.TubA2-1-1:1:1	526	Tubulin A2
P-SETit.TubA2-1-1:1:4	527	Tubulin A2
P-SETit.TubA2-2-1:1:1	528	Tubulin A2
P-SETit.TubA2-2-1:1:2	529	Tubulin A2
P-SETit.TubA3-1:1:1	530	Tubulin A3
P-SETit.TubA3-1:1:2	531	Tubulin A3
P-SETit.Ubq1-1:1:2	532	Ubiquitin 1
P-SETit.Ubq5-1:1:1	533	Ubiquitin 5
P-SETit.Ucc1-1:1:1	534	Uclacyanin
P-SETit.Ucc1-1:1:3	535	Uclacyanin
P-SETit.Ucc2-1:1:1	536	Uclacyanin
L-SETit.25509-1:1:1	537	Cluster 25509
L-SETit.Act1-1:1:1	538	Actin 1

L-SETit.Act2-1:1:1	539	Actin 2
L-SETit.Act8-1:1:1	540	Actin 8
L-SETit.Actin-1:1:1	541	Actin
L-SETit.AKR-1:1:1	542	Aspartate kinase reductase
L-SETit.Cab3-1:1:2	543	Chlorophyll a/b
L-SETit.Caffeoyl-CoA O-methyltransferase-1:1:1	544	Caffeoyl-CoA O-methyltransferase
L-SETit.Caffeoyl-CoAO-methyltransferase-1:1:2	545	Caffeoyl-CoA O-methyltransferase
L-SETit.Ccoamt-1:1:1	546	Caffeoyl-CoA O-methyltransferase
L-SETit.Chi-1:1:1	547	Chitinase
L-SETit.CP29-1:1:2	548	Chloroplast protein-29
L-SETit.CP29-1:1:3	549	Chloroplast protein-29
L-SETit.DRP-1:1:1	550	Dehydration responsive protein
L-SETit.DRP-1:1:2	551	Dehydration responsive protein
L-SETit.FStr1-1:1:1	552	Flavonol 4-sulfotransferase
L-SETit.FStr1-1:1:2	553	Flavonol 4-sulfotransferase
L-SETit.FStr1-1:1:3	554	Flavonol 4-sulfotransferase
L-SETit.gapdh-1:1:1	555	Glyceraldehyde-3-phosphate dehydrogenase
L-SETit.Gapdh-1:1:1	556	Glyceraldehyde-3-phosphate dehydrogenase
L-SETit.Gr-1:1:1	557	Putative growth-regulating factor
L-SETit.Gr-1:1:2	558	Putative growth-regulating factor
L-SETit.Isoflavone Reductase 2-1:1:1	559	Isoflavone Reductase
L-SETit.Isoflavone Reductase 1-1:1:1	560	Isoflavone Reductase
L-SETit.MOt4-1:1:1	561	O-methyltransferase
L-SETit.Mth-1:1:1	562	Metallothionein
L-SETit.Mth-1:1:2	563	Metallothionein
L-SETit.MTLP-1:1:1	564	Metallothionein like protein
L-SETit.MTLP-1:1:2	565	Metallothionein like protein
L-SETit.Nadp-me-1:1:1	566	NADP malate enzyme
L-SETit.NADP-Me-1:1:1	567	NADP malate enzyme
L-SETit.Nr1-1:1:1	568	Nitrate reductase
L-SETit.Nr1-1:1:2	569	Nitrate reductase
L-SETit.Nr1-1:1:3	570	Nitrate reductase
L-SETit.Nrt2-1:1:1	571	Nitrate reductase
L-SETit.OMt4-1:1:1	572	O-methyltransferase
L-SETit.Pip2-3-1:1:2	573	Aquaporin
L-SETit.POX-1:1:1	574	Peroxidase
L-SETit.Ppc-1:1:2	575	Phosphoenolpyruvate carboxylase

L-SETit.Ppc-1:1:3	576	Phosphoenolpyruvate carboxylase
L-SETit.Ppc-1:1:4	577	Phosphoenolpyruvate carboxylase
L-SETit.Ppdk-1:1:1	578	Pyruvate orthophosphate dikinase
L-SETit.Ppdk-1:1:3	579	Pyruvate orthophosphate dikinase
L-SETit.Pro2-1:1:1	580	Prolamine 2
L-SETit.Prx2-1:1:1	581	Peroxidase 2
L-SETit.Prx4-1:1:1	582	Peroxidase 4
L-SETit.Rcc3-1:1:3	583	Lipid Transfer Protein-RCc3
L-SETit.TGA6-1:1:1	584	Teosinte glume architecture
L-SETit.TIP-1:1:1	585	Tonoplast intrinsic protein
L-SETit.Tip-1:1:2	586	Tonoplast intrinsic protein
L-SETit.TubA-1:1:1	587	Tubulin A
L-SETit.TubA-1:1:2	588	Tubulin A
I-SETit.14-3-3A-3-1:1:1	589	14-3-3-like protein A
I-SETit.14-3-3A-4-1:1:1	590	14-3-3-like protein A
I-SETit.14-3-3A-5-1:1:1	591	14-3-3-like protein A
I-SETit.14-3-3B-3-1:1:1	592	14-3-3-like protein B
I-SETit.14-3-3B-4-1:1:1	593	14-3-3-like protein B
I-SETit.14-3-3B-5-1:1:1	594	14-3-3-like protein B
I-SETit.14-3-3C-3-1:1:1	595	14-3-3-like protein C
I-SETit.14-3-3C-4-1:1:1	596	14-3-3-like protein C
I-SETit.14-3-3C-5-1:1:1	597	14-3-3-like protein C
I-SETit.14-3-3D-1-1:1:1	598	14-3-3-like protein D
I-SETit.14-3-3D-3-1:1:1	599	14-3-3-like protein D
I-SETit.14-3-3D-4-1:1:1	600	14-3-3-like protein D
I-SETit.14-3-3D-4-1:1:2	601	14-3-3-like protein D
I-SETit.14-3-3D-5-1:1:1	602	14-3-3-like protein D
I-SETit.14-3-3E-3-1:1:1	603	14-3-3-like protein E
I-SETit.14-3-3E-4-1:1:1	604	14-3-3-like protein E
I-SETit.14-3-3E-5-1:1:1	605	14-3-3-like protein E
I-SETit.14-3-3lp-1:1:1	606	14-3-3-like protein
I-SETit.40S-7S-1_1-1:1:1	607	40S ribosomal protein S7
I-SETit.40S-7S-1_2-1:1:1	608	40S ribosomal protein S7
I-SETit.40S-7S-1_3-1:1:1	609	40S ribosomal protein S7
I-SETit.40S-7S-1_4-1:1:1	610	40S ribosomal protein S7
I-SETit.40S-7S-3_1-1:1:1	611	40S ribosomal protein S7
I-SETit.40S-7S-3_2-1:1:1	612	40S ribosomal protein S7
I-SETit.40S-7S-3_3-1:1:1	613	40S ribosomal protein S7
I-SETit.40S-7S-3_3p-1:1:1	614	40S ribosomal protein S7

I-SETit.60S_L10A1-1-1:1:1	615	60S ribosomal protein L10A1
I-SETit.60S_L10A1-2-1:1:1	616	60S ribosomal protein L10A1
I-SETit.60S_L10A1-3-1:1:1	617	60S ribosomal protein L10A1
I-SETit.60S_L10A1-5-1:1:1	618	60S ribosomal protein L10A1
I-SETit.Act1-1:1:1	619	Actin 1
I-SETit.Act1-1:1:2	620	Actin 1
I-SETit.Act1-1:1:3	621	Actin 1
I-SETit.Act1-1:1:4	622	Actin 1
I-SETit.Act2-1:1:1	623	Actin 2
I-SETit.Act3-1:1:1	624	Actin 3
I-SETit.Act3-1:1:2	625	Actin 3
I-SETit.Act4-1:1:1	626	Actin 4
I-SETit.Act4-1:1:2	627	Actin 4
I-SETit.Act6-1:1:1	628	Actin 6
I-SETit.Act6-1:1:2	629	Actin 6
I-SETit.Act6-1:1:3	630	Actin 6
I-SETit.Act6-1:1:4	631	Actin 6
I-SETit.Act7-1:1:1	632	Actin 7
I-SETit.Act7-1:1:2	633	Actin 7
I-SETit.Act7-1:1:3	634	Actin 7
I-SETit.Act8-1:1:1	635	Actin 8
I-SETit.Actin1-1:1:1	636	Actin 1
I-SETit.Arp1_1-1:1:1	637	60S acidic ribosomal protein P2A, putative, expressed
I-SETit.Arp1_1-1:1:2	638	60S acidic ribosomal protein P2A, putative, expressed
I-SETit.Arp1_1-1:1:3	639	60S acidic ribosomal protein P2A, putative, expressed
I-SETit.Arp1_2-1:1:1	640	60S acidic ribosomal protein P2A, putative, expressed
I-SETit.Arp1_2-1:1:2	641	60S acidic ribosomal protein P2A, putative, expressed
I-SETit.Arp1_3-1:1:1	642	60S acidic ribosomal protein P2A, putative, expressed
I-SETit.Arp1_3-1:1:2	643	60S acidic ribosomal protein P2A, putative, expressed
I-SETit.ASA2-3-1:1:1	644	Anthranilate Synthase alpha 2 subunit
I-SETit.CNT-1-1:1:1	645	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
I-SETit.CNT-2-1:1:1	646	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
I-SETit.CNT-3-1:1:1	647	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase



I-SETit.Cys-1-1:1:1	648	Cystein protease
I-SETit.Cys-2-1:1:1	649	Cystein protease
I-SETit.Cys-3-1:1:1	650	Cystein protease
I-SETit.DHDPS-1-1:1:1	651	Dihydrodipicolinate synthase precursor, chloroplastic
I-SETit.DHDPS-2-1:1:1	652	Dihydrodipicolinate synthase precursor, chloroplastic
I-SETit.DnaJ_1-1:1:1	653	Heat shock protein
I-SETit.DnaJ2-1:1:1	654	Heat shock protein
I-SETit.DnaJ2-1:1:2	655	Heat shock protein
I-SETit.DnaJ3-2-1:1:1	656	Heat shock protein
I-SETit.DnaK10-1:1:1	657	Heat shock protein
I-SETit.DnaK10-1:1:2	658	Heat shock protein
I-SETit.DnaK1-1:1:1	659	Heat shock protein
I-SETit.DnaK1-1:1:2	660	Heat shock protein
I-SETit.DnaK1-1:1:3	661	Heat shock protein
I-SETit.DnaK2-1:1:1	662	Heat shock protein
I-SETit.DnaK5-1:1:1	663	Heat shock protein
I-SETit.DnaK5-1:1:2	664	Heat shock protein
I-SETit.DnaK6-1:1:1	665	Heat shock protein
I-SETit.DnaK6-1:1:2	666	Heat shock protein
I-SETit.DnaK8-1:1:1	667	Heat shock protein
I-SETit.DnaK8-1:1:2	668	Heat shock protein
I-SETit.DnaK8-1:1:3	669	Heat shock protein
I-SETit.DnaK9-1:1:1	670	Heat shock protein
I-SETit.DnaK9-1:1:2	671	Heat shock protein
I-SETit.DnaK9-1-1:1:1	672	Heat shock protein
I-SETit.eEF1a_1-1:1:1	673	Elongation factor 1 alpha
I-SETit.eEF1a_1-1:1:2	674	Elongation factor 1 alpha
I-SETit.eEF1a_2-1:1:1	675	Elongation factor 1 alpha
I-SETit.eEF1a_2-1:1:2	676	Elongation factor 1 alpha
I-SETit.eEF1a_3-1:1:1	677	Elongation factor 1 alpha
I-SETit.eEF1a_3-1:1:2	678	Elongation factor 1 alpha
I-SETit.eEF1a_4-1:1:1	679	Elongation factor 1 alpha
I-SETit.eEF1a_4-1:1:2	680	Elongation factor 1 alpha
I-SETit.eEF1a_5-1:1:1	681	Elongation factor 1 alpha
I-SETit.eEF1a_5-1:1:2	682	Elongation factor 1 alpha
I-SETit.eEF1a_6-1:1:1	683	Elongation factor 1 alpha
I-SETit.eEF1a_6-1:1:2	684	Elongation factor 1 alpha
I-SETit.eEF1a_7-1:1:1	685	Elongation factor 1 alpha

I-SETit.eEF1a_7-1:1:2	686	Elongation factor 1 alpha
I-SETit.eEF1a_7-1:1:3	687	Elongation factor 1 alpha
I-SETit.eEF1g_1-1:1:1	688	Elongation Factor 1 gamma
I-SETit.eEF1g_2-1:1:1	689	Elongation Factor 1 gamma
I-SETit.eEF1g_2-1:1:2	690	Elongation Factor 1 gamma
I-SETit.eEF1g_2-1:1:3	691	Elongation Factor 1 gamma
I-SETit.eEF1g_3-1:1:1	692	Elongation Factor 1 gamma
I-SETit.eEF1g_3-1:1:2	693	Elongation Factor 1 gamma
I-SETit.eEF1g_4-1:1:1	694	Elongation Factor 1 gamma
I-SETit.eEF1g_4-1:1:2	695	Elongation Factor 1 gamma
I-SETit.eEF1g3-2-1:1:1	696	Elongation Factor 1 gamma
I-SETit.eEF1g3-3-1:1:1	697	Elongation Factor 1 gamma
I-SETit.eEF2-1:1:1	698	Elongation Factor 2
I-SETit.eEF2-1:1:2	699	Elongation Factor 2
I-SETit.eEF2-1:1:3	700	Elongation Factor 2
I-SETit.eIF5A1-2-1:1:1	701	Elongation Factor 5 alpha
I-SETit.eIF5A1-3-1:1:1	702	Elongation Factor 5 alpha
I-SETit.eIF5A1-4-1:1:1	703	Elongation Factor 5 alpha
I-SETit.eIF5A1-5-1:1:1	704	Elongation Factor 5 alpha
I-SETit.eIF5A2-1-1:1:1	705	Elongation Factor 5 alpha
I-SETit.eIF5A2-2-1:1:1	706	Elongation Factor 5 alpha
I-SETit.eIF5A2-2-1:1:2	707	Elongation Factor 5 alpha
I-SETit.eIF5A2-3-1:1:1	708	Elongation Factor 5 alpha
I-SETit.eIF5A2-4-1:1:1	709	Elongation Factor 5 alpha
I-SETit.eIF5A2-5-1:1:1	710	Elongation Factor 5 alpha
I-SETit.eIF5A3-2-1:1:1	711	Elongation Factor 5 alpha
I-SETit.eIF5A3-3-1:1:1	712	Elongation Factor 5 alpha
I-SETit.eIF5A3-4-1:1:1	713	Elongation Factor 5 alpha
I-SETit.eIF5A3-5-1:1:1	714	Elongation Factor 5 alpha
I-SETit.Ein3-1:1:1	715	Ethylene-insensitive 3-like protein
I-SETit.EIN3-1:1:1	716	Ethylene-insensitive 3-like protein
I-SETit.GAD_1-1:1:1	717	UDP-glucuronic acid decarboxylase
I-SETit.GAD_2-1:1:1	718	UDP-glucuronic acid decarboxylase
I-SETit.GAD_3-1:1:1	719	UDP-glucuronic acid decarboxylase
I-SETit.GAD_4-1:1:1	720	UDP-glucuronic acid decarboxylase
I-SETit.Grf1-1:1:1	721	Putative growth-regulating factor
I-SETit.Grf2-1:1:1	722	Putative growth-regulating factor
I-SETit.Grf3-1:1:1	723	Putative growth-regulating factor
I-SETit.Grp-1:1:1	724	Glycine-rich RNA binding protein

I-SETit.HDh-1:1:1	725	Aquaporin
I-SETit.I-SETit.PIP1-1_2-1:1:1	726	Aquaporin
I-SETit.I-SETit.PIP1-1_3-1:1:1	727	Aquaporin
I-SETit.I-SETit.PIP2-2_2-1:1:1-1:1:1	728	Aquaporin
I-SETit.LSm80-1-1:1:1	729	U6 snRNA-associated Sm-like protein LSm80
I-SETit.LSm80-2-1:1:1	730	U6 snRNA-associated Sm-like protein LSm80
I-SETit.LSm80-3-1:1:1	731	U6 snRNA-associated Sm-like protein LSm80
I-SETit.LSm80-4-1:1:1	732	U6 snRNA-associated Sm-like protein LSm80
I-SETit.LSm8-1-1:1:1	733	U6 snRNA-associated Sm-like protein LSm8
I-SETit.LSm8-4-1:1:1	734	U6 snRNA-associated Sm-like protein LSm8
I-SETit.MDH-1:1:1	735	Putative NAD-malate dehydrogenase
I-SETit.Nab-1:1:1	736	Nucleic acid binding protein
I-SETit.NAB-1:1:1	737	Nucleic acid binding protein
I-SETit.NADP-Me-1:1:1	738	NADP malate enzyme
I-SETit.NADP-Me-1:1:2	739	NADP malate enzyme
I-SETit.PGK1-1:1:1	740	Phosphoglycerate kinase
I-SETit.PGK1-1:1:2	741	Phosphoglycerate kinase
I-SETit.Pgk1-1-1:1:1	742	Phosphoglycerate kinase
I-SETit.Pgk1-2-1:1:1	743	Phosphoglycerate kinase
I-SETit.PGK3_1-1:1:1	744	Phosphoglycerate kinase
I-SETit.PIP1_3_2-1:1:1	745	Aquaporin
I-SETit.PIP1-1_2-1:1:1	746	Aquaporin
I-SETit.PIP1-1_3-1:1:1	747	Aquaporin
I-SETit.PIP1-3_3-1:1:1	748	Aquaporin
I-SETit.PIP1-4_3-1:1:1	749	Aquaporin
I-SETit.PIP2-2_2-1:1:1	750	Aquaporin
I-SETit.PIP2-2_3-1:1:1	751	Aquaporin
I-SETit.PIP2-5_2-1:1:1	752	Aquaporin
I-SETit.PIP2-5_3-1:1:1	753	Aquaporin
I-SETit.PPR-1:1:1	754	Pentatricopeptide repeat-containing protein
I-SETit.Prx17-1-1:1:1	755	Peroxidase 17
I-SETit.Prx3-1-1:1:1	756	Peroxidase 3
I-SETit.Prx3-2-1:1:1	757	Peroxidase 3
I-SETit.PSRP-3-1:1:1	758	Plastid and cyanobacterial ribosomal protein
I-SETit.RbcS_1-1:1:1	759	Small subunit RUBISCO
I-SETit.RbcS_2-1:1:1	760	Small subunit RUBISCO
I-SETit.RbcS_3-1:1:1	761	Small subunit RUBISCO
I-SETit.RbcS_4-1:1:1	762	Small subunit RUBISCO
I-SETit.Rps7-2_2-1:1:1	763	40S-7S-2 family protein

I-SETit.Rps7-2_3-1:1:1	764	40S-7S-2 family protein
I-SETit.Rps7-2_4-1:1:1	765	40S-7S-2 family protein
I-SETit.SBD-1-1:1:1	766	Shwachman-Bodian-Diamond syndrome protein
I-SETit.SBD-3-1:1:1	767	Shwachman-Bodian-Diamond syndrome protein
I-SETit.SBS-2-1:1:1	768	Shwachman-Bodian-Diamond syndrome protein
I-SETit.SETit.eEF1g_4-1:1:1	769	Elongation Factor 1 gamma
I-SETit.ThioR-1:1:1	770	Thioreductase
I-SETit.TubA2_3-1:1:2	771	Tubulin A2
I-SETit.TubA2-1:1:1	772	Tubulin A2
I-SETit.TubA2-2-1:1:1	773	Tubulin A2
I-SETit.TubA2-3-1:1:1	774	Tubulin A2
I-SETit.TubA3_1-1:1:2	775	Tubulin A3
I-SETit.TubA3_2-1:1:2	776	Tubulin A3
I-SETit.Ubq5-1:1:1	777	Ubiquitin 5
I-SETit.Wx1-1-1:1:1	778	Putative granule bound starch synthase
T-SETit.36384-1:1:1	779	Cluster 36384
T-SETit.37025-1:1:1	780	Cluster 37025
T-SETit.37470-1:1:1	781	Cluster 37470
T-SETit.Ams2-1:1:1	782	S-adenosylmethionine synthetase 2
T-SETit.Atps-1:1:1	783	ATP synthase subunit gamma
T-SETit.Cab-1:1:1	784	Chlorophyll a/b binding protein
T-SETit.Cab1-1:1:1	785	Chlorophyll a/b binding protein
T-SETit.Ctpt-1:1:1	786	Triose phosphate/phosphate translocator, chloroplast precursor
T-SETit.DnaK-1:1:1	787	Heat shock protein
T-SETit.Fba-1:1:2	788	Fructose-bisphosphate aldolase
T-SETit.Fba-1:1:3	789	Fructose-bisphosphate aldolase
T-SETit.Fba-1:1:4	790	Fructose-bisphosphate aldolase
T-SETit.Gapdh-1:1:1	791	Glyceraldehyde-3-phosphate dehydrogenase
T-SETit.MES2_nno-1:1:1	792	Methionine synthase 2
T-SETit.Oep-1:1:1	793	33kDa oxygen evolving protein
T-SETit.Pea-1:1:1	794	Proton-exporting ATPase
T-SETit.Pod-1:1:1	795	pyruvate orthophosphate dikinase
T-SETit.Ppc-1:1:1	796	Phosphoenolpyruvate carboxylase
T-SETit.Psi-K-1:1:1	797	Photosystem I reaction center subunit
T-SETit.Psi-L-1:1:1	798	Photosystem I reaction center subunit
T-SETit.Rfe-s-1:1:1	799	Rieske Fe-S
T-SETit.TubA-1:1:1	800	Tubulin A

T-contig00388	801	Root 3' UTR
T-contig05482	802	Root 3' UTR
T-contig08555	803	Root 3' UTR
T-contig08556	804	Root 3' UTR
T-contig09485	805	Root 3' UTR
T-contig13749	806	Root 3' UTR
T-contig16157	807	Root 3' UTR
T-contig18936	808	Root 3' UTR
T-contig18994	809	Root 3' UTR
T-contig21387	810	Root 3' UTR
T-contig23385	811	Root 3' UTR
T-contig24188	812	Root 3' UTR
T-contig24188	813	Root 3' UTR
T-contig24832	814	Root 3' UTR
T-contig24890	815	Root 3' UTR
T-contig24916	816	Root 3' UTR
T-contig25097	817	Root 3' UTR
T-contig25509	818	Root 3' UTR
T-contig25584	819	Root 3' UTR
T-contig26532	820	Root 3' UTR
T-contig28013	821	Root 3' UTR
T-contig29922	822	Root 3' UTR
T-contig34261	823	Root 3' UTR
T-contig34311	824	Root 3' UTR
T-contig34749	825	Root 3' UTR
T-contig35408	826	Root 3' UTR
T-contig35550	827	Root 3' UTR
T-contig35785	828	Root 3' UTR
T-contig35943	829	Root 3' UTR
T-contig36050	830	Root 3' UTR
T-contig36266	831	Root 3' UTR
T-contig36378	832	Root 3' UTR
T-contig36502	833	Root 3' UTR
T-contig36728	834	Root 3' UTR
T-contig36811	835	Root 3' UTR
T-contig36883	836	Root 3' UTR
T-contig37316	837	Root 3' UTR
T-contig37476	838	Root 3' UTR
T-contig37510	839	Root 3' UTR

T-contig37704	840	Root 3' UTR
T-contig37883	841	Root 3' UTR
T-contig37920	842	Root 3' UTR
T-contig37959	843	Root 3' UTR
T-contig37976	844	Root 3' UTR
T-contig38003	845	Root 3' UTR
T-SETIT-28JUL09- CLUS3016_12	846	Root 3' UTR
T-10SETIT-28JUL09- CLUS1332_4	847	Constitutive 3' UTR
T-17SETIT-28JUL09- CLUS1910_15	848	Constitutive 3' UTR
T-7SETIT-28JUL09- CLUS2844_11	849	Constitutive 3' UTR
T-contig00006	850	Constitutive 3' UTR
T-contig00142	851	Constitutive 3' UTR
T-contig00191	852	Constitutive 3' UTR
T-contig00205	853	Constitutive 3' UTR
T-contig00242	854	Constitutive 3' UTR
T-contig00263	855	Constitutive 3' UTR
T-contig01883	856	Constitutive 3' UTR
T-contig02157	857	Constitutive 3' UTR
T-contig02856	858	Constitutive 3' UTR
T-contig02883	859	Constitutive 3' UTR
T-contig04253	860	Constitutive 3' UTR
T-contig05397	861	Constitutive 3' UTR
T-contig05720	862	Constitutive 3' UTR
T-contig10626	863	Constitutive 3' UTR
T-contig10874	864	Constitutive 3' UTR
T-contig11193	865	Constitutive 3' UTR
T-contig14970	866	Constitutive 3' UTR
T-contig26892	867	Constitutive 3' UTR
T-contig32186	868	Constitutive 3' UTR
T-contig32187	869	Constitutive 3' UTR
T-contig33439	870	Constitutive 3' UTR
T-contig33682	871	Constitutive 3' UTR
T-contig34270	872	Constitutive 3' UTR
T-contig34378	873	Constitutive 3' UTR
T-contig35132	874	Constitutive 3' UTR
T-contig35270	875	Constitutive 3' UTR
T-contig35388	876	Constitutive 3' UTR

T-contig35412	877	Constitutive 3' UTR
T-contig35488	878	Constitutive 3' UTR
T-contig35982	879	Constitutive 3' UTR
T-contig36384	880	Constitutive 3' UTR
T-contig36588	881	Constitutive 3' UTR
T-contig36702	882	Constitutive 3' UTR
T-contig36980	883	Constitutive 3' UTR
T-contig36992	884	Constitutive 3' UTR
T-contig36993	885	Constitutive 3' UTR
T-contig37025	886	Constitutive 3' UTR
T-contig37162	887	Constitutive 3' UTR
T-contig37351	888	Constitutive 3' UTR
T-contig37386	889	Constitutive 3' UTR
T-contig37448	890	Constitutive 3' UTR
T-contig37456	891	Constitutive 3' UTR
T-contig37638	892	Constitutive 3' UTR
T-contig37732	893	Constitutive 3' UTR
T-contig37897	894	Constitutive 3' UTR
T-contig37927	895	Constitutive 3' UTR
T-contig37962	896	Constitutive 3' UTR
T-contig37980	897	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS11107_1	898	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS11705_1	899	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS11899_1	900	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS12698_2	901	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS13580_1	902	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS1404_1	903	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS14743_1	904	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS181186_1	905	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS19095_1	906	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS1910_13	907	Constitutive 3' UTR
T-SETIT-28JUL09- CLUS1910_14	908	Constitutive 3' UTR

T-SETIT28JUL09CLUS1910_16	909	Constitutive 3' UTR
T-SETIT28JUL09CLUS1910_17	910	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS1910_18	911	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS1910_19	912	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS2157_4	913	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS2166_1	914	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS243_3	915	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS3485_1	916	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS364_1	917	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS36567_1	918	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS42130_1	919	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS52844_1	920	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS7004_1	921	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS83_23	922	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS937_1	923	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS95524_1	924	Constitutive 3' UTR

Two size variants of the Foxtail millet Act8 (Actin 8) promoter are presented in Table 1. Alignment of the size variants for the Actin 8 promoter is provided in Figures 1a through 1c. The promoter, P-SETit.Act8-1:1:5 (SEQ ID NO: 24) is 1419 nucleotides in length. The promoter, P-SETit.Act8-1:1:6 (SEQ ID NO: 25) is comprised of a 5' deletion of P-SETit.Act8-1:1:5 (SEQ ID NO: 24) and is 902 nucleotides in length.

Two size variants of the Foxtail millet Alc1 promoter are presented in Table 1. Alignment of the size variants for the Alc1 promoter is provided in Figures 2a through 2c. The promoter, P-SETit.Alc1-1:1:1 (SEQ ID NO: 28) is 1577 nucleotides in length. The



promoter, P-SETit.Alc1-1:1:2 (SEQ ID NO: 29) is comprised of a 5' deletion of P-SETit.Alc1-1:1:1 (SEQ ID NO: 28) and is 412 nucleotides in length.

Two size variants of the Foxtail millet Cys promoter are presented in Table 1. Alignment of the size variants for the Cys promoter is provided in Figures 3a through 3f. The promoter, P-SETit.Cys-1:1:2 (SEQ ID NO: 45) is 3277 nucleotides in length. The promoter, P-SETit.Cys-1:1:3 (SEQ ID NO: 46) is comprised of a 5' deletion of P-SETit.Cys-1:1:2 (SEQ ID NO: 45) and is 2020 nucleotides in length.

Two size variants of the Foxtail millet Dzs promoter are presented in Table 1. Alignment of the size variants for the Dzs promoter is provided in Figures 4a through 4f. The promoter, P-SETit.Dzs-1:1:4 (SEQ ID NO: 47) is 3508 nucleotides in length. The promoter, P-SETit.Dzs-1:1:5 (SEQ ID NO: 48) is comprised of a 5' deletion of P-SETit.Dzs-1:1:4 (SEQ ID NO: 47) and is 1008 nucleotides in length.

Two size variants of the Foxtail millet Gst promoter are presented in Table 1. Alignment of the size variants for the Gst promoter is provided in Figures 5a through 5c. The promoter, P-SETit.Gst-1:1:1 (SEQ ID NO: 58) is 1681 nucleotides in length. The promoter, P-SETit.Gst-1:1:2 (SEQ ID NO: 59) is comprised of a 5' deletion of P-SETit.Gst-1:1:1 (SEQ ID NO: 58) and is 428 nucleotides in length.

Two size variants of the Foxtail millet Ifr promoter are presented in Table 1. Alignment of the size variants for the Ifr promoter is provided in Figures 6a through 6c. The promoter, P-SETit.Ifr-1:1:2 (SEQ ID NO: 60) is 1280 nucleotides in length. The promoter, P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) is comprised of a 5' deletion of P-SETit.Ifr-1:1:2 (SEQ ID NO: 60) and is 275 nucleotides in length.

Two size variants of the Foxtail millet Nrt2 promoter are presented in Table 1. Alignment of the size variants for the Nrt2 promoter is provided in Figures 7a through 7d. The promoter, P-SETit.Nrt2-1:1:2 (SEQ ID NO: 64) is 1866 nucleotides in length. The promoter, P-SETit.Nrt2-1:1:3 (SEQ ID NO: 65) is comprised of a 5' deletion of P-SETit.Nrt2-1:1:2 (SEQ ID NO: 64) and is 382 nucleotides in length.

Two size variants of the Foxtail millet Ppc promoter are presented in Table 1. Alignment of the size variants for the Ppc promoter is provided in Figures 8a through 8e. The promoter, P-SETit.Ppc-1:1:3 (SEQ ID NO: 74) is 2722 nucleotides in length. The promoter, P-SETit.Ppc-1:1:4 (SEQ ID NO: 75) is comprised of a 5' deletion of P-SETit.Ppc-1:1:3 (SEQ ID NO: 74) and is 1882 nucleotides in length.

Two size variants of the Foxtail millet Prx3 promoter are presented in Table 1. Alignment of the size variants for the Prx3 promoter is provided in Figures 9a through 9f.

The promoter, P-SETit.Prx3-1:1:4 (SEQ ID NO: 83) is 3354 nucleotides in length. The promoter, P-SETit.Prx3-1:1:3 (SEQ ID NO: 82) is comprised of a 5' deletion of P-SETit.Prx3-1:1:4 (SEQ ID NO: 83) and is 1908 nucleotides in length.

Three size variants of the Foxtail millet Rcc3 promoter are presented in Table 1. Alignment of the size variants for the Rcc3 promoter is provided in Figures 10a through 10f. The promoter, P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88) is 2062 nucleotides in length. The promoter, P-SETit.Rcc3-1:1:16 (SEQ ID NO: 91) is comprised of a 5' deletion of P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88) and is 2024 nucleotides in length. The promoter, P-SETit.Rcc3-1:1:10 (SEQ ID NO: 89) is comprised of a 5' deletion of P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88) and is 1563 nucleotides in length. The promoter, P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) is comprised of a 5' deletion of P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88) and is 915 nucleotides in length.

Two size variants of the Foxtail millet Ssp1 promoter are presented in Table 1. Alignment of the size variants for the Ssp1 promoter is provided in Figures 11a through 11b. The promoter, P-SETit.Ssp1-1:1:1 (SEQ ID NO: 93) is 1128 nucleotides in length. The promoter, P-SETit.Ssp1-1:1:2 (SEQ ID NO: 94) is comprised of a 5' deletion of P-SETit.Ssp1-1:1:1 (SEQ ID NO: 93) and is 479 nucleotides in length.

Two size variants of the Foxtail millet Tip promoter are presented in Table 1. Alignment of the size variants for the Tip promoter is provided in Figures 12a through 12d. The promoter, P-SETit.Tip-1:1:4 (SEQ ID NO: 97) is 2108 nucleotides in length. The promoter, P-SETit.Tip-1:1:1 (SEQ ID NO: 96) is comprised of a 5' deletion of P-SETit.Tip-1:1:4 (SEQ ID NO: 97) and is 917 nucleotides in length.

Two size variants of the Foxtail millet TubA2-1 promoter are presented in Table 1. Alignment of the size variants for the TubA2-1 promoter is provided in Figures 13a through 13d. The promoter, P-SETit.TubA2-1-1:1:2 (SEQ ID NO: 98) is 1593 nucleotides in length. The promoter, P-SETit.TubA2-1-1:1:3 (SEQ ID NO: 99) is comprised of a 5' deletion of P-SETit.TubA2-1-1:1:2 (SEQ ID NO: 98) and is 856 nucleotides in length.

Two size variants of the Foxtail millet Ubq1 promoter are presented in Table 1. Alignment of the size variants for the Ubq1 promoter is provided in Figures 14a through 14c. The promoter, P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102) is 1492 nucleotides in length. The promoter, P-SETit.Ubq1-1:1:3 (SEQ ID NO: 103) is comprised of a 5' deletion of P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102) and is 680 nucleotides in length.

Compositions derived from any of the promoters presented as SEQ ID NOS: 23 through 105 and SEQ ID NOS: 353 through 536 comprised of internal or 5' deletions can be

built using methods known in the art to improve expression, by removing elements that have either positive or negative effects on expression; or duplicating elements that have either positive or negative effects on expression; or duplicating or removing elements that have tissue or cell specific effects on expression. Compositions derived from any of the promoters presented as SEQ ID NOS: 23 through 105 and SEQ ID NOS: 353 through 536 comprised of 3' deletions in which the TATA box element or equivalent sequence thereof and downstream sequence is removed can be used to make enhancer elements. Further deletions can be made to remove any elements that have either positive or negative; or tissue specific; or cell specific; or timing specific (such as, but not limited to, circadian rhythms) effects on expression. Any of the promoters presented as SEQ ID NOS: 23 through 105 and SEQ ID NOS: 353 through 536 and the fragments or enhancers derived there from can be used to make chimeric transcriptional regulatory element compositions comprised of any of the promoters presented as SEQ ID NOS: 23 through 105 and SEQ ID NOS: 353 through 536 and the fragments or enhancers derived there from operably linked to other enhancers and promoters. The efficacy of the modifications, duplications or deletions described above on the desired expression aspects of a particular transgene are tested empirically in stable and transient plant assays, since the effect of such alterations to the native promoter composition are not readily predictable and require empirical testing to validate such effects.

The leader sequences (5' UTR) presented as SEQ ID NOS: 106 through 171 and SEQ ID NOS: 537 through 588 may be comprised of regulatory elements or may adopt secondary structures that can have an effect on transcription or translation of a transgene. The leader sequences presented as SEQ ID NOS: 106 through 171 and SEQ ID NOS: 537 through 588 can be used to make chimeric regulatory elements that affect transcription or translation of a transgene. In addition, the leader sequences presented as SEQ ID NOS: 106 through 171 and SEQ ID NOS: 537 through 588 can be used to make chimeric leader sequences which affect transcription or translation of a transgene.

Compositions derived from any of the introns presented as SEQ ID NOS: 172 through 267, SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778 can be comprised of internal deletions or duplications of cis regulatory elements; or alterations of the 5' and 3' sequences comprising the intron/exon splice junctions, can be used to improve expression or specificity of expression when operably linked to a promoter + leader or chimeric promoter + leader and coding sequence. Alterations of the 5' and 3' regions comprising the intron/exon splice junction can also be made to reduce the potential for introduction of false start and stop codons begin produced in the resulting transcript after processing and splicing of the

messenger RNA. The introns are tested empirically as described below to determine the intron's effect on expression of a transgene.

**Example 2: Analysis of Regulatory Elements driving GUS in Transgenic Corn**

Corn plants were transformed with plant expression vectors containing the test  
5 regulatory elements driving expression of the  $\beta$ -glucuronidase (GUS) transgene, and the resulting plants were analyzed for GUS protein expression.

Corn plants were transformed with the plant GUS expression constructs, listed in Table 2, below. Regulatory elements and chimeric regulatory elements presented in example 1 were cloned into a base plant expression vector using standard methods known in the art.  
10 The resulting plant expression vectors contained a right border region from *Agrobacterium tumefaciens*, a first transgene cassette to test the regulatory or chimeric regulatory element comprised of, a regulatory or chimeric regulatory element, operably linked to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS) that either possessed  
15 a processable intron (GUS-2, SEQ ID NO: 1091) or no intron (GUS-1, SEQ ID NO: 1090), operably linked to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088) or the 3' termination region from the rice lipid transfer protein gene (T-Os.LTP-1:1:1, SEQ ID NO: 1089); a second transgene selection cassette used for selection of transformed plant cells that conferred resistance to the herbicide  
20 glyphosate (driven by the rice Actin 1 transcriptional regulatory element group, SEQ ID NO: 1098), or alternatively, the antibiotic kanamycin (driven by the rice Actin 1 transcriptional regulatory element group, SEQ ID NO: 1098) and a left border region from *A. tumefaciens*. The resulting plasmids, pMON109728, pMON112215, pMON116789, pMON116816, pMON116818, pMON116820, pMON116829, pMON120698, pMON120699,  
25 pMON120700, pMON120701, pMON120702, pMON120703, pMON120704, pMON120705, pMON120706, pMON120709, pMON120710, pMON120711, pMON120712, pMON120713, pMON127440, pMON127441, pMON127442, pMON127443, pMON127444, pMON127445, pMON127446, pMON127447, pMON127448, pMON127449 and pMON132037 were used to transform corn plants.

**Table 2. Binary Plant Transformation Constructs, Regulatory or chimeric regulatory elements, GUS and 3' UTRs.**

Construct	Regulatory Elements	GUS	3' UTR
pMON109728	EXP-FMV.35S-SETit.Tip (SEQ ID NO: 8) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Tip-1:1:1 (SEQ ID NO: 96) L-SETit.Tip-1:1:1 (SEQ ID NO: 165)	GUS-1	T-AGRtu.nos-1:1:13
pMON112215	P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88) L-SETit.Rcc3-1:1:1 (SEQ ID NO: 161)	GUS-1	T-AGRtu.nos-1:1:13
pMON116789	P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	GUS-1	T-AGRtu.nos-1:1:13
pMON116816	P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	GUS-1	T-AGRtu.nos-1:1:13
pMON116818	P-SETit.Pox-1:1:1 (SEQ ID NO: 73) L-SETit.Pox-1:1:1 (SEQ ID NO: 147)	GUS-1	T-AGRtu.nos-1:1:13
pMON116820	P-SETit.Gst-1:1:1 (SEQ ID NO: 58) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	GUS-1	T-AGRtu.nos-1:1:13
pMON116829	P-SETit.Rcc3-1:1:10 (SEQ ID NO: 89) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	GUS-1	T-AGRtu.nos-1:1:13
pMON120698	EXP-FMV.35S-SETit.Rcc3:a (SEQ ID NO: 6) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Rcc3-1:1:10 (SEQ ID NO: 89) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	GUS-2	T-Os.LTP-1:1:1
pMON120699	EXP-FMV.35S-SETit.Gst:a (SEQ ID NO: 2) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Gst-1:1:1 (SEQ ID NO: 58) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	GUS-2	T-Os.LTP-1:1:1
pMON120700	EXP-FMV.35S-SETit.Rcc3:b (SEQ ID NO: 7) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	GUS-2	T-Os.LTP-1:1:1
pMON120701	EXP-FMV.35S-SETit.Pox (SEQ ID NO: 5) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Pox-1:1:1 (SEQ ID NO: 73)	GUS-2	T-Os.LTP-1:1:1

	L-SETit.Pox-1:1:1 (SEQ ID NO: 147)		
pMON120702	P-SETit.Ccoamt-1:1:2 (SEQ ID NO: 35) L-SETit.Ccoamt-1:1:2 (SEQ ID NO: 116)	GUS-2	T-Os.LTP-1:1:1
pMON120703	EXP-FMV.35S-SETit.Ccoamt (SEQ ID NO: 1) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Ccoamt-1:1:2 (SEQ ID NO: 35) L-SETit.Ccoamt-1:1:2 (SEQ ID NO: 116)	GUS-2	T-Os.LTP-1:1:1
pMON120704	P-SETit.Gst-1:1:2 (SEQ ID NO: 59) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	GUS-2	T-Os.LTP-1:1:1
pMON120705	EXP-FMV.35S-SETit.Gst:b (SEQ ID NO: 3) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Gst-1:1:2 (SEQ ID NO: 59) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	GUS-2	T-Os.LTP-1:1:1
pMON120706	EXP-FMV.35S-SETit.Ifr (SEQ ID NO: 4) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)	GUS-2	T-Os.LTP-1:1:1
pMON120709	P-SETit.Nrt2-1:1:3 (SEQ ID NO: 65) L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139)	GUS-2	T-Os.LTP-1:1:1
pMON120710	P-SETit.Nrt2-1:1:2 (SEQ ID NO: 64) L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139)	GUS-2	T-Os.LTP-1:1:1
pMON120711	P-SETit.Pip2-1:1:3 (SEQ ID NO: 71) L-SETit.Pip2-1:1:1 (SEQ ID NO: 145)	GUS-2	T-Os.LTP-1:1:1
pMON120712	P-SETit.Ifr-1:1:2 (SEQ ID NO: 60) L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)	GUS-2	T-Os.LTP-1:1:1
pMON120713	P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)	GUS-2	T-Os.LTP-1:1:1
pMON127440	P-SETit.Ppc-1:1:3 (SEQ ID NO: 74) L-SETit.Ppc-1:1:1 (SEQ ID NO: 148)	GUS-2	T-Os.LTP-1:1:1
pMON127441	P-SETit.Ppc-1:1:4 (SEQ ID NO: 75) L-SETit.Ppc-1:1:1 (SEQ ID NO: 148)	GUS-2	T-Os.LTP-1:1:1
pMON127442	P-SETit.Gapdh2-1:1:3 (SEQ ID NO: 55) L-SETit.Gapdh2-1:1:1 (SEQ ID NO: 133)	GUS-2	T-Os.LTP-1:1:1
pMON127443	EXP-SETit.Ppdk:1:1 (SEQ ID NO: 14)	GUS-	T-Os.LTP-1:1:1

	P-SETit.Ppdk-1:1:1 (SEQ ID NO: 76) L-SETit.Ppdk-1:1:4 (SEQ ID NO: 150) I-SETit.Ppdk-1:1:1 (SEQ ID NO: 175) L-SETit.Ppdk-1:1:2 (SEQ ID NO: 149)	2	
pMON127444	P-SETit.CP29-1:1:4 (SEQ ID NO: 42) L-SETit.CP29-1:1:1 (SEQ ID NO: 124)	GUS-2	T-Os.LTP-1:1:1
pMON127445	P-SETit.Cab3-1:1:3 (SEQ ID NO: 33) L-SETit.Cab3-1:1:1 (SEQ ID NO: 114)	GUS-2	T-Os.LTP-1:1:1
pMON127446	P-SETit.PSI-4a-1:1:1 (SEQ ID NO: 86) L-SETit.PSI-4a-1:1:1 (SEQ ID NO: 159)	GUS-2	T-Os.LTP-1:1:1
pMON127447	P-SETit.Rbcs-1:1:1 (SEQ ID NO: 87) L-SETit.Rbcs-1:1:1 (SEQ ID NO: 160)	GUS-2	T-Os.LTP-1:1:1
pMON127448	P-SETit.Cab1-1:1:1 (SEQ ID NO: 32) L-SETit.Cab1-1:1:1 (SEQ ID NO: 113)	GUS-2	T-Os.LTP-1:1:1
pMON127449	P-SETit.Fba-1:1:1 (SEQ ID NO: 51) L-SETit.Fba-1:1:1 (SEQ ID NO: 131)	GUS-2	T-Os.LTP-1:1:1
pMON132037	EXP-SETit.Ubq1-1:1:1 (SEQ ID NO: 20) P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102) L-SETit.Ubq1-1:1:1 (SEQ ID NO: 169) I-SETit.Ubq1-1:1:1 (SEQ ID NO: 177)	GUS-2	T-Os.LTP-1:1:1

The plant transformation vector, pMON109728 is comprised of the transcriptional regulatory element group, EXP-FMV.35S-SETit.Tip (SEQ ID NO: 8), which is further comprised of the enhancer element, E-FMV.35S-1:1:2 (SEQ ID NO: 351), operably linked 5' to the promoter element, P-SETit.Tip-1:1:1 (SEQ ID NO: 96), operably linked 5' to the leader element, L-SETit.Tip-1:1:1 (SEQ ID NO: 165). The plant transformation vector, pMON112215 is comprised of the promoter element, P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88), operably linked 5' to the leader element, L-SETit.Rcc3-1:1:1 (SEQ ID NO: 161). The plant transformation vector, pMON116789 is comprised of the promoter element, P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90), operably linked 5' to the leader element, L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162). The plant transformation vector, pMON116816 is comprised of the promoter element, P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90), operably linked 5' to the leader element, L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162). The plant transformation vector, pMON116818 is

comprised of the promoter element, P-SETit.Pox-1:1:1 (SEQ ID NO: 73), operably linked 5' to the leader element, L-SETit.Pox-1:1:1 (SEQ ID NO: 147). The plant transformation vector, pMON116820 is comprised of the promoter element, P-SETit.Gst-1:1:1 (SEQ ID NO: 58), operably linked 5' to the leader element, L-SETit.Gst-1:1:1 (SEQ ID NO: 135). The plant transformation vector, pMON116829 is comprised of the promoter element, P-SETit.Rcc3-1:1:10 (SEQ ID NO: 89), operably linked 5' to the leader element, L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162). The plant transformation vector, pMON120698 is comprised of the transcriptional regulatory element group, EXP-FMV.35S-SETit.Rcc3:a (SEQ ID NO: 6), which is further comprised of the enhancer element, E-FMV.35S-1:1:2 (SEQ ID NO: 351), operably linked 5' to the promoter element, P-SETit.Rcc3-1:1:10 (SEQ ID NO: 89), operably linked 5' to the leader element, L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162). The plant transformation vector, pMON120699 is comprised of the transcriptional regulatory element group, EXP-FMV.35S-SETit.Gst:a (SEQ ID NO: 2), which is further comprised of the enhancer element, E-FMV.35S-1:1:2 (SEQ ID NO: 351), operably linked 5' to the promoter element, P-SETit.Gst-1:1:1 (SEQ ID NO: 58), operably linked 5' to the leader element, L-SETit.Gst-1:1:1 (SEQ ID NO: 135). The plant transformation vector, pMON120700 is comprised of the transcriptional regulatory element group, EXP-FMV.35S-SETit.Rcc3:b (SEQ ID NO: 7), which is further comprised of the enhancer element, E-FMV.35S-1:1:2 (SEQ ID NO: 351), operably linked 5' to the promoter element, P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90), operably linked 5' to the leader element, L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162). The plant transformation vector, pMON120701 is comprised of the transcriptional regulatory element group, EXP-FMV.35S-SETit.Pox (SEQ ID NO: 5), which is further comprised of the enhancer element, E-FMV.35S-1:1:2 (SEQ ID NO: 351), operably linked 5' to the promoter element, P-SETit.Pox-1:1:1 (SEQ ID NO: 73), operably linked 5' to the leader element, L-SETit.Pox-1:1:1 (SEQ ID NO: 147). The plant transformation vector, pMON120702 is comprised of the promoter element, P-SETit.Ccoamt-1:1:2 (SEQ ID NO: 35), operably linked 5' to the leader element, L-SETit.Ccoamt-1:1:2 (SEQ ID NO: 116). The plant transformation vector, pMON120703 is comprised of the transcriptional regulatory element group, EXP-FMV.35S-SETit.Ccoamt (SEQ ID NO: 1), which is further comprised of the enhancer element, E-FMV.35S-1:1:2 (SEQ ID NO: 351), operably linked 5' to the promoter element, P-SETit.Ccoamt-1:1:2 (SEQ ID NO: 35), operably linked 5' to the leader element, L-SETit.Ccoamt-1:1:2 (SEQ ID NO: 116). The plant transformation vector, pMON120704 is comprised of the promoter element, P-SETit.Gst-1:1:2 (SEQ ID NO: 59), operably linked 5' to the leader element, L-SETit.Gst-1:1:1 (SEQ ID NO: 135). The plant transformation



vector, pMON120705 is comprised of the transcriptional regulatory element group, EXP-FMV.35S-SETit.Gst:b (SEQ ID NO: 3), which is further comprised of the enhancer element, E-FMV.35S-1:1:2 (SEQ ID NO: 351), operably linked 5' to the promoter element, P-SETit.Gst-1:1:2 (SEQ ID NO: 59), operably linked 5' to the leader element, L-SETit.Gst-1:1:1 (SEQ ID NO: 135). The plant transformation vector, pMON120706 is comprised of the transcriptional regulatory element group, EXP-FMV.35S-SETit.Ifr (SEQ ID NO: 4), which is further comprised of the enhancer element, E-FMV.35S-1:1:2 (SEQ ID NO: 351), operably linked 5' to the promoter element, P-SETit.Ifr-1:1:3 (SEQ ID NO: 61), operably linked 5' to the leader element, L-SETit.Ifr-1:1:1 (SEQ ID NO: 136). The plant transformation vector, pMON120709 is comprised of the promoter element, P-SETit.Nrt2-1:1:3 (SEQ ID NO: 65), operably linked 5' to the leader element, L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139). The plant transformation vector, pMON120710 is comprised of the promoter element, P-SETit.Nrt2-1:1:2 (SEQ ID NO: 64), operably linked 5' to the leader element, L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139). The plant transformation vector, pMON120711 is comprised of the promoter element, P-SETit.Pip2-1:1:3 (SEQ ID NO: 71), operably linked 5' to the leader element, L-SETit.Pip2-1:1:1 (SEQ ID NO: 145). The plant transformation vector, pMON120712 is comprised of the promoter element, P-SETit.Ifr-1:1:2 (SEQ ID NO: 60), operably linked 5' to the leader element, L-SETit.Ifr-1:1:1 (SEQ ID NO: 136). The plant transformation vector, pMON120713 is comprised of the promoter element, P-SETit.Ifr-1:1:3 (SEQ ID NO: 61), operably linked 5' to the leader element, L-SETit.Ifr-1:1:1 (SEQ ID NO: 136). The plant transformation vector, pMON127440 is comprised of the promoter element, P-SETit.Ppc-1:1:3 (SEQ ID NO: 74), operably linked 5' to the leader element, L-SETit.Ppc-1:1:1 (SEQ ID NO: 148). The plant transformation vector, pMON127441 is comprised of the promoter element, P-SETit.Ppc-1:1:4 (SEQ ID NO: 75), operably linked 5' to the leader element, L-SETit.Ppc-1:1:1 (SEQ ID NO: 148). The plant transformation vector, pMON127442 is comprised of the promoter element, P-SETit.Gapdh2-1:1:3 (SEQ ID NO: 55), operably linked 5' to the leader element, L-SETit.Gapdh2-1:1:1 (SEQ ID NO: 133). The plant transformation vector, pMON127443 is comprised of the transcriptional regulatory element group, EXP-SETit.Ppdk:1:1 (SEQ ID NO: 14), promoter element, P-SETit.Ppdk-1:1:1 (SEQ ID NO: 76), operably linked 5' to the leader element, L-SETit.Ppdk-1:1:4 (SEQ ID NO: 150), operably linked 5' to the intron element, I-SETit.Ppdk-1:1:1 (SEQ ID NO: 175), operably linked 5' to the leader element, L-SETit.Ppdk-1:1:2 (SEQ ID NO: 149). The plant transformation vector, pMON127444 is comprised of the promoter element, P-SETit.CP29-1:1:4 (SEQ ID NO: 42), operably linked 5' to the leader element, L-SETit.CP29-1:1:1 (SEQ

ID NO: 124). The plant transformation vector, pMON127445 promoter element, P-SETit.Cab3-1:1:3 (SEQ ID NO: 33), operably linked 5' to the leader element, L-SETit.Cab3-1:1:1 (SEQ ID NO: 114). The plant transformation vector, pMON127446 is comprised of the promoter element, P-SETit.PSI-4a-1:1:1 (SEQ ID NO: 86), operably linked 5' to the leader element, L-SETit.PSI-4a-1:1:1 (SEQ ID NO: 159). The plant transformation vector, pMON127447 is comprised of the promoter element, P-SETit.Rbcs-1:1:1 (SEQ ID NO: 87), operably linked 5' to the leader element, L-SETit.Rbcs-1:1:1 (SEQ ID NO: 160). The plant transformation vector, pMON127448 is comprised of the promoter element, P-SETit.Cab1-1:1:1 (SEQ ID NO: 32), operably linked 5' to the leader element, L-SETit.Cab1-1:1:1 (SEQ ID NO: 113). The plant transformation vector, pMON127449 is comprised of the promoter element, P-SETit.Fba-1:1:1 (SEQ ID NO: 51), operably linked 5' to the leader element, L-SETit.Fba-1:1:1 (SEQ ID NO: 131). The plant transformation vector, pMON132037 is comprised of the transcriptional regulatory element group, EXP-SETit.Ubq1:1:1 (SEQ ID NO: 20), which is further comprised of the promoter element, P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102), operably linked 5' to the leader element, L-SETit.Ubq1-1:1:1 (SEQ ID NO: 169), operably linked 5' to the intron element, I-SETit.Ubq1-1:1:1 (SEQ ID NO: 177).

Corn plants were transformed with plant GUS expression constructs, pMON109728, pMON112215, pMON116789, pMON116816, pMON116818, pMON116820, pMON116829, pMON120698, pMON120699, pMON120700, pMON120701, pMON120702, pMON120703, pMON120704, pMON120705, pMON120706, pMON120709, pMON120710, pMON120711, pMON120712, pMON120713, pMON127440, pMON127441, pMON127442, pMON127443, pMON127444, pMON127445, pMON127446, pMON127447, pMON127448, pMON127449 and pMON132037.

Plants were transformed using *Agrobacterium*-mediated transformations known to those skilled in the art. Briefly, LH244 corn seed embryos are extracted from surface-sterilized, developing corn kernels approximately 9 to 13 days after pollination. The embryos are co-cultured with *Agrobacterium tumefaciens*, transformed with the GUS expression constructs for 18 to 28 hours in the dark. The embryos are then transferred to selective media and cultured in the dark for approximately 3 weeks to induce the formation of callus. Following callus induction, the embryo-derived callus tissue is transferred to new media and cultured under light for 5 to 10 days. The callus tissue is then transferred to new media to induce the formation of shoots. After 2 to 3 weeks, the transformed shoots are transferred to rooting medium and cultured to permit the formation of roots. Once sufficient root formation

has occurred, the transformed plants are transferred to soil and transferred to the greenhouse. Events containing one or two copies of the transgene cassette are selected for study using real-time PCR methods known to those skilled in the art.

Histochemical GUS analysis was used for qualitative expression analysis of transformed plants. Whole tissue sections were incubated with GUS staining solution X-Gluc (5-bromo-4-chloro-3-indolyl-b-glucuronide) (1 milligram/milliliter) for an appropriate length of time, rinsed, and visually inspected for blue coloration. GUS activity was qualitatively determined by direct visual inspection or inspection under a microscope using selected plant organs and tissues. The R0 plants were inspected for expression in the roots and leaves.

For quantitative analysis, total protein was extracted from selected tissues of transformed corn plants. One microgram of total protein was used with the fluorogenic substrate 4-methyleumbelliferyl-β-D-glucuronide (MUG) in a total reaction volume of 50 microliters. The reaction product, 4-methlyumbelliferone (4-MU), is maximally fluorescent at high pH, where the hydroxyl group is ionized. Addition of a basic solution of sodium carbonate simultaneously stops the assay and adjusts the pH for quantifying the fluorescent product. Fluorescence was measured with excitation at 365 nm, emission at 445 nm using a Fluoromax-3 with Micromax Reader, with slit width set at excitation 2 nm and emission 3nm.

The average R<sub>0</sub> GUS expression observed for each transformation is presented in Table 3 and 4 below.

**Table 3. Average R<sub>0</sub> GUS Leaf and Root expression in transgenic corn plants, transformed with listed construct.**

Construct	Regulatory Elements	V3 Root	V7 Root	VT Root	V3 Leaf	V7 Leaf	VT Leaf
pMON109728	EXP-FMV.35S-SETit.Tip (SEQ ID NO: 8) E-FMV.35S-1:1:2 (SEQ ID NO: 177) P-SETit.Tip-1:1:1 (SEQ ID NO: 95) L-SETit.Tip-1:1:1 (SEQ ID NO: 163)	11.98	nd	14.78	423.85	nd	11.38
pMON112215	P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88) L-SETit.Rcc3-	nd	nd	nd	nd	nd	nd

	1:1:1 (SEQ ID NO: 161)						
pMON116789	P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	43.43	nd	363.90	19.09	12.16	6.58
pMON116816	P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	5.77	nd	0.00	35.02	nd	0.00
pMON116818	P-SETit.Pox-1:1:1 (SEQ ID NO: 73) L-SETit.Pox-1:1:1 (SEQ ID NO: 147)	8.10	nd	0.00	22.96	0.00	0.00
pMON116820	P-SETit.Gst-1:1:1 (SEQ ID NO: 58) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	0.00	nd	12.06	5.21	0.00	8.85
PMON116829	P-SETit.Rcc3-1:1:10 (SEQ ID NO: 89) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	20.67	nd	0.00	0.00	0.00	0.00
pMON120698	EXP-FMV.35S-SETit.Rcc3:a (SEQ ID NO: 6) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Rcc3-1:1:10 (SEQ ID NO: 89) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	0.00	nd	12.90	0.00	0.00	0.00
pMON120699	EXP-FMV.35S-SETit.Gst:a (SEQ ID NO: 2) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Gst-1:1:1	19.42	nd	147.75	47.82	26.76	116.51

	(SEQ ID NO: 58) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)						
pMON120700	EXP-FMV.35S- SETit.Rcc3:b (SEQ ID NO: 7) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Rcc3- 1:1:11 (SEQ ID NO: 90) L-SETit.Rcc3- 1:1:2 (SEQ ID NO: 162)	nd	11.97	12.18	0.00	5.16	0.00
pMON120701	EXP-FMV.35S- SETit.Pox (SEQ ID NO: 5) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Pox-1:1:1 (SEQ ID NO: 73) L-SETit.Pox-1:1:1 (SEQ ID NO: 147)	10.42	35.53	22.82	0.00	0.00	0.00
pMON120702	P-SETit.Ccoamt- 1:1:2 (SEQ ID NO: 35) L-SETit.Ccoamt- 1:1:2 (SEQ ID NO: 116)	0.00	nd	14.98	0.00	0.00	8.81
pMON120703	EXP-FMV.35S- SETit.Ccoamt (SEQ ID NO: 1) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Ccoamt- 1:1:2 (SEQ ID NO: 35) L-SETit.Ccoamt- 1:1:2 (SEQ ID NO: 116)	9.79	nd	93.87	12.14	67.80	22.52
pMON120704	P-SETit.Gst-1:1:2 (SEQ ID NO: 59) L-SETit.Gst-1:1:1 (SEQ ID NO:	7.12	nd	24.05	0.00	8.78	0.00

	135)						
pMON120705	EXP-FMV.35S-SETit.Gst:b (SEQ ID NO: 3) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Gst-1:1:2 (SEQ ID NO: 59) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	19.48	nd	38.27	34.28	136.79	104.30
pMON120706	EXP-FMV.35S-SETit.Ifr (SEQ ID NO: 4) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)	25.62	nd	92.11	32.18	191.22	107.41
pMON120709	P-SETit.Nrt2-1:1:3 (SEQ ID NO: 65) L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139)	10.40	nd	7.20	0.00	0.00	0.00
pMON120710	P-SETit.Nrt2-1:1:2 (SEQ ID NO: 64) L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139)	17.29	nd	11.89	0.00	0.00	0.00
pMON120711	P-SETit.Pip2-1:1:3 (SEQ ID NO: 71) L-SETit.Pip2-1:1:1 (SEQ ID NO: 145)	168.44	36.18	14.61	22.48	0.00	7.04
pMON120712	P-SETit.Ifr-1:1:2 (SEQ ID NO: 60) L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)	64.92	nd	15.97	0.00	0.00	0.00
pMON120713	P-SETit.Ifr-1:1:3 (SEQ ID NO: 61)	0.00	nd	0.00	0.00	0.00	6.38

	L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)						
pMON127440	P-SETit.Ppc-1:1:3 (SEQ ID NO: 74) L-SETit.Ppc-1:1:1 (SEQ ID NO: 148)	0.00	nd	0.47	0.00	0.00	37.59
pMON127441	P-SETit.Ppc-1:1:4 (SEQ ID NO: 75) L-SETit.Ppc-1:1:1 (SEQ ID NO: 148)	0.00	0.00	0.00	101.10	521.14	179.12
pMON127442	P-SETit.Gapdh2- 1:1:3 (SEQ ID NO: 55) L-SETit.Gapdh2- 1:1:1 (SEQ ID NO: 133)	0.00	0.00	0.00	13.12	0.00	0.00
pMON127443	EXP- SETit.Ppdk:1:1 (SEQ ID NO: 14) P-SETit.Ppdk- 1:1:1 (SEQ ID NO: 76) L-SETit.Ppdk- 1:1:4 (SEQ ID NO: 150) I-SETit.Ppdk- 1:1:1 (SEQ ID NO: 175) L-SETit.Ppdk- 1:1:2 (SEQ ID NO: 149)	0.00	0.00	5.66	32.56	21.04	58.48
pMON127444	P-SETit.CP29- 1:1:4 (SEQ ID NO: 42) L-SETit.CP29- 1:1:1 (SEQ ID NO: 124)	0.00	6.17	0.00	165.74	348.96	249.42
pMON127445	P-SETit.Cab3- 1:1:3 (SEQ ID NO: 33) L-SETit.Cab3- 1:1:1 (SEQ ID NO: 114)	<0.1	<0.1	<0.1	299.16	136.85	179.80
pMON127446	P-SETit.PSI-4a- 1:1:1 (SEQ ID NO: 86) L-SETit.PSI-4a-	0.00	0.00	0.00	101.30	83.44	110.81

	1:1:1 (SEQ ID NO: 159)						
pMON127447	P-SETit.Rbcs-1:1:1 (SEQ ID NO: 87) L-SETit.Rbcs-1:1:1 (SEQ ID NO: 160)	0.00	22.74	18.69	160.14	172.65	264.89
pMON127448	P-SETit.Cab1-1:1:1 (SEQ ID NO: 32) L-SETit.Cab1-1:1:1 (SEQ ID NO: 113)	0.16	0.00	0.00	158.08	109.59	48.01
pMON127449	P-SETit.Fba-1:1:1 (SEQ ID NO: 51) L-SETit.Fba-1:1:1 (SEQ ID NO: 131)	<0.1	<0.1	<0.1	81.41	82.57	83.95
pMON132037	EXP-SETit.Ubq1:1:1 (SEQ ID NO: 20) P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102) L-SETit.Ubq1-1:1:1 (SEQ ID NO: 169) I-SETit.Ubq1-1:1:1 (SEQ ID NO: 177)	0.00	28.54	57.31	57.95	36.71	45.62

The average level of GUS expression amongst the constructs varied. Those constructs demonstrating the highest level of root expression, particularly at VT stage were: pMON116789 ((P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) + L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)); pMON120699 ((EXP-FMV.35S-SETit.Gst:a (SEQ ID NO: 2), comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Gst-1:1:1 (SEQ ID NO: 58) + L-SETit.Gst-1:1:1 (SEQ ID NO: 135)); pMON120703 ((EXP-FMV.35S-SETit.Ccoamt (SEQ ID NO: 1), comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Ccoamt-1:1:2 (SEQ ID NO: 35) + L-SETit.Ccoamt-1:1:2 (SEQ ID NO: 116)); pMON120706 ((EXP-FMV.35S-SETit.Ifr (SEQ ID NO: 4) comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) + L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)) and pMON132037 ((EXP-SETit.Ubq1:1:1 (SEQ ID NO: 20) comprised of P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102) + L-SETit.Ubq1-1:1:1 (SEQ ID NO: 169) + I-SETit.Ubq1-1:1:1 (SEQ ID NO: 177)).



Those constructs demonstrating the highest level of leaf expression were: pMON127447 ((P-SETit.Rbcs-1:1:1 (SEQ ID NO: 87) + L-SETit.Rbcs-1:1:1 (SEQ ID NO: 160)); pMON127444 ((P-SETit.CP29-1:1:4 (SEQ ID NO: 42) + L-SETit.CP29-1:1:1 (SEQ ID NO: 124)); pMON127445 ((P-SETit.Cab3-1:1:3 (SEQ ID NO: 33) + L-SETit.Cab3-1:1:1 (SEQ ID NO: 114)); pMON127441 ((P-SETit.Ppc-1:1:4 (SEQ ID NO: 75) + L-SETit.Ppc-1:1:1 (SEQ ID NO: 148)); pMON120699 ((EXP-FMV.35S-SETit.Gst:a (SEQ ID NO: 2) comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Gst-1:1:1 (SEQ ID NO: 58) + L-SETit.Gst-1:1:1 (SEQ ID NO: 135)); pMON127446 ((P-SETit.PSI-4a-1:1:1 (SEQ ID NO: 86) + L-SETit.PSI-4a-1:1:1 (SEQ ID NO: 159)); pMON120706 ((EXP-FMV.35S-SETit.Ifr (SEQ ID NO: 4) comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) + L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)); pMON120705 ((EXP-FMV.35S-SETit.Gst:b (SEQ ID NO: 3) comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Gst-1:1:2 (SEQ ID NO: 59) + L-SETit.Gst-1:1:1 (SEQ ID NO: 135)) and pMON127449 ((P-SETit.Fba-1:1:1 (SEQ ID NO: 51) + L-SETit.Fba-1:1:1 (SEQ ID NO: 131)).

**Table 4. Average R<sub>0</sub> GUS Anther, Silk, Endosperm and Embryo expression in transgenic corn plants, transformed with listed construct.**

Construct	Regulatory Elements	VT Anther	VT Silk	21 DAP Embryo	21 DAP Endosperm
pMON109728	EXP-FMV.35S-SETit.Tip (SEQ ID NO: 8) E-FMV.35S-1:1:2 (SEQ ID NO: 177) P-SETit.Tip-1:1:1 (SEQ ID NO: 95) L-SETit.Tip-1:1:1 (SEQ ID NO: 163)	nd	74.07	nd	nd
pMON112215	P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88) L-SETit.Rcc3-1:1:1 (SEQ ID NO: 161)	nd	nd	nd	nd
pMON116789	P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	289.88	11.83	nd	nd
pMON116816	P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	9.66	0.00	17.06	20.16

pMON116818	P-SETit.Pox-1:1:1 (SEQ ID NO: 73) L-SETit.Pox-1:1:1 (SEQ ID NO: 147)	0.00	10.12	0.00	0.00
pMON116820	P-SETit.Gst-1:1:1 (SEQ ID NO: 58) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	19.62	8.08	14.09	15.25
PMON116829	P-SETit.Rcc3-1:1:10 (SEQ ID NO: 89) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	8.58	0.00	0.00	12.52
pMON120698	EXP-FMV.35S-SETit.Rcc3:a (SEQ ID NO: 6) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Rcc3-1:1:10 (SEQ ID NO: 89) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	0.00	0.00	13.62	23.07
pMON120699	EXP-FMV.35S-SETit.Gst:a (SEQ ID NO: 2) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Gst-1:1:1 (SEQ ID NO: 58) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	41.63	21.74	27.03	92.79
pMON120700	EXP-FMV.35S-SETit.Rcc3:b (SEQ ID NO: 7) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	7.65	0.00	0.00	11.30
pMON120701	EXP-FMV.35S-SETit.Pox (SEQ ID NO: 5) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Pox-1:1:1 (SEQ ID NO: 73) L-SETit.Pox-1:1:1 (SEQ ID NO: 147)	55.20	77.97	0.00	10.70
pMON120702	P-SETit.Ccoamt-1:1:2 (SEQ ID NO: 35) L-SETit.Ccoamt-1:1:2 (SEQ ID NO: 116)	33.96	0.00	0.00	6.93

pMON120703	EXP-FMV.35S-SETit.Ccoamt (SEQ ID NO: 1) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Ccoamt-1:1:2 (SEQ ID NO: 35) L-SETit.Ccoamt-1:1:2 (SEQ ID NO: 116)	22.01	36.65	9.23	28.99
pMON120704	P-SETit.Gst-1:1:2 (SEQ ID NO: 59) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	16.43	0.00	nd	nd
pMON120705	EXP-FMV.35S-SETit.Gst:b (SEQ ID NO: 3) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Gst-1:1:2 (SEQ ID NO: 59) L-SETit.Gst-1:1:1 (SEQ ID NO: 135)	156.37	46.06	39.48	82.83
pMON120706	EXP-FMV.35S-SETit.Ifr (SEQ ID NO: 4) E-FMV.35S-1:1:2 (SEQ ID NO: 351) P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)	77.35	307.35	14.27	100.43
pMON120709	P-SETit.Nrt2-1:1:3 (SEQ ID NO: 65) L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139)	6.90	132.25	138.75	20.39
pMON120710	P-SETit.Nrt2-1:1:2 (SEQ ID NO: 64) L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139)	8.60	0.00	12.49	6.52
pMON120711	P-SETit.Pip2-1:1:3 (SEQ ID NO: 71) L-SETit.Pip2-1:1:1 (SEQ ID NO: 145)	15.13	6.65	10.49	0.00
pMON120712	P-SETit.Ifr-1:1:2 (SEQ ID NO: 60) L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)	9.64	0.00	8.26	0.00
pMON120713	P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)	11.02	0.00	23.91	5.94

pMON127440	P-SETit.Ppc-1:1:3 (SEQ ID NO: 74) L-SETit.Ppc-1:1:1 (SEQ ID NO: 148)	18.44	1.15	0.00	0.00
pMON127441	P-SETit.Ppc-1:1:4 (SEQ ID NO: 75) L-SETit.Ppc-1:1:1 (SEQ ID NO: 148)	181.79	0.00	4.10	1.17
pMON127442	P-SETit.Gapdh2-1:1:3 (SEQ ID NO: 55) L-SETit.Gapdh2-1:1:1 (SEQ ID NO: 133)	28.05	0.00	155.68	400.86
pMON127443	EXP-SETit.Ppdk:1:1 (SEQ ID NO: 14) P-SETit.Ppdk-1:1:1 (SEQ ID NO: 76) L-SETit.Ppdk-1:1:4 (SEQ ID NO: 150) I-SETit.Ppdk-1:1:1 (SEQ ID NO: 175) L-SETit.Ppdk-1:1:2 (SEQ ID NO: 149)	0.00	0.00	2.62	0.67
pMON127444	P-SETit.CP29-1:1:4 (SEQ ID NO: 42) L-SETit.CP29-1:1:1 (SEQ ID NO: 124)	87.67	24.63	0.00	0.00
pMON127445	P-SETit.Cab3-1:1:3 (SEQ ID NO: 33) L-SETit.Cab3-1:1:1 (SEQ ID NO: 114)	35.79	35.94	<0.1	<0.1
pMON127446	P-SETit.PSI-4a-1:1:1 (SEQ ID NO: 86) L-SETit.PSI-4a-1:1:1 (SEQ ID NO: 159)	56.44	194.45	1.64	3.24
pMON127447	P-SETit.Rbcs-1:1:1 (SEQ ID NO: 87) L-SETit.Rbcs-1:1:1 (SEQ ID NO: 160)	102.30	125.45	3.25	6.58
pMON127448	P-SETit.Cab1-1:1:1 (SEQ ID NO: 32) L-SETit.Cab1-1:1:1 (SEQ ID NO: 113)	61.33	22.61	2.32	4.47
pMON127449	P-SETit.Fba-1:1:1 (SEQ ID NO: 51) L-SETit.Fba-1:1:1 (SEQ ID NO: 131)	12.62	<0.1	<0.1	<0.1

pMON132037	EXP-SETit.Ubq1-1:1:1 (SEQ ID NO: 20) P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102) L-SETit.Ubq1-1:1:1 (SEQ ID NO: 169) I-SETit.Ubq1-1:1:1 (SEQ ID NO: 177)	131.65	85.35	59.09	67.31
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The average level of GUS expression in the seed and reproductive tissues varied amongst the constructs. Highest levels of anther expression at VT stage were observed for pMON116789 ((P-SETit.Rcc3-1:1:11 (SEQ ID NO: 90) + L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)); pMON127441 ((P-SETit.Ppc-1:1:4 (SEQ ID NO: 75) + L-SETit.Ppc-1:1:1 (SEQ ID NO: 148)); pMON120705 ((EXP-FMV.35S-SETit.Gst:b (SEQ ID NO: 3) comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Gst-1:1:2 (SEQ ID NO: 59) + L-SETit.Gst-1:1:1 (SEQ ID NO: 135)); pMON132037 ((EXP-SETit.Ubq1:1:1 (SEQ ID NO: 20) comprised of P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102) + L-SETit.Ubq1-1:1:1 (SEQ ID NO: 169) + I-SETit.Ubq1-1:1:1 (SEQ ID NO: 177)); pMON127447 ((P-SETit.Rbcs-1:1:1 (SEQ ID NO: 87) + L-SETit.Rbcs-1:1:1 (SEQ ID NO: 160)); pMON127444 ((P-SETit.CP29-1:1:4 (SEQ ID NO: 42) + L-SETit.CP29-1:1:1 (SEQ ID NO: 124)) and pMON120706 ((EXP-FMV.35S-SETit.Ifr (SEQ ID NO: 4) comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) + L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)).

The highest levels of average GUS expression in the silk was observed in plants transformed with pMON120706 ((EXP-FMV.35S-SETit.Ifr (SEQ ID NO: 4) comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Ifr-1:1:3 (SEQ ID NO: 61) + L-SETit.Ifr-1:1:1 (SEQ ID NO: 136)); pMON127446 ((P-SETit.PSI-4a-1:1:1 (SEQ ID NO: 86) + L-SETit.PSI-4a-1:1:1 (SEQ ID NO: 159)); pMON120709 ((P-SETit.Nrt2-1:1:3 (SEQ ID NO: 65) + L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139)); pMON127447 ((P-SETit.Rbcs-1:1:1 (SEQ ID NO: 87) + L-SETit.Rbcs-1:1:1 (SEQ ID NO: 160)) and pMON132037 ((EXP-SETit.Ubq1:1:1 (SEQ ID NO: 20) comprised of P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102) + L-SETit.Ubq1-1:1:1 (SEQ ID NO: 169) + I-SETit.Ubq1-1:1:1 (SEQ ID NO: 177)). Average GUS expression in the developing embryo 21 DAP was observed in plants transformed with pMON127442 ((P-SETit.Gapdh2-1:1:3 (SEQ ID NO: 55) + L-SETit.Gapdh2-1:1:1 (SEQ ID NO: 133)); pMON120709 ((P-SETit.Nrt2-1:1:3 (SEQ ID NO: 65) + L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139)); pMON132037 ((EXP-SETit.Ubq1:1:1 (SEQ ID NO: 20) comprised of P-SETit.Ubq1-1:1:1 (SEQ ID NO: 102) + L-SETit.Ubq1-1:1:1 (SEQ ID NO: 169) + I-SETit.Ubq1-1:1:1 (SEQ ID NO: 177)).

(SEQ ID NO: 177)); pMON120705 ((EXP-FMV.35S-SETit.Gst:b (SEQ ID NO: 3) comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Gst-1:1:2 (SEQ ID NO: 59) + L-SETit.Gst-1:1:1 (SEQ ID NO: 135)) and pMON120699 ((EXP-FMV.35S-SETit.Gst:a (SEQ ID NO: 2) comprised of E-FMV.35S-1:1:2 (SEQ ID NO: 179) + P-SETit.Gst-1:1:1 (SEQ ID NO: 58) + L-SETit.Gst-1:1:1 (SEQ ID NO: 135)). Average GUS expression was also highest in plants transformed with pMON127442 ((P-SETit.Gapdh2-1:1:3 (SEQ ID NO: 55) + L-SETit.Gapdh2-1:1:1 (SEQ ID NO: 133)) and pMON120709 ((P-SETit.Nrt2-1:1:3 (SEQ ID NO: 65) + L-SETit.Nrt2-1:1:2 (SEQ ID NO: 139))

Plants transformed with the GUS expression vectors, pMON112215 ((P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88) + L-SETit.Rcc3-1:1:1 (SEQ ID NO: 161)) were crossed with non-transformed LH244 plants to produce an F1 population of transformants. GUS expression levels were measured in selected tissues over the course of development. The F1 tissues used for this study included: imbibed seed embryo, imbibed seed endosperm, root (3 days after germination), coleoptiles (3 days after germination), V3 root, V3 leaf, V7 root, V7 mature leaf, VT (at tasseling, prior to reproduction) seminal root, VT internode, VT cob, VT anther, VT pollen, VT silk, kernel 7 days after pollination, embryo 21 days after pollination, endosperm 21 days after pollination, embryo 35 days after pollination, endosperm 35 days after pollination.

Drought stress was induced in R<sub>0</sub> V3 plants and in F1 V3 plants by withholding watering for 4 days allowing the water content to be reduced by at least 50% of the original water content of the fully watered plant. The drought protocol was comprised essentially of the following steps. V3 stage plants were deprived of water. As a corn plant experiences drought, the shape of the leaf will change from the usual healthy and unfolded appearance to a leaf demonstrating folding at the mid-rib vascular bundle and appearing V-shaped when viewed from the leaf tip to the stem. This change in morphology usually began to occur by about 2 days after the cessation of watering and was shown in earlier experiments to be associated with water loss of around 50% as measured by weight of pots prior to cessation of watering and weight of pots when the leaf curl morphology was observed in un-watered plants. Plants were considered to be under drought conditions, when the leaves showed wilting as evidenced by an inward curling (V-shape) of the leaf. This level of stress is considered to be a form of sub-lethal stress. Control leaf samples were taken from each plant for GUS testing prior to the induction of drought. Drought (indicated as “Des” in Table 5 below) was then induced and once each plant demonstrated drought induction as defined above, the plant was destroyed to acquire both root and leaf samples. GUS expression levels

in the leaves were compared to the control tissue samples from the same plants prior to drought. For R<sub>0</sub> generation plants, fourteen plants for each vector were used. For F1 analysis, eight plants for each vector were used and GUS measures taken as described above. Four of the F1 plants were destroyed for tissue sampling for GUS assay (Des) after drought-  
 5 induction. The other two F1 plants were allowed to recover and then destructively sampled to determine if the pattern of GUS expression under recovery was the same as that before drought was imposed.

In addition to drought, F1 germinating seedlings and F1 V3 stage plants transformed with the vectors presented in Table 2 were also exposed to conditions of cold to determine if  
 10 the regulatory elements and chimeric regulatory elements demonstrated cold-induced expression of GUS. Sixty seeds, comprised of 6 seeds from each of 10 transformation events for each regulatory element or chimeric regulatory element, were tested for induction of gene expression under cold conditions. The seeds were germinated in petri plates on water saturated filter paper. Three days after germination, the seedlings were exposed to cold  
 15 stress by placing the Petri dishes containing the germinated seedlings in a dark growth chamber set to 10 degrees Celsius for 24 hours. At the end of the 24 hour period, the root and coleoptiles tissues were sampled for quantitative GUS expression as described above. Whole plants were tested for induction of GUS expression under cold stress at V3 stage. Twenty V3 stage corn plants, comprised of 2 plants from each of 10 transformation events for each  
 20 regulatory element or chimeric regulatory element, were exposed to a temperature of 12 degrees Celsius in a growth chamber for 24 hours. Plants in the growth chamber were grown under a white light fluence of 800 micro moles per meter squared per second with a light cycle of ten hours of white light and fourteen hours of darkness. After cold exposure, leaf and root tissues were sampled for quantitative GUS exposure as described above. Table 5  
 25 below shows the level of GUS expression in selected tissues in F1 plants transformed with pMON112215.

**Table 5. F1 GUS expression in transgenic corn plants, transformed with pMON112215.**

Stages	Organ	Inducer	Mean	SE
Imbibed seed	Embryo	-	22.6	1.54
Imbibed seed	Endosperm	-	7.48	1.64
3 DAG	Root	-	0	0
V3	Root main	Unstressed	0	0
V3	Root crown	-	0	0
V3	Root main	Cold	0	0
V3	Root crown	-	0	0

V3	Root main	Des	0	0
V3	Root crown	-	nd	nd
V7	Root seminal	-	0	0
V7	Root crown	-	0	0
VT	Root seminal	-	8.26	1.38
3 DAG	Coleoptile	-	0	0
V3	Leaf	Unstressed	0	0
V3	Leaf	Cold	0	0
V3	Leaf	Des	6.18	0.3
V7	Leaf - Mature	-	0	0
VT	Internode	-	9.83	3.47
VT	Cob	-	7.38	1.78
VT	Anther	-	1060.86	849.27
VT	Pollen	-	200.74	101.84
VT	Silk	-	16.63	9.45
21 DAP	Embryo	-	18.6	2.18
35 DAP	Embryo	-	4.16	1.35
10 DAP	Kernal	-	0	0
21 DAP	Endosperm	-	11.14	0
35 DAP	Endosperm	-	3.26	2.27

F1 corn plants, transformed with pMON112215 ((P-SETit.Rcc3-1:1:1 (SEQ ID NO: 88) + L-SETit.Rcc3-1:1:1 (SEQ ID NO: 161)) demonstrated high levels of expression in VT anther tissue. Expression in pollen was also observed to be higher than in tissues other than anther. Expression was observed to be around background levels in developing embryo and endosperm, VT silk, VT seminal root and VT internode. Using more sensitive assay methods such as ELISA of TIC809 expression, the SETit.Rcc3 promoter and leader has been shown previously to drive expression of a transgene in stably transformed, corn root tissues (WO 2009/126470).

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### Example 3: Analysis of Regulatory Elements driving GUS in Transgenic Corn

Corn root and leaf tissue from 12 to 13 day old seedlings is bombarded with plant GUS expression and control vectors to determine the capacity of transcriptional regulatory elements derived from *Setaria italica* to drive expression of a transgene, GUS.

Corn plant tissues were transformed with the plant GUS expression constructs, listed in Table 6, below. Regulatory elements presented in example 1 were cloned into a base plant expression vector using standard methods known in the art. The resulting plant expression vectors contained a right border region from *Agrobacterium tumefaciens*, a first transgene



cassette to test the regulatory or chimeric regulatory element comprised of, a regulatory or chimeric regulatory element, operably linked to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS) that possessed a processable intron (GUS-2, SEQ ID NO: 1091), operably linked to the 3' termination region from the rice lipid transfer protein gene (T-Os.LTP-1:1:1, SEQ ID NO: 1089); a second transgene selection cassette used for selection of transformed plant cells that conferred resistance to the herbicide glyphosate (driven by the rice Actin 1 transcriptional regulatory element group, SEQ ID NO: 1098), and a left border region from *A. tumefaciens*. The resulting plasmids, pMON129227, pMON129228, pMON129229, pMON129230, pMON129231, pMON129232, pMON129233, pMON129234, pMON129235, pMON129236, pMON129237, pMON129238, pMON129239, pMON129240, pMON129241, pMON129242, pMON129243, pMON129244, pMON129245, pMON129246, pMON129247, pMON129248, pMON129249, pMON129250, pMON129251, pMON129252, pMON129253, pMON129254, pMON129255, pMON129256, pMON129257, pMON129258 and pMON129259 were used to transform corn plant tissue using particle bombardment.

**Table 6. Binary Plant Transformation Vectors, Regulatory or chimeric regulatory elements, GUS and 3' UTRs.**

Construct	Regulatory Elements	3' UTR
pMON129227	P-SETit.Cyp-1-1:1:1 (SEQ ID NO: 43) L-SETit.Cyp-1-1:1:1 (SEQ ID NO: 125)	T-Os.LTP-1:1:1
pMON129228	P-SETit.Cyp78a-1:1:2 (SEQ ID NO: 44) L-SETit.Cyp78a-1:1:1 (SEQ ID NO: 126)	T-Os.LTP-1:1:1
pMON129229	P-SETit.OMT2.1-1:1:2 (SEQ ID NO: 66) L-SETit.OMT2.1-1:1:1 (SEQ ID NO: 140)	T-Os.LTP-1:1:1
pMON129230	P-SETit.OMT2.2-1:1:2 (SEQ ID NO: 67) L-SETit.OMT2.2-1:1:2 (SEQ ID NO: 142)	T-Os.LTP-1:1:1
pMON129231	P-SETit.OMT2.3-1:1:1 (SEQ ID NO: 68) L-SETit.OMT2.2-1:1:1 (SEQ ID NO: 141)	T-Os.LTP-1:1:1
pMON129232	P-SETit.Grcw2-1:1:1 (SEQ ID NO: 56) L-SETit.Grcw2-1:1:1 (SEQ ID NO: 134)	T-Os.LTP-1:1:1
pMON129233	P-SETit.Prx2-1:1:3 (SEQ ID NO: 81)	T-Os.LTP-1:1:1

	L-SETit.Prx2-1:1:2 (SEQ ID NO: 155)	
pMON129234	P-SETit.Srp-1:1:2 (SEQ ID NO: 92) L-SETit.Srp-1:1:1 (SEQ ID NO: 163)	T-Os.LTP-1:1:1
pMON129235	P-SETit.LaDo-1:1:2 (SEQ ID NO: 62) L-SETit.LaDo-1:1:1 (SEQ ID NO: 137)	T-Os.LTP-1:1:1
pMON129236	P-SETit.Aip-1:1:1 (SEQ ID NO: 27) L-SETit.Aip-1:1:1 (SEQ ID NO: 109)	T-Os.LTP-1:1:1
pMON129237	P-SETit.Prx-1:1:1 (SEQ ID NO: 79) L-SETit.Prx-1:1:1 (SEQ ID NO: 153)	T-Os.LTP-1:1:1
pMON129238	P-SETit.Cbl7-1:1:1 (SEQ ID NO: 34) L-SETit.Cbl7-1:1:1 (SEQ ID NO: 115)	T-Os.LTP-1:1:1
pMON129239	P-SETit.Fst-1:1:1 (SEQ ID NO: 54) L-SETit.Fst-1:1:1 (SEQ ID NO: 132)	T-Os.LTP-1:1:1
pMON129240	P-SETit.Cda-1:1:1 (SEQ ID NO: 36) L-SETit.Cda-1:1:1 (SEQ ID NO: 117)	T-Os.LTP-1:1:1
pMON129241	P-SETit.Prx3-1:1:4 (SEQ ID NO: 83) L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)	T-Os.LTP-1:1:1
pMON129242	P-SETit.Prx3-1:1:3 (SEQ ID NO: 82) L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)	T-Os.LTP-1:1:1
pMON129243	P-SETit.Prx47-1:1:2 (SEQ ID NO: 84) L-SETit.Prx47-1:1:1 (SEQ ID NO: 157)	T-Os.LTP-1:1:1
pMON129244	P-SETit.Eie-1:1:1 (SEQ ID NO: 49) L-SETit.Eie-1:1:1 (SEQ ID NO: 129)	T-Os.LTP-1:1:1
pMON129245	P-SETit.Omt3-1:1:3 (SEQ ID NO: 69) L-SETit.Omt3-1:1:1 (SEQ ID NO: 143)	T-Os.LTP-1:1:1
pMON129246	P-SETit.Cys-1:1:2 (SEQ ID NO: 45) L-SETit.Cys-1:1:1 (SEQ ID NO: 127)	T-Os.LTP-1:1:1
pMON129247	P-SETit.Cys-1:1:3 (SEQ ID NO: 46) L-SETit.Cys-1:1:1 (SEQ ID NO: 127)	T-Os.LTP-1:1:1
pMON129248	P-SETit.Ucc1-1:1:2 (SEQ ID NO: 105) L-SETit.Ucc1-1:1:1 (SEQ ID NO: 170)	T-Os.LTP-1:1:1
pMON129249	P-SETit.Tip-1:1:4 (SEQ ID NO: 97)	T-Os.LTP-

	L-SETit.Tip-1:1:1 (SEQ ID NO: 165)	1:1:1
pMON129250	P-SETit.Prx72-1:1:2 (SEQ ID NO: 85) L-SETit.Prx72-1:1:1 (SEQ ID NO: 158)	T-Os.LTP-1:1:1
pMON129251	P-SETit.Prx17-1:1:2 (SEQ ID NO: 80) L-SETit.Prx17-1:1:1 (SEQ ID NO: 154)	T-Os.LTP-1:1:1
pMON129252	P-SETit.Mt1-1:1:2 (SEQ ID NO: 63) L-SETit.Mt1-1:1:1 (SEQ ID NO: 138)	T-Os.LTP-1:1:1
pMON129253	P-SETit.Ali1-1:1:3 (SEQ ID NO: 31) L-SETit.Ali1-1:1:1 (SEQ ID NO: 112)	T-Os.LTP-1:1:1
pMON129254	P-SETit.Rcc3-1:1:16 (SEQ ID NO: 91) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	T-Os.LTP-1:1:1
pMON129255	P-SETit.Pip2-3-1:1:1 (SEQ ID NO: 72) L-SETit.Pip2-3-1:1:1 (SEQ ID NO: 146)	T-Os.LTP-1:1:1
pMON129256	P-SETit.Tga6-1:1:2 (SEQ ID NO: 95)	T-Os.LTP-1:1:1
pMON129257	P-SETit.25509-1:1:3 (SEQ ID NO: 23)	T-Os.LTP-1:1:1
pMON129258	P-SETit.Grf-1:1:2 (SEQ ID NO: 57)	T-Os.LTP-1:1:1
pMON129259	P-SETit.Omt4_2-1:1:2 (SEQ ID NO: 70) L-SETit.Omt4_2-1:1:1 (SEQ ID NO: 144)	T-Os.LTP-1:1:1

The plant transformation vector, pMON129227 is comprised of the promoter element, P-SETit.Cyp-1-1:1:1 (SEQ ID NO: 43), operably linked 5' to the leader element, L-SETit.Cyp-1-1:1:1 (SEQ ID NO: 125). The plant transformation vector, pMON129228 is

5 comprised of the promoter element, P-SETit.Cyp78a-1:1:2 (SEQ ID NO: 44), operably linked 5' to the leader element, L-SETit.Cyp78a-1:1:1 (SEQ ID NO: 126). The plant transformation vector, pMON129229 is comprised of the promoter element, P-SETit.OMT2.1-1:1:2 (SEQ ID NO: 66), operably linked 5' to the leader element, L-SETit.OMT2.1-1:1:1 (SEQ ID NO: 140). The plant transformation vector, pMON129230 is comprised of the promoter element,

10 P-SETit.OMT2.2-1:1:2 (SEQ ID NO: 67), operably linked 5' to the leader element, L-SETit.OMT2.2-1:1:2 (SEQ ID NO: 142). The plant transformation vector, pMON129231 is comprised of the promoter element, P-SETit.OMT2.3-1:1:1 (SEQ ID NO: 68), operably linked 5' to the leader element, L-SETit.OMT2.2-1:1:1 (SEQ ID NO: 141). The plant

transformation vector, pMON129232 is comprised of the promoter element, P-SETit.Grcw2-1:1:1 (SEQ ID NO: 56), operably linked 5' to the leader element, L-SETit.Grcw2-1:1:1 (SEQ ID NO: 134). The plant transformation vector, pMON129233 is comprised of the promoter element, P-SETit.Prx2-1:1:3 (SEQ ID NO: 81), operably linked 5' to the leader element, L-SETit.Prx2-1:1:2 (SEQ ID NO: 155). The plant transformation vector, pMON129234 is comprised of the promoter element, P-SETit.Srp-1:1:2 (SEQ ID NO: 92), operably linked 5' to the leader element, L-SETit.Srp-1:1:1 (SEQ ID NO: 163). The plant transformation vector, pMON129235 is comprised of the promoter element, P-SETit.LaDo-1:1:2 (SEQ ID NO: 62), operably linked 5' to the leader element, L-SETit.LaDo-1:1:1 (SEQ ID NO: 137). The plant transformation vector, pMON129236 is comprised of the promoter element, P-SETit.Aip-1:1:1 (SEQ ID NO: 27), operably linked 5' to the leader element, L-SETit.Aip-1:1:1 (SEQ ID NO: 109). The plant transformation vector, pMON129237 is comprised of the promoter element, P-SETit.Prx-1:1:1 (SEQ ID NO: 79), operably linked 5' to the leader element, L-SETit.Prx-1:1:1 (SEQ ID NO: 153). The plant transformation vector, pMON129238 is comprised of the promoter element, P-SETit.Cbl7-1:1:1 (SEQ ID NO: 34), operably linked 5' to the leader element, L-SETit.Cbl7-1:1:1 (SEQ ID NO: 115). The plant transformation vector, pMON129239 is comprised of the promoter element, P-SETit.Fst-1:1:1 (SEQ ID NO: 54), operably linked 5' to the leader element, L-SETit.Fst-1:1:1 (SEQ ID NO: 132). The plant transformation vector, pMON129240 is comprised of the promoter element, P-SETit.Cda-1:1:1 (SEQ ID NO: 36), operably linked 5' to the leader element, L-SETit.Cda-1:1:1 (SEQ ID NO: 117). The plant transformation vector, pMON129241 is comprised of the promoter element, P-SETit.Prx3-1:1:4 (SEQ ID NO: 83), operably linked 5' to the leader element, L-SETit.Prx3-1:1:1 (SEQ ID NO: 156). The plant transformation vector, pMON129242 is comprised of the promoter element, P-SETit.Prx3-1:1:3 (SEQ ID NO: 82), operably linked 5' to the leader element, L-SETit.Prx3-1:1:1 (SEQ ID NO: 156). The plant transformation vector, pMON129243 is comprised of the promoter element, P-SETit.Prx47-1:1:2 (SEQ ID NO: 84), operably linked 5' to the leader element, L-SETit.Prx47-1:1:1 (SEQ ID NO: 157). The plant transformation vector, pMON129244 is comprised of the promoter element, P-SETit.Eie-1:1:1 (SEQ ID NO: 49), operably linked 5' to the leader element, L-SETit.Eie-1:1:1 (SEQ ID NO: 129). The plant transformation vector, pMON129245 is comprised of the promoter element, P-SETit.Omt3-1:1:3 (SEQ ID NO: 69), operably linked 5' to the leader element, L-SETit.Omt3-1:1:1 (SEQ ID NO: 143). The plant transformation vector, pMON129246 is comprised of the promoter element, P-SETit.Cys-1:1:2 (SEQ ID NO: 45), operably linked 5' to the leader element, L-SETit.Cys-

1:1:1 (SEQ ID NO: 127). The plant transformation vector, pMON129247 is comprised of the promoter element, P-SETit.Cys-1:1:3 (SEQ ID NO: 46), operably linked 5' to the leader element, L-SETit.Cys-1:1:1 (SEQ ID NO: 127). The plant transformation vector, pMON129248 is comprised of the promoter element, P-SETit.Ucc1-1:1:2 (SEQ ID NO: 105), operably linked 5' to the leader element, L-SETit.Ucc1-1:1:1 (SEQ ID NO: 170). The plant transformation vector, pMON129249 is comprised of the promoter element, P-SETit.Tip-1:1:4 (SEQ ID NO: 97), operably linked 5' to the leader element, L-SETit.Tip-1:1:1 (SEQ ID NO: 165). The plant transformation vector, pMON129250 is comprised of the promoter element, P-SETit.Prx72-1:1:2 (SEQ ID NO: 85), operably linked 5' to the leader element, L-SETit.Prx72-1:1:1 (SEQ ID NO: 158). The plant transformation vector, pMON129251 is comprised of the promoter element, P-SETit.Prx17-1:1:2 (SEQ ID NO: 80), operably linked 5' to the leader element, L-SETit.Prx17-1:1:1 (SEQ ID NO: 154). The plant transformation vector, pMON129252 is comprised of the promoter element, P-SETit.Mt1-1:1:2 (SEQ ID NO: 63), operably linked 5' to the leader element, L-SETit.Mt1-1:1:1 (SEQ ID NO: 138). The plant transformation vector, pMON129253 is comprised of the promoter element, P-SETit.Ali1-1:1:3 (SEQ ID NO: 31), operably linked 5' to the leader element, L-SETit.Ali1-1:1:1 (SEQ ID NO: 112). The plant transformation vector, pMON129254 is comprised of the promoter element, P-SETit.Rcc3-1:1:16 (SEQ ID NO: 91), operably linked 5' to the leader element, L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162). The plant transformation vector, pMON129255 is comprised of the promoter element, P-SETit.Pip2-3-1:1:1 (SEQ ID NO: 72), operably linked 5' to the leader element, L-SETit.Pip2-3-1:1:1 (SEQ ID NO: 146). The plant transformation vector, pMON129256 is comprised of the promoter element, P-SETit.Tga6-1:1:2 (SEQ ID NO: 95). The plant transformation vector, pMON129257 is comprised of the promoter element, P-SETit.25509-1:1:3 (SEQ ID NO: 23). The plant transformation vector, pMON129258 is comprised of the promoter element, P-SETit.Grf-1:1:2 (SEQ ID NO: 57). The plant transformation vector, pMON129259 is comprised of the promoter element, P-SETit.Omt4\_2-1:1:2 (SEQ ID NO: 70), operably linked 5' to the leader element, L-SETit.Omt4\_2-1:1:1 (SEQ ID NO: 144).

Corn plant tissues were transformed with plant GUS expression constructs,

30	pMON129227,	pMON129228,	pMON129229,	pMON129230,	pMON129231,
	pMON129232,	pMON129233,	pMON129234,	pMON129235,	pMON129236,
	pMON129237,	pMON129238,	pMON129239,	pMON129240,	pMON129241,
	pMON129242,	pMON129243,	pMON129244,	pMON129245,	pMON129246,
	pMON129247,	pMON129248,	pMON129249,	pMON129250,	pMON129251,

pMON129252, pMON129253, pMON129254, pMON129255, pMON129256, pMON129257, pMON129258 and pMON129259, using particle bombardment.

Corn plant tissue was transformed using particle bombardment methods known to those skilled in the art with the vectors described above. Briefly, LH244 corn seeds are surface sterilized and allowed to germinate in trays with a photoperiod of 16 hours light and 8 hours of darkness. After 12 to 13 days, tissue is harvested under sterile conditions from the seedlings and used for bombardment. Approximately 10 leaf and 15 root explants are used for bombardment of each experimental construct. The tissue samples are randomly placed on a petri dish containing plant culture medium. Ten micrograms of plasmid DNA is used to coat 0.6 micron gold particles (Catalog #165-2262 Bio-Rad, Hercules, CA) for bombardment. Macro-carriers were loaded with the DNA-coated gold particles (Catalog #165-2335 Bio-Rad, Hercules CA). A PDS 1000/He biolistic gun was used for transformation (Catalog #165-2257 Bio-Rad, Hercules CA). The bombarded root and leaf tissues were allowed to incubate in the dark for 24 hours at 26 degrees Celsius. Following this overnight incubation, the tissues are stained in solution for GUS expression overnight at 37 degrees Celsius. After staining overnight, the tissues are soaked in 70% ethanol overnight to remove chlorophyll and reveal the GUS staining. The tissues were then photographed and a rating scale of "0" to "4" reflecting the level of GUS expression is assigned to each construct.

Four control plasmids were also used for bombardment designated, pMON19469, pMON59327, pMON30098 and pMON103758. The plasmid vectors, pMON19469, pMON59327, and pMON103758 contained known transcriptional regulatory elements driving GUS expression and were used as comparators for expression. The plasmid vector, pMON30098 was comprised of a transgene cassette used for the expression of green fluorescent protein and served as a negative control in the bombardment assay. The plasmid vector, pMON19469 is comprised of a transgene cassette comprised of the P-CaMV.35S-enh-1:1:9 promoter (SEQ ID NO: 1096), derived from the Cauliflower mosaic virus 35S promoter, operably linked 5' to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS-3, SEQ ID NO: 1092), operably linked 5' to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). The plasmid vector, pMON59327 is comprised of a transgene cassette used for the expression of GUS which is comprised of the rice Rcc3 promoter (P-Os.Rcc3-1:1:24, SEQ ID NO: 1093) and leader (L-Os.Rcc3-1:1:1, SEQ ID NO: 1094), operably linked 5' to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102),

operably linked 5' to a coding sequence for  $\beta$ -glucuronidase (GUS-3, SEQ ID NO: 1092), operably linked 5' to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). The plasmid vector, pMON103758 is comprised of a transgene cassette used for the expression of GUS which is comprised of the rice Rcc3 promoter (P-Os.Rcc3-1:1:24, SEQ ID NO: 1093) and leader (L-Os.Rcc3-1:1:1, SEQ ID NO: 1094), operably linked 5' to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably linked 5' to a coding sequence for  $\beta$ -glucuronidase (GUS-3, SEQ ID NO: 1092), operably linked 5' to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). The plasmid vector, pMON30098 was comprised of a green fluorescent protein transgene cassette and served as a negative control in the bombardment assay and was comprised of a transgene cassette comprised of the transcriptional regulatory element group, EXP-CaMV.35S-enh (SEQ ID NO: 1095) which was further comprised of the promoter, P-CaMV.35S-enh-1:1:9 (SEQ ID NO: 1096), operably linked 5' to the leader element, L-CaMV.35S-1:1:2 (SEQ ID NO: 1097), operably linked 5' to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably 5' linked to a coding sequence for GFP (CR-Av.GFP.nno, SEQ ID NO: 1103), operably linked 5' to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088).

The average GUS expression ratings from the particle bombarded assay are shown in Table 7 below.

**Table 7. Average GUS expression ratings for bombarded corn root and leaf tissue transformed with listed plant GUS expression constructs.**

Construct	Regulatory Elements	Root	Leaf
pMON19469	P-CaMV.35S-enh-1:1:9 promoter (SEQ ID NO: 1096)	4	4
pMON59327	P-Os.Rcc3-1:1:24 (SEQ ID NO: 1093) L-Os.Rcc3-1:1:1 (SEQ ID NO: 1094)	4	0
pMON30098 (Neg. Control)	EXP-CaMV.35S-enh (SEQ ID NO: 1095)	0	0
pMON103758	P-Os.Rcc3-1:1:24 (SEQ ID NO: 1093) L-Os.Rcc3-1:1:1 (SEQ ID NO: 1094)	3	0
pMON129227	P-SETit.Cyp-1-1:1:1 (SEQ ID NO: 43) L-SETit.Cyp-1-1:1:1 (SEQ ID NO: 125)	0	0
pMON129228	P-SETit.Cyp78a-1:1:2 (SEQ ID NO: 44) L-SETit.Cyp78a-1:1:1 (SEQ ID NO: 126)	1	0
pMON129229	P-SETit.OMT2.1-1:1:2 (SEQ ID NO: 66) L-SETit.OMT2.1-1:1:1 (SEQ ID NO: 140)	1	0

pMON129230	P-SETit.OMT2.2-1:1:2 (SEQ ID NO: 67) L-SETit.OMT2.2-1:1:2 (SEQ ID NO: 142)	2	0
pMON129231	P-SETit.OMT2.3-1:1:1 (SEQ ID NO: 68) L-SETit.OMT2.2-1:1:1 (SEQ ID NO: 141)	3	0
pMON129232	P-SETit.Grew2-1:1:1 (SEQ ID NO: 56) L-SETit.Grew2-1:1:1 (SEQ ID NO: 134)	2	0
pMON129233	P-SETit.Prx2-1:1:3 (SEQ ID NO: 81) L-SETit.Prx2-1:1:2 (SEQ ID NO: 155)	1	0
pMON129234	P-SETit.Srp-1:1:2 (SEQ ID NO: 92) L-SETit.Srp-1:1:1 (SEQ ID NO: 163)	3	0
pMON129235	P-SETit.LaDo-1:1:2 (SEQ ID NO: 62) L-SETit.LaDo-1:1:1 (SEQ ID NO: 137)	1	0
pMON129236	P-SETit.Aip-1:1:1 (SEQ ID NO: 27) L-SETit.Aip-1:1:1 (SEQ ID NO: 109)	2	0
pMON129237	P-SETit.Prx-1:1:1 (SEQ ID NO: 79) L-SETit.Prx-1:1:1 (SEQ ID NO: 153)	3	1
pMON129238	P-SETit.Cbl7-1:1:1 (SEQ ID NO: 34) L-SETit.Cbl7-1:1:1 (SEQ ID NO: 115)	0	0
pMON129239	P-SETit.Fst-1:1:1 (SEQ ID NO: 54) L-SETit.Fst-1:1:1 (SEQ ID NO: 132)	0	0
pMON129240	P-SETit.Cda-1:1:1 (SEQ ID NO: 36) L-SETit.Cda-1:1:1 (SEQ ID NO: 117)	0	0
pMON129241	P-SETit.Prx3-1:1:4 (SEQ ID NO: 83) L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)	3	2
pMON129242	P-SETit.Prx3-1:1:3 (SEQ ID NO: 82) L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)	3	1
pMON129243	P-SETit.Prx47-1:1:2 (SEQ ID NO: 84) L-SETit.Prx47-1:1:1 (SEQ ID NO: 157)	3	0
pMON129244	P-SETit.Eie-1:1:1 (SEQ ID NO: 49) L-SETit.Eie-1:1:1 (SEQ ID NO: 129)	1	0
pMON129245	P-SETit.Omt3-1:1:3 (SEQ ID NO: 69) L-SETit.Omt3-1:1:1 (SEQ ID NO: 143)	0	0
pMON129246	P-SETit.Cys-1:1:2 (SEQ ID NO: 45) L-SETit.Cys-1:1:1 (SEQ ID NO: 127)	2	0
pMON129247	P-SETit.Cys-1:1:3 (SEQ ID NO: 46) L-SETit.Cys-1:1:1 (SEQ ID NO: 127)	3	0
pMON129248	P-SETit.Ucc1-1:1:2 (SEQ ID NO: 105) L-SETit.Ucc1-1:1:1 (SEQ ID NO: 170)	3	1
pMON129249	P-SETit.Tip-1:1:4 (SEQ ID NO: 97) L-SETit.Tip-1:1:1 (SEQ ID NO: 165)	3	1
pMON129250	P-SETit.Prx72-1:1:2 (SEQ ID NO: 85) L-SETit.Prx72-1:1:1 (SEQ ID NO: 158)	3	2



pMON129251	P-SETit.Prx17-1:1:2 (SEQ ID NO: 80) L-SETit.Prx17-1:1:1 (SEQ ID NO: 154)	0	0
pMON129252	P-SETit.Mt1-1:1:2 (SEQ ID NO: 63) L-SETit.Mt1-1:1:1 (SEQ ID NO: 138)	2	0
pMON129253	P-SETit.Ali1-1:1:3 (SEQ ID NO: 31) L-SETit.Ali1-1:1:1 (SEQ ID NO: 112)	2	0
pMON129254	P-SETit.Rcc3-1:1:16 (SEQ ID NO: 91) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	3	1
pMON129255	P-SETit.Pip2-3-1:1:1 (SEQ ID NO: 72) L-SETit.Pip2-3-1:1:1 (SEQ ID NO: 146)	3	0
pMON129256	P-SETit.Tga6-1:1:2 (SEQ ID NO: 95)	0	0
pMON129257	P-SETit.25509-1:1:3 (SEQ ID NO: 23)	0	0
pMON129258	P-SETit.Grf-1:1:2 (SEQ ID NO: 57)	0	0
pMON129259	P-SETit.Omt4_2-1:1:2 (SEQ ID NO: 70) L-SETit.Omt4_2-1:1:1 (SEQ ID NO: 144)	0	0

The highest average level of GUS expression for root tissues transformed by particle bombardment were observed using the constructs, pMON129231 ((P-SETit.OMT2.3-1:1:1 (SEQ ID NO: 68) + L-SETit.OMT2.2-1:1:1 (SEQ ID NO: 141)); pMON129234 ((P-SETit.Srp-1:1:2 (SEQ ID NO: 92) + L-SETit.Srp-1:1:1 (SEQ ID NO: 163)); pMON129237 ((P-SETit.Prx-1:1:1 (SEQ ID NO: 79) + L-SETit.Prx-1:1:1 (SEQ ID NO: 153)); pMON129241 ((P-SETit.Prx3-1:1:4 (SEQ ID NO: 83) + L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)); pMON129242 ((P-SETit.Prx3-1:1:3 (SEQ ID NO: 82) + L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)); pMON129243 ((P-SETit.Prx47-1:1:2 (SEQ ID NO: 84) + L-SETit.Prx47-1:1:1 (SEQ ID NO: 157)); pMON129247 ((P-SETit.Cys-1:1:3 (SEQ ID NO: 46) + L-SETit.Cys-1:1:1 (SEQ ID NO: 127)); pMON129248 ((P-SETit.Ucc1-1:1:2 (SEQ ID NO: 105) + L-SETit.Ucc1-1:1:1 (SEQ ID NO: 170)); pMON129249 ((P-SETit.Tip-1:1:4 (SEQ ID NO: 97) + L-SETit.Tip-1:1:1 (SEQ ID NO: 165)); pMON129250 ((P-SETit.Prx72-1:1:2 (SEQ ID NO: 85) + L-SETit.Prx72-1:1:1 (SEQ ID NO: 158)); pMON129254 ((P-SETit.Rcc3-1:1:16 (SEQ ID NO: 91) + L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)) and pMON129255 ((P-SETit.Pip2-3-1:1:1 (SEQ ID NO: 72) + L-SETit.Pip2-3-1:1:1 (SEQ ID NO: 146)). Leaf expression was seen most highest in tissues bombarded with the contstructs pMON129241 ((P-SETit.Prx3-1:1:4 (SEQ ID NO: 83) + L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)) and pMON129250 ((P-SETit.Prx72-1:1:2 (SEQ ID NO: 85) + L-SETit.Prx72-1:1:1 (SEQ ID NO: 158)).

**Example 4: Analysis of Regulatory Elements driving GUS in Transgenic Corn**

Corn plants were transformed with plant expression vectors containing the test regulatory elements driving expression of the  $\beta$ -glucuronidase (GUS) transgene, and the resulting plants were analyzed for GUS protein expression.

5 Corn plants were transformed with the plant GUS expression constructs, listed in Table 8, below. Regulatory elements presented in Example 1 for monocot expression were cloned into a base plant expression vector using standard methods known in the art. The resulting plant expression vectors contained a right border region from *Agrobacterium tumefaciens*, a first transgene cassette to test the regulatory or chimeric regulatory element

10 comprised of, a regulatory or chimeric regulatory element, operably linked to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS) that possessed a processable intron (GUS-2, SEQ ID NO: 1091), operably linked to the 3' termination region from the rice lipid transfer protein gene (T-Os.LTP-1:1:1, SEQ ID NO: 1089); a second

15 transgene selection cassette used for selection of transformed plant cells that conferred resistance to the herbicide glyphosate (driven by the rice Actin 1 promoter, SEQ ID NO: 1098), and a left border region from *A. tumefaciens*. The resulting plasmids, pMON129227, pMON129228, pMON129229, pMON129230, pMON129231, pMON129232, pMON129233, pMON129234, pMON129235, pMON129236, pMON129237, pMON129238, pMON129239, pMON129240, pMON129241, pMON129242, pMON129243, pMON129244, pMON129245, pMON129246, pMON129247, pMON129249, pMON129250, pMON129251, pMON129252, pMON129253, pMON129254, pMON129255, pMON129256, pMON129257, pMON129258 and pMON129259 were used to transform corn plants.

25 **Table 8. Binary Plant Transformation Vectors, Regulatory or chimeric regulatory elements, GUS and 3' UTRs.**

Construct	Regulatory Elements	3' UTR
pMON129227	P-SETit.Cyp-1-1:1:1 (SEQ ID NO: 43) L-SETit.Cyp-1-1:1:1 (SEQ ID NO: 125)	T-Os.LTP-1:1:1
pMON129228	P-SETit.Cyp78a-1:1:2 (SEQ ID NO: 44) L-SETit.Cyp78a-1:1:1 (SEQ ID NO: 126)	T-Os.LTP-1:1:1
pMON129229	P-SETit.OMT2.1-1:1:2 (SEQ ID NO: 66) L-SETit.OMT2.1-1:1:1 (SEQ ID NO: 140)	T-Os.LTP-1:1:1
pMON129230	P-SETit.OMT2.2-1:1:2 (SEQ ID NO: 67) L-SETit.OMT2.2-1:1:2 (SEQ ID NO: 141)	T-Os.LTP-1:1:1

	142)	
pMON129231	P-SETit.OMT2.3-1:1:1 (SEQ ID NO: 68) L-SETit.OMT2.2-1:1:1 (SEQ ID NO: 141)	T-Os.LTP-1:1:1
pMON129232	P-SETit.Grew2-1:1:1 (SEQ ID NO: 56) L-SETit.Grew2-1:1:1 (SEQ ID NO: 134)	T-Os.LTP-1:1:1
pMON129233	P-SETit.Prx2-1:1:3 (SEQ ID NO: 81) L-SETit.Prx2-1:1:2 (SEQ ID NO: 155)	T-Os.LTP-1:1:1
pMON129234	P-SETit.Srp-1:1:2 (SEQ ID NO: 92) L-SETit.Srp-1:1:1 (SEQ ID NO: 163)	T-Os.LTP-1:1:1
pMON129235	P-SETit.LaDo-1:1:2 (SEQ ID NO: 62) L-SETit.LaDo-1:1:1 (SEQ ID NO: 137)	T-Os.LTP-1:1:1
pMON129236	P-SETit.Aip-1:1:1 (SEQ ID NO: 27) L-SETit.Aip-1:1:1 (SEQ ID NO: 109)	T-Os.LTP-1:1:1
pMON129237	P-SETit.Prx-1:1:1 (SEQ ID NO: 79) L-SETit.Prx-1:1:1 (SEQ ID NO: 153)	T-Os.LTP-1:1:1
pMON129238	P-SETit.Cbl7-1:1:1 (SEQ ID NO: 34) L-SETit.Cbl7-1:1:1 (SEQ ID NO: 115)	T-Os.LTP-1:1:1
pMON129239	P-SETit.Fst-1:1:1 (SEQ ID NO: 54) L-SETit.Fst-1:1:1 (SEQ ID NO: 132)	T-Os.LTP-1:1:1
pMON129240	P-SETit.Cda-1:1:1 (SEQ ID NO: 36) L-SETit.Cda-1:1:1 (SEQ ID NO: 117)	T-Os.LTP-1:1:1
pMON129241	P-SETit.Prx3-1:1:4 (SEQ ID NO: 83) L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)	T-Os.LTP-1:1:1
pMON129242	P-SETit.Prx3-1:1:3 (SEQ ID NO: 82) L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)	T-Os.LTP-1:1:1
pMON129243	P-SETit.Prx47-1:1:2 (SEQ ID NO: 84) L-SETit.Prx47-1:1:1 (SEQ ID NO: 157)	T-Os.LTP-1:1:1
pMON129244	P-SETit.Eie-1:1:1 (SEQ ID NO: 49) L-SETit.Eie-1:1:1 (SEQ ID NO: 129)	T-Os.LTP-1:1:1
pMON129245	P-SETit.Omt3-1:1:3 (SEQ ID NO: 69) L-SETit.Omt3-1:1:1 (SEQ ID NO: 143)	T-Os.LTP-1:1:1
pMON129246	P-SETit.Cys-1:1:2 (SEQ ID NO: 45) L-SETit.Cys-1:1:1 (SEQ ID NO: 127)	T-Os.LTP-1:1:1
pMON129247	P-SETit.Cys-1:1:3 (SEQ ID NO: 46) L-SETit.Cys-1:1:1 (SEQ ID NO: 127)	T-Os.LTP-1:1:1
pMON129249	P-SETit.Tip-1:1:4 (SEQ ID NO: 97) L-SETit.Tip-1:1:1 (SEQ ID NO: 165)	T-Os.LTP-1:1:1
pMON129250	P-SETit.Prx72-1:1:2 (SEQ ID NO: 85) L-SETit.Prx72-1:1:1 (SEQ ID NO: 158)	T-Os.LTP-1:1:1
pMON129251	P-SETit.Prx17-1:1:2 (SEQ ID NO: 80) L-SETit.Prx17-1:1:1 (SEQ ID NO: 154)	T-Os.LTP-1:1:1
pMON129252	P-SETit.Mt1-1:1:2 (SEQ ID NO: 63)	T-Os.LTP-1:1:1

	L-SETit.Mt1-1:1:1 (SEQ ID NO: 138)	
pMON129253	P-SETit.Ali1-1:1:3 (SEQ ID NO: 31) L-SETit.Ali1-1:1:1 (SEQ ID NO: 112)	T-Os.LTP-1:1:1
pMON129254	P-SETit.Rcc3-1:1:16 (SEQ ID NO: 91) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	T-Os.LTP-1:1:1
pMON129255	P-SETit.Pip2-3-1:1:1 (SEQ ID NO: 72) L-SETit.Pip2-3-1:1:1 (SEQ ID NO: 146)	T-Os.LTP-1:1:1
pMON129256	P-SETit.Tga6-1:1:2 (SEQ ID NO: 95)	T-Os.LTP-1:1:1
pMON129257	P-SETit.25509-1:1:3 (SEQ ID NO: 23)	T-Os.LTP-1:1:1
pMON129258	P-SETit.Grf-1:1:2 (SEQ ID NO: 57)	T-Os.LTP-1:1:1
pMON129259	P-SETit.Omt4_2-1:1:2 (SEQ ID NO: 70) L-SETit.Omt4_2-1:1:1 (SEQ ID NO: 144)	T-Os.LTP-1:1:1

The plant transformation vector, pMON129227 is comprised of the promoter element, P-SETit.Cyp-1-1:1:1 (SEQ ID NO: 43), operably linked 5' to the leader element, L-SETit.Cyp-1-1:1:1 (SEQ ID NO: 125). The plant transformation vector, pMON129228 is comprised of the promoter element, P-SETit.Cyp78a-1:1:2 (SEQ ID NO: 44), operably linked 5' to the leader element, L-SETit.Cyp78a-1:1:1 (SEQ ID NO: 126). The plant transformation vector, pMON129229 is comprised of the promoter element, P-SETit.OMT2.1-1:1:2 (SEQ ID NO: 66), operably linked 5' to the leader element, L-SETit.OMT2.1-1:1:1 (SEQ ID NO: 140). The plant transformation vector, pMON129230 is comprised of the promoter element, P-SETit.OMT2.2-1:1:2 (SEQ ID NO: 67), operably linked 5' to the leader element, L-SETit.OMT2.2-1:1:2 (SEQ ID NO: 142). The plant transformation vector, pMON129231 is comprised of the promoter element, P-SETit.OMT2.3-1:1:1 (SEQ ID NO: 68), operably linked 5' to the leader element, L-SETit.OMT2.2-1:1:1 (SEQ ID NO: 141). The plant transformation vector, pMON129232 is comprised of the promoter element, P-SETit.Grcw2-1:1:1 (SEQ ID NO: 56), operably linked 5' to the leader element, L-SETit.Grcw2-1:1:1 (SEQ ID NO: 134). The plant transformation vector, pMON129233 is comprised of the promoter element, P-SETit.Prx2-1:1:3 (SEQ ID NO: 81), operably linked 5' to the leader element, L-SETit.Prx2-1:1:2 (SEQ ID NO: 155). The plant transformation vector, pMON129234 is comprised of the promoter element, P-SETit.Srp-1:1:2 (SEQ ID NO: 92), operably linked 5' to the leader element, L-SETit.Srp-1:1:1 (SEQ ID NO: 163). The plant transformation vector, pMON129235 is comprised of the promoter element, P-SETit.LaDo-1:1:2 (SEQ ID NO: 62), operably linked 5' to the leader element, L-SETit.LaDo-1:1:1 (SEQ ID NO: 137). The plant transformation vector, pMON129236 is comprised of the promoter element, P-SETit.Aip-1:1:1 (SEQ ID NO: 27), operably linked 5' to the leader element, L-SETit.Aip-1:1:1 (SEQ ID NO: 109). The plant transformation vector, pMON129237 is comprised of the

promoter element, P-SETit.Prx-1:1:1 (SEQ ID NO: 79), operably linked 5' to the leader element, L-SETit.Prx-1:1:1 (SEQ ID NO: 153). The plant transformation vector, pMON129238 is comprised of the promoter element, P-SETit.Cbl7-1:1:1 (SEQ ID NO: 34), operably linked 5' to the leader element, L-SETit.Cbl7-1:1:1 (SEQ ID NO: 115). The plant transformation vector, pMON129239 is comprised of the promoter element, P-SETit.Fst-1:1:1 (SEQ ID NO: 54), operably linked 5' to the leader element, L-SETit.Fst-1:1:1 (SEQ ID NO: 132). The plant transformation vector, pMON129240 is comprised of the promoter element, P-SETit.Cda-1:1:1 (SEQ ID NO: 36), operably linked 5' to the leader element, L-SETit.Cda-1:1:1 (SEQ ID NO: 117). The plant transformation vector, pMON129241 is comprised of the promoter element, P-SETit.Prx3-1:1:4 (SEQ ID NO: 83), operably linked 5' to the leader element, L-SETit.Prx3-1:1:1 (SEQ ID NO: 156). The plant transformation vector, pMON129242 is comprised of the promoter element, P-SETit.Prx3-1:1:3 (SEQ ID NO: 82), operably linked 5' to the leader element, L-SETit.Prx3-1:1:1 (SEQ ID NO: 156). The plant transformation vector, pMON129243 is comprised of the promoter element, P-SETit.Prx47-1:1:2 (SEQ ID NO: 84), operably linked 5' to the leader element, L-SETit.Prx47-1:1:1 (SEQ ID NO: 157). The plant transformation vector, pMON129244 is comprised of the promoter element, P-SETit.Eie-1:1:1 (SEQ ID NO: 49), operably linked 5' to the leader element, L-SETit.Eie-1:1:1 (SEQ ID NO: 129). The plant transformation vector, pMON129245 is comprised of the promoter element, P-SETit.Omt3-1:1:3 (SEQ ID NO: 69), operably linked 5' to the leader element, L-SETit.Omt3-1:1:1 (SEQ ID NO: 143). The plant transformation vector, pMON129246 is comprised of the promoter element, P-SETit.Cys-1:1:2 (SEQ ID NO: 45), operably linked 5' to the leader element, L-SETit.Cys-1:1:1 (SEQ ID NO: 127). The plant transformation vector, pMON129247 is comprised of the promoter element, P-SETit.Cys-1:1:3 (SEQ ID NO: 46), operably linked 5' to the leader element, L-SETit.Cys-1:1:1 (SEQ ID NO: 127). The plant transformation vector, pMON129249 is comprised of the promoter element, P-SETit.Tip-1:1:4 (SEQ ID NO: 97), operably linked 5' to the leader element, L-SETit.Tip-1:1:1 (SEQ ID NO: 165). The plant transformation vector, pMON129250 is comprised of the promoter element, P-SETit.Prx72-1:1:2 (SEQ ID NO: 85), operably linked 5' to the leader element, L-SETit.Prx72-1:1:1 (SEQ ID NO: 158). The plant transformation vector, pMON129251 is comprised of the promoter element, P-SETit.Prx17-1:1:2 (SEQ ID NO: 80), operably linked 5' to the leader element, L-SETit.Prx17-1:1:1 (SEQ ID NO: 154). The plant transformation vector, pMON129252 is comprised of the promoter element, P-SETit.Mt1-1:1:2 (SEQ ID NO: 63), operably linked 5' to the leader element, L-SETit.Mt1-1:1:1 (SEQ ID NO: 138). The plant transformation

vector, pMON129253 is comprised of the promoter element, P-SETit.Ali1-1:1:3 (SEQ ID NO: 31), operably linked 5' to the leader element, L-SETit.Ali1-1:1:1 (SEQ ID NO: 112). The plant transformation vector, pMON129254 is comprised of the promoter element, P-SETit.Rcc3-1:1:16 (SEQ ID NO: 91), operably linked 5' to the leader element, L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162). The plant transformation vector, pMON129255 is comprised of the promoter element, P-SETit.Pip2-3-1:1:1 (SEQ ID NO: 72), operably linked 5' to the leader element, L-SETit.Pip2-3-1:1:1 (SEQ ID NO: 146). The plant transformation vector, pMON129256 is comprised of the promoter element, P-SETit.Tga6-1:1:2 (SEQ ID NO: 95). The plant transformation vector, pMON129257 is comprised of the promoter element, P-SETit.25509-1:1:3 (SEQ ID NO: 23). The plant transformation vector, pMON129258 is comprised of the promoter element, P-SETit.Grf-1:1:2 (SEQ ID NO: 57). The plant transformation vector, pMON129259 is comprised of the promoter element, P-SETit.Omt4\_2-1:1:2 (SEQ ID NO: 70), operably linked 5' to the leader element, L-SETit.Omt4\_2-1:1:1 (SEQ ID NO: 144).

Corn plants were transformed with plant GUS expression constructs, pMON129227, pMON129228, pMON129229, pMON129230, pMON129231, pMON129232, pMON129233, pMON129234, pMON129235, pMON129236, pMON129237, pMON129238, pMON129239, pMON129240, pMON129241, pMON129242, pMON129243, pMON129244, pMON129245, pMON129246, pMON129247, pMON129249, pMON129250, pMON129251, pMON129252, pMON129253, pMON129254, pMON129255, pMON129256, pMON129257, pMON129258 and pMON129259.

Plants were transformed using *Agrobacterium*-mediated transformations and LH244 corn seed embryos as outlined in Example 2. Leaf and root tissue were harvested from 1 to 5 transformants and assayed for GUS expression. Histochemical GUS analysis was used for qualitative expression analysis of transformed plants. Whole tissue sections were incubated with GUS staining solution X-Gluc (5-bromo-4-chloro-3-indolyl-b-glucuronide) (1 milligram/milliliter) for an appropriate length of time, rinsed, and visually inspected for blue coloration. GUS activity was qualitatively determined by direct visual inspection or inspection under a microscope using selected plant organs and tissues. The R0 plants were inspected for expression in the roots and leaves.

For quantitative analysis, total protein was extracted from selected tissues of transformed corn plants. One microgram of total protein was used with the fluorogenic

substrate 4-methyleumbelliferyl- $\beta$ -D-glucuronide (MUG) in a total reaction volume of 50 microliters. The reaction product, 4-methyumbelliferone (4-MU), is maximally fluorescent at high pH, where the hydroxyl group is ionized. Addition of a basic solution of sodium carbonate simultaneously stops the assay and adjusts the pH for quantifying the fluorescent product. Fluorescence was measured with excitation at 365 nm, emission at 445 nm using a

The average R<sub>0</sub> GUS expression for transgenic plants transformed with the constructs described in Table 8 are shown in Table 9 below.

**Table 9. Average R<sub>0</sub> GUS V3 Leaf and Root expression in transgenic corn plants, transformed with listed constructs.**

Construct	Regulatory Elements	V3 Root	V3 Leaf
pMON129227	P-SETit.Cyp-1-1:1:1 (SEQ ID NO: 43) L-SETit.Cyp-1-1:1:1 (SEQ ID NO: 125)	0.00	0.00
pMON129228	P-SETit.Cyp78a-1-1:1:2 (SEQ ID NO: 44) L-SETit.Cyp78a-1-1:1:1 (SEQ ID NO: 126)	7.91	0.00
pMON129229	P-SETit.OMT2.1-1-1:1:2 (SEQ ID NO: 66) L-SETit.OMT2.1-1-1:1:1 (SEQ ID NO: 140)	0.00	0.00
pMON129230	P-SETit.OMT2.2-1-1:1:2 (SEQ ID NO: 67) L-SETit.OMT2.2-1-1:1:2 (SEQ ID NO: 142)	0.00	0.00
pMON129231	P-SETit.OMT2.3-1-1:1:1 (SEQ ID NO: 68) L-SETit.OMT2.2-1-1:1:1 (SEQ ID NO: 141)	0.00	0.00
pMON129232	P-SETit.Grew2-1-1:1:1 (SEQ ID NO: 56) L-SETit.Grew2-1-1:1:1 (SEQ ID NO: 134)	0.00	0.00
pMON129233	P-SETit.Prx2-1-1:1:3 (SEQ ID NO: 81) L-SETit.Prx2-1-1:1:2 (SEQ ID NO: 155)	5.96	nd
pMON129234	P-SETit.Srp-1-1:1:2 (SEQ ID NO: 92) L-SETit.Srp-1-1:1:1 (SEQ ID NO: 163)	0.00	0.00
pMON129235	P-SETit.LaDo-1-1:1:2 (SEQ ID NO: 62) L-SETit.LaDo-1-1:1:1 (SEQ ID NO: 137)	0.00	0.00
pMON129236	P-SETit.Aip-1-1:1:1 (SEQ ID NO: 27) L-SETit.Aip-1-1:1:1 (SEQ ID NO: 109)	0.00	0.00
pMON129237	P-SETit.Prx-1-1:1:1 (SEQ ID NO: 79) L-SETit.Prx-1-1:1:1 (SEQ ID NO: 153)	10.66	0.00
pMON129238	P-SETit.Cbl7-1-1:1:1 (SEQ ID NO: 34) L-SETit.Cbl7-1-1:1:1 (SEQ ID NO: 115)	0.00	0.00
pMON129239	P-SETit.Fst-1-1:1:1 (SEQ ID NO: 54) L-SETit.Fst-1-1:1:1 (SEQ ID NO: 132)	2.86	0.00

pMON129240	P-SETit.Cda-1:1:1 (SEQ ID NO: 36) L-SETit.Cda-1:1:1 (SEQ ID NO: 117)	0.00	0.00
pMON129241	P-SETit.Prx3-1:1:4 (SEQ ID NO: 83) L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)	7.49	0.00
pMON129242	P-SETit.Prx3-1:1:3 (SEQ ID NO: 82) L-SETit.Prx3-1:1:1 (SEQ ID NO: 156)	1.90	0.00
pMON129243	P-SETit.Prx47-1:1:2 (SEQ ID NO: 84) L-SETit.Prx47-1:1:1 (SEQ ID NO: 157)	9.26	0.00
pMON129244	P-SETit.Eie-1:1:1 (SEQ ID NO: 49) L-SETit.Eie-1:1:1 (SEQ ID NO: 129)	2.35	0.00
pMON129245	P-SETit.Omt3-1:1:3 (SEQ ID NO: 69) L-SETit.Omt3-1:1:1 (SEQ ID NO: 143)	0.00	0.00
pMON129246	P-SETit.Cys-1:1:2 (SEQ ID NO: 45) L-SETit.Cys-1:1:1 (SEQ ID NO: 127)	0.00	0.00
pMON129247	P-SETit.Cys-1:1:3 (SEQ ID NO: 46) L-SETit.Cys-1:1:1 (SEQ ID NO: 127)	0.00	0.00
pMON129249	P-SETit.Tip-1:1:4 (SEQ ID NO: 97) L-SETit.Tip-1:1:1 (SEQ ID NO: 165)	34.79	0.00
pMON129250	P-SETit.Prx72-1:1:2 (SEQ ID NO: 85) L-SETit.Prx72-1:1:1 (SEQ ID NO: 158)	0.00	0.00
pMON129251	P-SETit.Prx17-1:1:2 (SEQ ID NO: 80) L-SETit.Prx17-1:1:1 (SEQ ID NO: 154)	0.00	0.00
pMON129252	P-SETit.Mt1-1:1:2 (SEQ ID NO: 63) L-SETit.Mt1-1:1:1 (SEQ ID NO: 138)	0.00	24.92
pMON129253	P-SETit.Ali1-1:1:3 (SEQ ID NO: 31) L-SETit.Ali1-1:1:1 (SEQ ID NO: 112)	0.00	11.58
pMON129254	P-SETit.Rcc3-1:1:16 (SEQ ID NO: 91) L-SETit.Rcc3-1:1:2 (SEQ ID NO: 162)	9.39	0.00
pMON129255	P-SETit.Pip2-3-1:1:1 (SEQ ID NO: 72) L-SETit.Pip2-3-1:1:1 (SEQ ID NO: 146)	79.81	0.00
pMON129256	P-SETit.Tga6-1:1:2 (SEQ ID NO: 95)	6.91	0.00
pMON129257	P-SETit.25509-1:1:3 (SEQ ID NO: 23)	0.00	0.00
pMON129258	P-SETit.Grf-1:1:2 (SEQ ID NO: 57)	0.00	0.00
pMON129259	P-SETit.Omt4_2-1:1:2 (SEQ ID NO: 70) L-SETit.Omt4_2-1:1:1 (SEQ ID NO: 144)	0.00	0.00

The highest average levels of GUS expression in the roots of V3 stage plants was observed in plants transformed with the constructs pMON129255 ((P-SETit.Pip2-3-1:1:1 (SEQ ID NO: 72) + L-SETit.Pip2-3-1:1:1 (SEQ ID NO: 146)) and pMON129249 ((P-SETit.Tip-1:1:4 (SEQ ID NO: 97) + L-SETit.Tip-1:1:1 (SEQ ID NO: 165)).



### **Example 5: Analysis of Actin and Tubulin Regulatory Elements driving GUS in Corn Protoplasts**

5 Corn leaf protoplasts are transformed with plant expression vectors containing a test transcriptional regulatory element or transcriptional regulatory expression element group, driving expression of the  $\beta$ -glucuronidase (GUS) transgene, and compared to leaf protoplast in which expression of GUS is driven by known constitutive promoters.

10 Corn protoplast cells, derived from leaf tissue are transformed using methods known in the art with plant expression vectors to compare expression of a transgene driven by the transcriptional regulatory expression element groups, EXP-SETit.TubA3:1:3 (SEQ ID NO: 19), EXP-SETit.TubA2:1:3 (SEQ ID NO: 16), EXP-SETit.TubA2-2:1:1 (SEQ ID NO: 18), EXP-SETit.Act8:1:1 (SEQ ID NO: 9), EXP-SETit.Act8:1:2 (SEQ ID NO: 10), EXP-SETit.Act8:c (SEQ ID NO: 11), EXP-SETit.TubA2-1:1:2 (SEQ ID NO: 17) and EXP-SETit.TubA2:1:1 (SEQ ID NO: 15) with that of known constitutive promoters. Each  
15 transcriptional regulatory expression element group is cloned using methods known in the art into a plant expression vector shown in Table 10 below. The resulting plant expression vectors are comprised of a transgene cassette comprised of a transcriptional regulatory expression element group, operably linked 5' to a coding sequence for  $\beta$ -glucuronidase (GUS) (GUS-1 or GUS-3, represented by SEQ ID NOS: 1090 and 1092, respectively), which  
20 is operably linked 5' to a 3' termination region derived from the *A. tumefaciens* Nopaline synthase gene (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088) or the wheat Hsp17 gene (T-Ta.Hsp17-1:1:1, SEQ ID NO: 1108).

Control plasmids used for comparison are constructed as described above and are comprised of a known constitutive, transcriptional regulatory expression element groups.  
25 The control plasmid vector, pMON19469 is comprised of the transcriptional regulatory element group, EXP-CaMV.35S-enh/I-Zm.DnaK-1:1:1 (SEQ ID NO: 1104). The control plasmid vector, pMON65328 is comprised of the transcriptional regulatory element group, EXP-CaMV.35S-enh-Lhcb1/I-Os.Act1-1:1:9 (SEQ ID NO: 1105). The control plasmid vector, pMON25455 is comprised of the transcriptional regulatory element group, EXP-  
30 Os.Act1:1:1 (SEQ ID NO: 1098). The control plasmid vector, pMON122605 is comprised of the transcriptional regulatory element group, EXP-Os.TubA-3:1:1 (SEQ ID NO: 1107). Each control vector transcriptional regulatory element group is operably linked 5' to a coding sequence for  $\beta$ -glucuronidase (GUS) (GUS-1 or GUS-3, represented by SEQ ID NOS: 1090

and 1092, respectively), which is operably linked 5' to a 3' termination region derived from the *A. tumefaciens* Nopaline synthase gene (T-AGR<sub>tu.nos-1:1:13</sub>, SEQ ID NO: 1088) or the wheat Hsp17 gene (T-Ta.Hsp17-1:1:1, SEQ ID NO: 1108). In addition, three controls are provided as controls for background GUS and luciferase expression, a no DNA control, an empty vector which is not designed for transgene expression and an expression vector used to express green fluorescent protein (GFP).

**Table 10. GUS plant expression vectors and corresponding transcriptional regulatory expression element groups and constituent promoters, leaders and introns, and 3' UTR used for transformation of corn leaf protoplasts.**

Construct	Regulatory Elements	3' UTR
pMON136270	EXP-SETit.TubA3:1:3 (SEQ ID NO: 19) P-SETit.TubA3-1:1:3 (SEQ ID NO: 101) L-SETit.TubA3-1:1:1 (SEQ ID NO: 168)	T-AGR <sub>tu.nos-1:1:13</sub>
pMON136272	EXP-SETit.TubA2:1:3 (SEQ ID NO: 16) P-SETit.TubA2-1-1:1:3 (SEQ ID NO: 99) L-SETit.TubA2-1-1:1:1 (SEQ ID NO: 166) I-SETit.TubA2_1-1:1:2 (SEQ ID NO: 176)	T-AGR <sub>tu.nos-1:1:13</sub>
pMON136275	EXP-SETit.TubA2-2:1:1 (SEQ ID NO: 18) P-SETit.TubA2-2-1:1:3 (SEQ ID NO: 100) L-SETit.TubA2-2-1:1:1 (SEQ ID NO: 167)	T-AGR <sub>tu.nos-1:1:13</sub>
pMON136276	EXP-SETit.Act8:1:1 (SEQ ID NO: 9) P-SETit.Act8-1:1:5 (SEQ ID NO: 24) L-SETit.Act8-1:1:2 (SEQ ID NO: 106) I-SETit.Act8-1:1:2 (SEQ ID NO: 172) L-SETit.Act8-1:1:3 (SEQ ID NO: 107)	T-AGR <sub>tu.nos-1:1:13</sub>
pMON136277	EXP-SETit.Act8:1:2 (SEQ ID NO: 10) P-SETit.Act8-1:1:6 (SEQ ID NO: 25) L-SETit.Act8-1:1:2 (SEQ ID NO: 106) I-SETit.Act8-1:1:2 (SEQ ID NO: 172) L-SETit.Act8-1:1:3 (SEQ ID NO: 107)	T-AGR <sub>tu.nos-1:1:13</sub>
pMON136278	EXP-SETit.Act8:c (SEQ ID NO: 11) P-SETit.Act8-1-1:1:2 (SEQ ID NO: 26) L-SETit.Act8-1:1:4 (SEQ ID NO: 108) I-SETit.Act8-1:1:2 (SEQ ID NO: 172) L-SETit.Act8-1:1:3 (SEQ ID NO: 107)	T-AGR <sub>tu.nos-1:1:13</sub>
pMON136279	EXP-SETit.TubA2-1:1:2 (SEQ ID NO: 17) P-SETit.TubA2-1-1:1:2 (SEQ ID NO: 98) L-SETit.TubA2-1-1:1:1 (SEQ ID NO: 166)	T-AGR <sub>tu.nos-1:1:13</sub>
pMON136280	EXP-SETit.TubA2:1:1 (SEQ ID NO: 15) P-SETit.TubA2-1-1:1:2 (SEQ ID NO: 98)	T-AGR <sub>tu.nos-1:1:13</sub>

L-SETit.TubA2-1-1:1:1 (SEQ ID NO: 166)
I-SETit.TubA2_1-1:1:2 (SEQ ID NO: 176)

The plant transformation vector, pMON136270 is comprised of the transcriptional regulatory element group, EXP-SETit.TubA3:1:3 (SEQ ID NO: 19), which is further comprised of the promoter element, P-SETit.TubA3-1:1:3 (SEQ ID NO: 101), operably linked 5' to the leader element, L-SETit.TubA3-1:1:1 (SEQ ID NO: 168). The plant transformation vector, pMON136272 is comprised of the transcriptional regulatory element group, EXP-SETit.TubA2:1:3 (SEQ ID NO: 16), which is further comprised of the promoter element, P-SETit.TubA2-1-1:1:3 (SEQ ID NO: 99), operably linked 5' to the leader element, L-SETit.TubA2-1-1:1:1 (SEQ ID NO: 166), operably linked 5' to the intron element, I-SETit.TubA2\_1-1:1:2 (SEQ ID NO: 176). The plant transformation vector, pMON136275 is comprised of the transcriptional regulatory element group, EXP-SETit.TubA2-2:1:1 (SEQ ID NO: 18), which is further comprised of the promoter element, P-SETit.TubA2-2-1:1:3 (SEQ ID NO: 100), operably linked 5' to the leader element, L-SETit.TubA2-2-1:1:1 (SEQ ID NO: 167). The plant transformation vector, pMON136276 is comprised of the transcriptional regulatory element group, EXP-SETit.Act8:1:1 (SEQ ID NO: 9), which is further comprised of the promoter element, P-SETit.Act8-1:1:5 (SEQ ID NO: 24), operably linked 5' to the leader element, L-SETit.Act8-1:1:2 (SEQ ID NO: 106), operably linked 5' to the intron element, I-SETit.Act8-1:1:2 (SEQ ID NO: 172), operably linked 5' to the leader element, L-SETit.Act8-1:1:3 (SEQ ID NO: 107). The plant transformation vector, pMON136277 is comprised of the transcriptional regulatory element group, EXP-SETit.Act8:1:2 (SEQ ID NO: 10), which is further comprised of the promoter element, P-SETit.Act8-1:1:6 (SEQ ID NO: 25), operably linked 5' to the leader element, L-SETit.Act8-1:1:2 (SEQ ID NO: 106), operably linked 5' to the intron element, I-SETit.Act8-1:1:2 (SEQ ID NO: 172), operably linked 5' to the leader element, L-SETit.Act8-1:1:3 (SEQ ID NO: 107). The plant transformation vector, pMON136278 is comprised of the transcriptional regulatory element group, EXP-SETit.Act8:c (SEQ ID NO: 11), which is further comprised of the promoter element, P-SETit.Act8-1-1:1:2 (SEQ ID NO: 26), operably linked 5' to the leader element, L-SETit.Act8-1:1:4 (SEQ ID NO: 108), operably linked 5' to the intron element, I-SETit.Act8-1:1:2 (SEQ ID NO: 172), operably linked 5' to the leader element, L-SETit.Act8-1:1:3 (SEQ ID NO: 107). The plant transformation vector, pMON136279 is comprised of the transcriptional regulatory element group, EXP-SETit.TubA2-1:1:2 (SEQ ID NO: 17), which is further comprised of the promoter element, P-SETit.TubA2-1-1:1:2 (SEQ ID NO: 98),

operably linked 5' to the leader element, L-SETit.TubA2-1-1:1:1 (SEQ ID NO: 166). The plant transformation vector, pMON136280 is comprised of the transcriptional regulatory element group, EXP-SETit.TubA2:1:1 (SEQ ID NO: 15), which is further comprised of the promoter element, P-SETit.TubA2-1-1:1:2 (SEQ ID NO: 98), operably linked 5' to the leader  
5 element, L-SETit.TubA2-1-1:1:1 (SEQ ID NO: 166), operably linked 5' to the intron element, I-SETit.TubA2\_1-1:1:2 (SEQ ID NO: 176).

Two plasmids, for use in co-transformation and normalization of data, are also constructed using methods known in the art. Each plasmid contains a specific luciferase coding sequence which is driven by a constitutive transcriptional regulatory expression  
10 element group. The plant vector, pMON19437 is comprised of a transgene cassette comprised of a constitutive promoter (EXP-CaMV.35S-enh, SEQ ID NO: 1095), operably linked 5' to an intron, (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably linked 5' to a firefly (*Photinus pyralis*) luciferase coding sequence (LUCIFERASE:1:3, SEQ ID NO: 1109), operably linked 5' to a 3' termination region from the *Agrobacterium tumefaciens* nopaline  
15 synthase gene (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). The plant vector, pMON63934 is comprised of a transgene cassette comprised of a constitutive transcriptional regulatory expression element group, (EXP-CaMV.35S-enh-Lhcb1, SEQ ID NO: 1106), operably linked 5' to a sea pansy (*Renilla reniformis*) luciferase coding sequence (CR-Ren.hRenilla Lucife-0:0:1, SEQ ID NO: 1110), operably linked 5' to a 3' termination region from the  
20 *Agrobacterium tumefaciens* nopaline synthase gene (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088).

Corn leaf protoplasts are transformed using a PEG-based transformation method, similar to those known in the art. Protoplast cells are transformed with a DNA prep comprised of equimolar quantities of the two luciferase expression plasmids, pMON19437  
25 and pMON63934 and one of the test plasmids and incubated overnight in total darkness. After incubation, the cells are rinsed, resuspended and lysed. Measurements of both GUS and luciferase are conducted using aliquots of each lysis preparation. Essentially, the collected, transformed protoplast cells are lysed in 5X passive lysis buffer (Promega). After allowing for lysis, aliquots of the lysed preparation are placed into two different small-well  
30 trays. One tray is used for GUS measurements. For quantitative analysis of GUS expression, total protein is extracted from lysis preparation. One microgram of total protein is used with the fluorogenic substrate 4-methyleumbelliferyl- $\beta$ -D-glucuronide (MUG) in a total reaction volume of 50 microliters. The reaction product, 4-methlyumbelliferone (4-MU), is

maximally fluorescent at high pH, where the hydroxyl group is ionized. Addition of a basic solution of sodium carbonate simultaneously stops the assay and adjusts the pH for quantifying the fluorescent product. Fluorescence is measured with excitation at 365 nm, emission at 445 nm using a Fluoromax-3 with Micromax Reader, with slit width set at  
5 excitation 2 nm and emission 3nm. GUS values are expressed as pmol of 4-MU protein per minute per milligram protein ( $\text{pmol 4-MU min}^{-1} \text{mg}^{-1} \text{protein}$ ).

The second tray is used to perform a dual luciferase assay using the dual luciferase reporter assay system (Promega, Madison, WI). All luciferase detection reagents are prepared as described by the manufacturer and assays conducted following the  
10 manufacturer's protocol (See for example, Promega Notes Magazine, No: 57, 1996, p.02). Firefly luciferase reagent (LARII) is added to each sample and assay of the firefly luciferase activity recorded. Upon completion of the firefly luciferase assay, the firefly luminescence is quenched and luminescence of the *Renilla reniformis* luciferase simultaneously activated by adding Stop & Glo<sup>tm</sup> reagent to the sample. Measurement of the *Renilla reniformis* luciferase  
15 activity is recorded following activation of the *Renilla* luciferase. One or two transformations for each transcriptional regulatory expression element group are performed and the mean expression values for each transcriptional regulatory expression element group determined from several samples from each transformation experiment. Sample measurements are made using four replicates of each test transcriptional regulatory expression element group  
20 construct transformation, or alternatively, three replicates of each test transcriptional regulatory expression element group construct per one of two transformation experiments. The mean GUS and luciferase expression levels are provided in Tables 11 and 12. The firefly luciferase values are provided in the column labeled "Fluc" and the *Renilla* luciferase values are provided as in the column labeled "Rluc".

25 To compare the relative activity of each transcriptional regulatory expression element group, GUS values are expressed as a ratio of the mean GUS expression to the mean luciferase activity and normalized with respect to the expression levels observed for the transcriptional regulatory expression element groups EXP-Os.Act1:1:1 (SEQ ID NO: 1098) and EXP-Os.TubA-3:1:1 (SEQ ID NO: 1107). Table 11 below shows the mean GUS/Rluc  
30 ratios normalized with respect to EXP-Os.Act1:1: and EXP-Os.TubA-3:1:1 expression in corn protoplasts.

**Table 11. Mean GUS/RLuc fold expression relative to EXP-Os.TubA-3:1:1 expression in corn leaf protoplast cells.**

<b>Construct</b>	<b>Transcriptional Regulatory Element Group</b>	<b>Mean Gus/RLuc</b>	<b>Mean Gus/RLuc Normalized with respect to EXP-Os.TubA-3:1:1</b>	<b>Mean Gus/RLuc Normalized with respect to EXP-Os.Act1:1:1</b>
pMON19469	EXP-CaMV.35S-enh/I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102)	2.640	2.718	1.054
pMON65328	EXP-CaMV.35S-enh-Lhcb1/I-Os.Act1-1:1:9 (SEQ ID NO: 1105)	3.213	3.307	1.283
pMON25455	EXP-Os.Act1:1:1 (SEQ ID NO: 1098)	2.504	2.578	1.000
pMON122605	EXP-Os.TubA-3:1:1 (SEQ ID NO: 1107)	0.971	1.000	0.388
pMON136270	EXP-SETit.TubA3:1:3 (SEQ ID NO: 19)	1.909	1.965	0.762
pMON136272	EXP-SETit.TubA2:1:3 (SEQ ID NO: 16)	0.144	0.148	0.057
pMON136275	EXP-SETit.TubA2-2:1:1 (SEQ ID NO: 18)	0.001	0.001	0.000
pMON136276	EXP-SETit.Act8:1:1 (SEQ ID NO: 9)	0.353	0.364	0.141
pMON136277	EXP-SETit.Act8:1:2 (SEQ ID NO: 10)	0.271	0.279	0.108
pMON136278	EXP-SETit.Act8:c (SEQ ID NO: 11)	0.005	0.005	0.002
pMON136279	EXP-SETit.TubA2-1:1:2 (SEQ ID NO: 17)	0.003	0.004	0.001
pMON136280	EXP-SETit.TubA2:1:1 (SEQ ID NO: 15)	0.155	0.159	0.062

**Table 12. Mean GUS/Fluc fold expression relative to EXP-Os.TubA-3:1:1 expression in corn leaf protoplast cells.**

<b>Construct</b>	<b>Transcriptional Regulatory Element Group</b>	<b>Mean Gus/FLuc</b>	<b>Gus/Fluc Normlized with respect to EXP-Os.TubA-3:1:1</b>	<b>Gus/Fluc Normlized with respect to EXP-Os.Act1:1:1</b>
pMON19469	EXP-CaMV.35S-	21.580	18.072	2.521

5

	enh/I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102)			
pMON65328	EXP-CaMV.35S-enh-Lhcb1/I-Os.Act1-1:1:9 (SEQ ID NO: 1105)	29.566	24.759	3.454
pMON25455	EXP-Os.Act1:1:1 (SEQ ID NO: 1098)	8.559	7.167	1.000
pMON122605	EXP-Os.TubA-3:1:1 (SEQ ID NO: 1107)	1.194	1.000	0.140
pMON136270	EXP-SETit.TubA3:1:3 (SEQ ID NO: 19)	2.222	1.861	0.260
pMON136272	EXP-SETit.TubA2:1:3 (SEQ ID NO: 16)	0.302	0.253	0.035
pMON136275	EXP-SETit.TubA2-2:1:1 (SEQ ID NO: 18)	0.002	0.002	0.000
pMON136276	EXP-SETit.Act8:1:1 (SEQ ID NO: 9)	0.678	0.568	0.079
pMON136277	EXP-SETit.Act8:1:2 (SEQ ID NO: 10)	0.550	0.460	0.064
pMON136278	EXP-SETit.Act8:c (SEQ ID NO: 11)	0.009	0.008	0.001
pMON136279	EXP-SETit.TubA2-1:1:2 (SEQ ID NO: 17)	0.004	0.004	0.001
pMON136280	EXP-SETit.TubA2:1:1 (SEQ ID NO: 15)	0.378	0.317	0.044

The normalized GUS/Rluc and GUS/Fluc ratios provided in Tables 11 and 12 provide evidence that most of the expression elements are capable of driving GUS expression in corn leaf protoplasts. The constructs, pMON136275 (EXP-SETit.TubA2-2:1:1 (SEQ ID NO: 18)) and pMON136279 (EXP-SETit.TubA2-1:1:2 (SEQ ID NO: 17)) demonstrated the least amount of expression. The construct, pMON136270 (EXP-SETit.TubA3:1:3 (SEQ ID NO: 19)) provided the highest level of expression amongst the test elements relative to the constitutive controls.

### Example 6: Analysis of Actin and Tubulin Regulatory Elements driving GUS in Wheat Protoplasts

Wheat leaf protoplasts were transformed with plant expression vectors containing a test transcriptional regulatory expression element group, driving expression of the  $\beta$ -glucuronidase (GUS) transgene, and compared to leaf protoplast in which expression of GUS is driven by known constitutive promoters.

Wheat protoplast cells, derived from leaf tissue were transformed using PEG based transformation methods known in the art with plant expression vectors to compare expression of a transgene driven by the transcriptional regulatory expression element groups, EXP-SETit.TubA3:1:3 (SEQ ID NO: 19), EXP-SETit.TubA2:1:3 (SEQ ID NO: 16), EXP-SETit.TubA2-2:1:1 (SEQ ID NO: 18), EXP-SETit.Act8:1:1 (SEQ ID NO: 9), EXP-SETit.Act8:1:2 (SEQ ID NO: 10), EXP-SETit.Act8:c (SEQ ID NO: 11), EXP-SETit.TubA2-1:1:2 (SEQ ID NO: 17) and EXP-SETit.TubA2:1:1 (SEQ ID NO: 15) with that of known constitutive promoters. Each transcriptional regulatory expression element group is cloned using methods known in the art into a plant expression vector shown in Table 10 in example 5 above. The resulting plant expression vectors are comprised of a transgene cassette comprised of a transcriptional regulatory expression element group, operably linked 5' to a coding sequence for  $\beta$ -glucuronidase (GUS) (GUS-1 or GUS-3, represented by SEQ ID NOS: 1090 and 1092, respectively), which is operably linked 5' to a 3' termination region derived from the *A. tumefaciens* Nopaline synthase gene (T-AGRtu.nos-1:1:13, SEQ ID NO: 56) or the wheat Hsp17 gene (T-Ta.Hsp17-1:1:1, SEQ ID NO: 57).

Control plasmid vector constructs and luciferase transformation control plasmid constructs were the same as those described in example 5. Measurements of both GUS and luciferase activity were conducted as described in example 5.

To compare the relative activity of each transcriptional regulatory expression element group, GUS values were expressed as a ratio of GUS to luciferase activity and normalized with respect to the expression levels observed for the transcriptional regulatory expression element group EXP-Os.TubA-3:1:1 (SEQ ID NO: 1107). Table 13 below shows the GUS/Rluc ratios normalized with respect to EXP-Os.TubA-3:1:1 expression in corn protoplasts.

GUS and luciferase activity are measured as described in Example 2 with replicate assays to determine the average level of GUS and luciferase expression in wheat protoplast cells. Mean GUS values are compared to the mean luciferase values and normalized with respect to expression seen in wheat protoplast cells transformed with a GUS expression



vector in which GUS is driven by the transcriptional regulatory expression element group, EXP-Os.TubA-3:1:1 (SEQ ID NO: 65) to determine the relative fold activity of GUS expression driven by the transcriptional regulatory expression element groups, EXP-SETit.TubA3:1:3 (SEQ ID NO: 19), EXP-SETit.TubA2:1:3 (SEQ ID NO: 16), EXP-SETit.TubA2-2:1:1 (SEQ ID NO: 18), EXP-SETit.Act8:1:1 (SEQ ID NO: 9), EXP-SETit.Act8:1:2 (SEQ ID NO: 10), EXP-SETit.Act8:c (SEQ ID NO: 11), EXP-SETit.TubA2-1:1:2 (SEQ ID NO: 17) and EXP-SETit.TubA2:1:1 (SEQ ID NO: 15).

The mean GUS and luciferase expression levels are provided in Table 13. The *Renilla* luciferase values are provided in the column labeled “Rluc”.

10 **Table 13. Mean GUS/Rluc fold expression relative to EXP-Os.TubA-3:1:1 expression in corn leaf protoplast cells.**

Construct	Transcriptional Regulatory Element Group	Mean Gus/RLuc	Gus/Rluc Normalized with respect to EXP-Os.TubA-3:1:1	Gus/Rluc Normalized with respect to EXP-Os.Act1:1:1
pMON19469	EXP-CaMV.35S-enh/I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102)	12.540	22.423	1.030
pMON65328	EXP-CaMV.35S-enh-Lhcb1/I-Os.Act1-1:1:9 (SEQ ID NO: 1105)	16.371	29.274	1.345
pMON25455	EXP-Os.Act1:1:1 (SEQ ID NO: 1098)	12.175	21.770	1.000
pMON122605	EXP-Os.TubA-3:1:1 (SEQ ID NO: 1107)	0.559	1.000	0.046
pMON136270	EXP-SETit.TubA3:1:3 (SEQ ID NO: 19)	2.300	4.112	0.189
pMON136272	EXP-SETit.TubA2:1:3 (SEQ ID NO: 16)	0.933	1.669	0.077
pMON136275	EXP-SETit.TubA2-2:1:1 (SEQ ID NO: 18)	0.028	0.051	0.002
pMON136276	EXP-SETit.Act8:1:1 (SEQ ID NO: 9)	1.606	2.873	0.132
pMON136277	EXP-SETit.Act8:1:2 (SEQ ID NO: 10)	1.281	2.291	0.105
pMON136278	EXP-SETit.Act8:c (SEQ ID NO: 11)	0.051	0.091	0.004
pMON136279	EXP-SETit.TubA2-1:1:2 (SEQ ID NO: 17)	0.037	0.066	0.003

	17)			
pMON136280	EXP-SETit.TubA2:1:1 (SEQ ID NO: 15)	1.516	2.710	0.125

The highest level of GUS expression in wheat protoplast was observed in cells transformed with the constructs pMON136270 (EXP-SETit.TubA3:1:3 (SEQ ID NO: 19)); pMON136276 (EXP-SETit.Act8:1:1 (SEQ ID NO: 9)); pMON136277 (EXP-SETit.Act8:1:2 (SEQ ID NO: 10)) and pMON136280 (EXP-SETit.TubA2:1:1 (SEQ ID NO: 15)).

**Example 7: Identification of transcriptional regulatory elements used for seed expression.**

Transcriptional regulatory elements comprising promoters, leaders, introns and 3' UTRs useful in providing expression of a transgene in plant seed and reproductive tissues are identified based upon the expression of expressed sequence tags (ESTs) in cDNA libraries made from messenger RNA isolated from seed, flower and other tissues derived from Foxtail millet (*Setaria italica* (L.) Beauv). Libraries of cDNA are made from tissues isolated from *S. italica* using methods known to those skilled in the art from flower tissue at 0, 4, 7, 14, 21 and 31 days after pollination (DAP) as well as leaf and root. The resulting cDNAs are sequenced using various sequencing methods known in the art. The resulting ESTs are assembled into clusters using bioinformatics software such as `clc_ref_assemble_complete` version 2.01.37139 (CLC bio USA, Cambridge, Massachusetts 02142). Transcript abundance of each cluster is determined by counting the number of cDNA reads for each cluster. Table 14 below shows cluster assemblies that have been produced using cDNAs from libraries made from *S. italica* tissue isolated from leaf, root and flower at 0, 4, 7, 14, 21 and 31 days after pollination (DAP) that demonstrate expression in specific windows of developing of the developing seed and were either not observed or minimally observed in the leaf and root. Each cluster is annotated using bioinformatics analysis methods such as nucleotide and protein BLAST against public and proprietary data of genes expressed in monocots and dicots. In many cases, a homolog to the cluster was not identified and is indicated in Table 14 as "No homolog".

**Table 14. Foxtail millet EST clusters and annotations.**

<b>Cluster ID</b>	<b>SEQ ID NO:</b>	<b>Annotation</b>
SETIT-28JUL09-CLUS10381_5	925	hypothetical protein SORBIDRAFT_03g000770
SETIT-28JUL09-CLUS101265_2	926	No homolog
SETIT-28JUL09-CLUS6475_-5	927	Putative uncharacterized protein
SETIT-28JUL09- CLUS1019870_1	928	Putative uncharacterized protein
SETIT-28JUL09-CLUS680767_- 4	929	Plastidial ADP-glucose transporter
SETIT-28JUL09-CLUS343678_3	930	No homolog
SETIT-28JUL09-CLUS7568_3	931	No homolog
SETIT-28JUL09-CLUS771450_2	932	No homolog
SETIT-28JUL09- CLUS1164825_1	933	No homolog
SETIT-28JUL09-CLUS1406_-46	934	No homolog
SETIT-28JUL09- CLUS1165324_1	935	No homolog
SETIT-28JUL09- CLUS1140244_1	936	No homolog
SETIT-28JUL09-CLUS153853_- 4	937	No homolog
SETIT-28JUL09-CLUS19108_-4	938	No homolog
SETIT-28JUL09-CLUS23464_-6	939	Putative uncharacterized protein OS= <i>Oryza</i>
SETIT-28JUL09- CLUS1180442_1	940	No homolog
SETIT-28JUL09- CLUS675196_13	941	Zein-like seed storage protein (Fragment)
SETIT-28JUL09-CLUS83_2	942	Granule-bound starch synthase
SETIT-28JUL09-CLUS16759_4	943	No homolog
SETIT-28JUL09-CLUS5145_3	944	No homolog
SETIT-28JUL09- CLUS1193060_1	945	No homolog
SETIT-28JUL09- CLUS1187352_1	946	No homolog
SETIT-28JUL09-CLUS733_4	947	No homolog
SETIT-28JUL09-CLUS4206_-30	948	No homolog
SETIT-28JUL09-CLUS4114_2	949	No homolog
SETIT-28JUL09-CLUS674506_2	950	Globulin-1 S allele
SETIT-28JUL09-CLUS674506_3	951	Vicilin-like embryo storage protein OS= <i>Zea</i>
SETIT-28JUL09-CLUS53110_1	952	No homolog
SETIT-28JUL09-CLUS2505_8	953	No homolog

SETIT-28JUL09-CLUS2888_5	954	Putative uncharacterized protein
SETIT-28JUL09-CLUS2219_3	955	No homolog
SETIT-28JUL09-CLUS12533_-3	956	Nucleoside diphosphate kinase
SETIT-28JUL09-CLUS12533_-6	957	Nucleoside diphosphate kinase
SETIT-28JUL09-CLUS2300_3	958	hypothetical protein SORBIDRAFT_01g043300
SETIT-28JUL09-CLUS696559_1	959	No homolog
SETIT-28JUL09-CLUS681829_1	960	No homolog
SETIT-28JUL09-CLUS680981_1	961	No homolog
SETIT-28JUL09-CLUS2305_6	962	No homolog
SETIT-28JUL09-CLUS685018_1	963	No homolog
SETIT-28JUL09-CLUS12299_7	964	Polyprotein (Fragment); Ubiquitin 5
SETIT-28JUL09-CLUS295335_-4	965	No homolog
SETIT-28JUL09-CLUS206694_6	966	No homolog
SETIT-28JUL09- CLUS1102871_1	967	hypothetical protein SORBIDRAFT_03g001990
SETIT-28JUL09- CLUS1104561_1	968	No homolog
SETIT-28JUL09-CLUS2723_-8	969	No homolog
SETIT-28JUL09-CLUS387500_3	970	No homolog
SETIT-28JUL09-CLUS6331_-8	971	hypothetical protein SORBIDRAFT_03g007310
SETIT-28JUL09- CLUS1103723_1	972	Os08g0402800 protein
SETIT-28JUL09-CLUS482_-8	973	No homolog
SETIT-28JUL09- CLUS1096748_1	974	No homolog
SETIT-28JUL09- CLUS1127439_1	975	No homolog
SETIT-28JUL09- CLUS1115180_1	976	No homolog
SETIT-28JUL09- CLUS1108597_1	977	Putative hydrolase OS= <i>Oryza sativa</i> subsp.
SETIT-28JUL09-CLUS96386_-2	978	No homolog
SETIT-28JUL09-CLUS373929_-2	979	hypothetical protein SORBIDRAFT_09g021920
SETIT-28JUL09- CLUS1090880_1	980	Protection of telomeres 1a protein
SETIT-28JUL09- CLUS1130991_1	981	No homolog
SETIT-28JUL09- CLUS1131180_1	982	No homolog
SETIT-28JUL09-CLUS5112_3	983	No homolog

SETIT-28JUL09-CLUS1020178_2	984	No homolog
SETIT-28JUL09-CLUS1437_14	985	Anther-specific proline-rich protein APG
SETIT-28JUL09-CLUS4707_5	986	Putative uncharacterized protein
SETIT-28JUL09-CLUS1130710_1	987	No homolog
SETIT-28JUL09-CLUS880479_1	988	No homolog
SETIT-28JUL09-CLUS879579_1	989	No homolog
SETIT-28JUL09-CLUS17065_3	990	Putative uncharacterized protein OS=Zea mays
SETIT-28JUL09-CLUS1703_5	991	hypothetical protein SORBIDRAFT_01g038035
SETIT-28JUL09-CLUS878855_1	992	No homolog
SETIT-28JUL09-CLUS35651_8	993	No homolog
SETIT-28JUL09-CLUS533810_3	994	Putative uncharacterized protein
SETIT-28JUL09-CLUS888639_1	995	hypothetical protein SORBIDRAFT_08g019910
SETIT-28JUL09-CLUS8608_2	996	Putative uncharacterized protein
SETIT-28JUL09-CLUS48534_2	997	hypothetical protein SORBIDRAFT_04g001090
SETIT-28JUL09-CLUS8620_17	998	No homolog
SETIT-28JUL09-CLUS31891_4	999	No homolog
SETIT-28JUL09-CLUS6173_1	1000	Os09g0539100 protein
SETIT-28JUL09-CLUS884159_1	1001	hypothetical protein SORBIDRAFT_05g019890
SETIT-28JUL09-CLUS112639_5	1002	No homolog
SETIT-28JUL09-CLUS886862_1	1003	No homolog
SETIT-28JUL09-CLUS52311_2	1004	No homolog
SETIT-28JUL09-CLUS886157_1	1005	No homolog
SETIT-28JUL09-CLUS4920_5	1006	Phosphatidylinositol 4-kinase OS=Oryza
SETIT-28JUL09-CLUS1697_9	1007	No homolog
SETIT-28JUL09-CLUS18000_5	1008	hypothetical protein SORBIDRAFT_10g022890
SETIT-28JUL09-CLUS10981_4	1009	6-phosphofructokinase
SETIT-28JUL09-CLUS880709_1	1010	Ribosomal RNA apurinic site specific lyase
SETIT-28JUL09-CLUS882664_1	1011	Os10g0374600 protein
SETIT-28JUL09-CLUS19159_2	1012	hypothetical protein SORBIDRAFT_06g017240
SETIT-28JUL09-CLUS5475_-2	1013	No homolog
SETIT-28JUL09-CLUS1194621_1	1014	22kD alpha canein 5 OS=Saccharum officinarum; Seed storage protein
SETIT-28JUL09-CLUS675196_9	1015	Zein-like seed storage protein (Fragment)
SETIT-28JUL09-CLUS719393_1	1016	10kD delta canein; Delta zein storage protein

SETIT-28JUL09-CLUS722936_1	1017	10kD delta canein
SETIT-28JUL09-CLUS722936_2	1018	10kD delta canein
SETIT-28JUL09-CLUS684877_1	1019	Alpha kafirin OS=Sorghum bicolor
SETIT-28JUL09-CLUS675196_2	1020	Zein-like seed storage protein (Fragment); Alpha-coixin
SETIT-28JUL09-CLUS691558_2	1021	27 kDa pennisetin
SETIT-28JUL09-CLUS764553_1	1022	22 kDa pennisetin OS=Pennisetum americanum
SETIT-28JUL09-CLUS675787_1	1023	21 kDa pennisetin OS=Pennisetum americanum; Alpha-coixin
SETIT-28JUL09-CLUS691558_3	1024	27 kDa pennisetin
SETIT-28JUL09-CLUS675196_11	1025	22 kDa pennisetin OS=Pennisetum americanum
SETIT-28JUL09-CLUS722936_3	1026	No homolog
SETIT-28JUL09-CLUS695757_1	1027	Putative uncharacterized protein
SETIT-28JUL09-CLUS681682_1	1028	Delta-coixin OS=Coix lachryma-jobi PE=2 SV=1; Prolamine
SETIT-28JUL09-CLUS675531_1	1029	N-methyltransferase
SETIT-28JUL09-CLUS674096_1	1030	No homolog
SETIT-28JUL09-CLUS674121_1	1031	Prolamine
SETIT-28JUL09-CLUS675389_2	1032	No homolog
SETIT-28JUL09-CLUS677324_1	1033	hypothetical protein SORBIDRAFT_01g012345

An analysis of the expression of cDNAs for each cluster presented in Table 14 is provided in Table 15 below. For flower tissue, the following total numbers of EST reads were performed; flower 0 DAP, 251341; flower 4 DAP, 39277; flower 7 DAP, 34330; flower 14 DAP, 34920; flower 21 DAP, 42321; flower 31 DAP, 257327. For leaf and root tissue, the following total numbers of EST reads were performed; leaf, 478570 and root, 434180.

**Table 15. Count of cDNAs expressed corresponding to EST clusters.**

Cluster Annotation	DAP 0	DAP 4	DAP 7	DAP 14	DAP 21	DAP 31	Leaf	Root	Expression Window and Organ
SETIT-28JUL09-CLUS10381_5	17	331	0	0	0	0	0	0	0-7
SETIT-28JUL09-CLUS101265_2	12	152	87	0	0	0	0	0	0-7
SETIT-28JUL09-CLUS6475_5	11	143	82	0	0	0	0	0	0-7
SETIT-28JUL09-CLUS1019870_1	14	119	34	0	0	0	0	0	0-7

SETIT-28JUL09- CLUS680767_-4	0	0	0	239	100	16	0	0	14 -31
SETIT-28JUL09- CLUS343678_3	0	0	0	491	0	0	0	0	14
SETIT-28JUL09- CLUS7568_3	0	0	0	486	0	0	0	0	14
SETIT-28JUL09- CLUS771450_2	0	0	0	481	0	0	0	0	14
SETIT-28JUL09- CLUS1164825_1	0	0	0	419	0	0	0	0	14
SETIT-28JUL09- CLUS1406_-46	0	0	0	324	0	0	0	0	14
SETIT-28JUL09- CLUS1165324_1	0	0	0	323	0	0	0	0	14
SETIT-28JUL09- CLUS1140244_1	0	0	0	310	0	0	0	0	14
SETIT-28JUL09- CLUS153853_-4	0	0	0	304	0	0	0	0	14
SETIT-28JUL09- CLUS19108_-4	0	0	0	138	115	0	0	0	14-21
SETIT-28JUL09- CLUS23464_-6	0	0	0	132	111	0	0	0	14-21
SETIT-28JUL09- CLUS1180442_1	0	0	0	294	247	0	0	0	14-21
SETIT-28JUL09- CLUS675196_13	0	0	0	169	142	0	0	0	14-21
SETIT-28JUL09- CLUS83_2	0	0	0	111	187	15	0	0	14-31
SETIT-28JUL09- CLUS16759_4	0	0	0	0	525	0	0	0	21
SETIT-28JUL09- CLUS5145_3	0	0	0	0	448	0	0	0	21
SETIT-28JUL09- CLUS1193060_1	0	0	0	0	390	0	0	0	21
SETIT-28JUL09- CLUS1187352_1	0	0	0	0	385	0	0	0	21
SETIT-28JUL09- CLUS733_4	0	0	0	0	356	0	0	0	21
SETIT-28JUL09- CLUS4206_-30	0	0	0	0	352	0	0	0	21
SETIT-28JUL09- CLUS4114_2	0	0	0	0	324	0	0	0	21
SETIT-28JUL09- CLUS674506_2	0	0	0	0	772	12	0	0	21-31
SETIT-28JUL09- CLUS674506_3	0	0	0	0	545	9	0	0	21-31
SETIT-28JUL09- CLUS53110_1	0	0	0	0	0	167	0	9	31
SETIT-28JUL09- CLUS2505_8	0	0	0	0	0	547	0	0	31
SETIT-28JUL09- CLUS2888_5	0	0	0	0	0	475	0	0	31
SETIT-28JUL09- CLUS2219_3	0	0	0	0	0	369	0	0	31
SETIT-28JUL09- CLUS12533_-3	0	0	0	0	0	363	0	0	31
SETIT-28JUL09- CLUS12533_-6	0	0	0	0	0	311	0	0	31
SETIT-28JUL09-	0	0	0	0	0	276	0	0	31

CLUS2300_3									
SETIT-28JUL09- CLUS696559_1	0	0	0	0	0	250	0	0	31
SETIT-28JUL09- CLUS681829_1	0	0	0	0	0	246	0	0	31
SETIT-28JUL09- CLUS680981_1	0	0	0	0	0	235	0	0	31
SETIT-28JUL09- CLUS2305_6	0	0	0	0	0	220	0	0	31
SETIT-28JUL09- CLUS685018_1	0	0	0	0	0	217	0	0	31
SETIT-28JUL09- CLUS12299_7	0	295	0	0	0	0	0	0	4
SETIT-28JUL09- CLUS295335_-4	0	569	0	0	0	0	0	0	4
SETIT-28JUL09- CLUS206694_6	0	387	0	0	0	0	0	0	4
SETIT-28JUL09- CLUS1102871_1	0	350	0	0	0	0	0	0	4
SETIT-28JUL09- CLUS1104561_1	0	348	0	0	0	0	0	0	4
SETIT-28JUL09- CLUS2723_-8	0	338	0	0	0	0	0	0	4
SETIT-28JUL09- CLUS387500_3	0	330	0	0	0	0	0	0	4
SETIT-28JUL09- CLUS6331_-8	0	323	0	0	0	0	0	0	4
SETIT-28JUL09- CLUS1103723_1	0	302	0	0	0	0	0	0	4
SETIT-28JUL09- CLUS482_-8	0	90	208	0	0	0	0	0	4-7
SETIT-28JUL09- CLUS1096748_1	0	112	129	0	0	0	0	0	4-7
SETIT-28JUL09- CLUS1127439_1	0	110	127	0	0	0	0	0	4-7
SETIT-28JUL09- CLUS1115180_1	0	104	120	0	0	0	0	0	4-7
SETIT-28JUL09- CLUS1108597_1	0	98	113	0	0	0	0	0	4-7
SETIT-28JUL09- CLUS96386_-2	0	96	110	0	0	0	0	0	4-7
SETIT-28JUL09- CLUS373929_-2	0	91	104	0	0	0	0	0	4-7
SETIT-28JUL09- CLUS1090880_1	0	90	104	0	0	0	0	0	4-7
SETIT-28JUL09- CLUS1130991_1	0	0	562	0	0	0	0	0	7
SETIT-28JUL09- CLUS1131180_1	0	0	495	0	0	0	0	0	7
SETIT-28JUL09- CLUS5112_3	0	0	484	0	0	0	0	0	7
SETIT-28JUL09- CLUS1020178_2	0	0	471	0	0	0	0	0	7
SETIT-28JUL09- CLUS1437_14	0	0	461	0	0	0	0	0	7
SETIT-28JUL09- CLUS4707_5	0	0	446	0	0	0	0	0	7
SETIT-28JUL09- CLUS1130710_1	0	0	427	0	0	0	0	0	7



SETIT-28JUL09- CLUS880479_1	549	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS879579_1	405	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS17065_3	382	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS1703_5	287	0	0	0	0	0	0	8	Ovule pollen
SETIT-28JUL09- CLUS878855_1	280	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS35651_8	225	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS533810_3	203	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS888639_1	198	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS8608_2	196	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS48534_2	175	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS8620_17	172	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS31891_4	171	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS6173_1	159	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS884159_1	155	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS112639_5	149	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS886862_1	144	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS52311_2	138	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS886157_1	136	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS4920_5	133	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS1697_9	132	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS18000_5	129	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS10981_4	129	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS880709_1	129	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS882664_1	128	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS19159_2	128	0	0	0	0	0	0	0	Ovule pollen
SETIT-28JUL09- CLUS5475_-2	0	0	1721	12386	34684	11782	0	0	Seed
SETIT-28JUL09- CLUS1194621_1	0	0	1386	9267	23798	8799	0	0	Seed
SETIT-28JUL09- CLUS675196_9	0	0	1232	17098	22397	17083	0	0	Seed
SETIT-28JUL09- CLUS719393_1	0	0	1184	13872	15107	13799	0	0	Seed

SETIT-28JUL09-CLUS722936_1	0	0	1184	23792	30815	25296	0	0	Seed
SETIT-28JUL09-CLUS722936_-2	0	0	1179	20955	22818	17587	0	0	Seed
SETIT-28JUL09-CLUS684877_1	0	58	1003	5775	18118	5089	0	0	Seed
SETIT-28JUL09-CLUS675196_-2	0	0	955	27322	31248	23018	0	0	Seed
SETIT-28JUL09-CLUS691558_2	0	0	878	18204	29860	18448	0	0	Seed
SETIT-28JUL09-CLUS764553_1	0	0	771	10897	12055	6685	0	0	Seed
SETIT-28JUL09-CLUS675787_1	0	48	547	11309	12229	11428	0	0	Seed
SETIT-28JUL09-CLUS691558_-3	0	0	511	7149	9793	4697	0	0	Seed
SETIT-28JUL09-CLUS675196_11	0	0	478	5648	6182	5752	0	0	Seed
SETIT-28JUL09-CLUS722936_-3	0	0	255	4537	4906	5131	0	0	Seed
SETIT-28JUL09-CLUS695757_1	0	0	240	2803	3438	2313	0	0	Seed
SETIT-28JUL09-CLUS681682_1	0	0	210	5982	6290	5382	0	0	Seed
SETIT-28JUL09-CLUS675531_1	0	58	178	573	346	458	0	0	Seed
SETIT-28JUL09-CLUS674096_1	0	0	114	1648	2816	1837	0	0	Seed
SETIT-28JUL09-CLUS675389_2	0	0	102	6728	7852	7164	0	0	Seed
SETIT-28JUL09-CLUS677324_1	0	56	65	133	74	59	0	0	Seed

As can be seen in Table 15 above, many of the identified clusters demonstrate expression in specific windows of seed development; at 0 DAP in which expression is inferred to be in both ovule and pollen, or during the seed development window from 4 to 31 DAP. The identified cDNA clusters were used to design primers, which were then used with GenomeWalker™ (Clontech Laboratories, Inc, Mountain View, CA) libraries constructed following the manufacturer's protocol to clone the 5' region of the corresponding genomic DNA sequence. In the case of promoters leaders and introns, this cloned region contained the 5'transcriptional regulatory, 5' UTR and if present, intron sequence upstream of the protein-coding region for each gene from *S. italica*. Using this sequence, regulatory elements were bioinformatically identified within the 5' region for each gene. Bioinformatic analysis was used to identify the transcriptional start site (TSS) and any bi-directionality, introns, or upstream coding sequence present in the sequence. Using the results of this analysis, regulatory elements were defined within the 5' sequence upstream of the coding sequence of the gene. Primers were then designed to amplify the regulatory elements. The corresponding

DNA molecule for each regulatory element was amplified using standard polymerase chain reaction conditions with primers containing unique restriction enzyme sites and genomic DNA isolated from *S. italica*. The resulting DNA fragments were ligated into a base plant expression vector using standard restriction enzyme digestion of compatible restriction sites and DNA ligation methods. In some cases, high sequence identity between some leaders provided the discovery of regulatory elements from homologous genes. The resulting transcriptional regulatory element groups, promoters, leaders and introns identified through this analysis are presented in Table 16 below:

**Table 16. Transcriptional regulatory element groups (EXP), Promoters (P), leaders (L) and introns (I) identified using expression analysis.**

Annotation	SEQ ID NO:	Description
EXP-SETit.CLUS120796-1	12	Cluster 120796-1
EXP-SETit.CLUS19108	13	Cluster 19108
EXP-SETit.Ubq5	22	Ubiquitin 5
P-SETit.Alc1-1:1:1	28	Alpha-coixin
P-SETit.Alc1-1:1:2	29	Alpha-coixin
P-SETit.Alc2-1:1:2	30	Alpha-coixin
P-SETit.CLUS1164825-1-1:1:1	37	Cluster 1164825-1
P-SETit.CLUS1165324-1:1:1	38	Cluster 1165324-1
P-SETit.CLUS120796-1-1:1:1	39	Cluster 120796-1
P-SETit.CLUS19108-1:1:2	40	Cluster 19108
P-SETit.CLUS882664-1-1:1:2	41	Cluster 882664-1
P-SETit.Dzs-1:1:4	47	Delta zein storage protein
P-SETit.Dzs-1:1:5	48	Delta zein storage protein
P-SETit.EST CLUS675389-2-1:1:2	50	Cluster 675389-2
P-SETit.FM54-1:1:2	52	Cluster 1102871_1
P-SETit.FM63-1:1:2	53	Cluster 1019870_1
P-SETit.Pro1-1:1:2	77	Prolamin
P-SETit.Pro2-1:1:3	78	Prolamin
P-SETit.Ssp1-1:1:1	93	Seed storage protein
P-SETit.Ssp1-1:1:2	94	Seed storage protein
P-SETit.Ubq5-1:1:2	104	Ubiquitin 5
L-SETit.Alc1-1:1:1	110	Alpha-coixin
L-SETit.Alc2-1:1:1	111	Alpha-coixin
L-SETit.CLUS1164825-1-1:1:1	118	Cluster 1164825-1
L-SETit.CLUS120796-1-1:1:1	119	Cluster 120796-1
L-SETit.CLUS120796-1-1:1:2	120	Cluster 120796-1
L-SETit.CLUS19108-1:1:1	121	Cluster 19108

L-SETit.CLUS19108-1:1:2	122	Cluster 19108
L-SETit.CLUS882664-1-1:1:1	123	Cluster 882664-1
L-SETit.Dzs-1:1:1	128	Delta zein storage protein
L-SETit.EST CLUS675389-2-1:1:1	130	Cluster 675389-2
L-SETit.Pro1-1:1:1	151	Prolamin
L-SETit.Pro2-1:1:2	152	Prolamin
L-SETit.Ssp1-1:1:1	164	Seed storage protein
L-SETit.Ubq5-1:1:1	170	Ubiquitin 5
I-SETit.CLUS120796-1-1:1:1	173	Cluster 120796-1
I-SETit.CLUS19108-1:1:1	174	Cluster 19108
I-SETit.Ubq5-1:1:2	178	Ubiquitin 5

In some instances, the transcriptional start site could not be identified. The transcriptional regulatory elements, P-SETit.FM54-1:1:2 (SEQ ID NO: 52), P-SETit.FM63-1:1:2 (SEQ ID NO: 53) and P-SETit.CLUS1165324-1:1:1 (SEQ ID NO: 38) may be further  
5 comprised of a promoter element operably linked to a leader element or fragment of a leader element.

#### **Example 8: Analysis of Seed Regulatory Elements driving GUS in Bombarded Corn Tissues.**

10 Seed, root and leaf tissue isolated from corn plants is bombarded with plant GUS expression and control vectors to determine the capacity of transcriptional regulatory elements derived from *Setaria italica* to drive expression of a transgene, GUS.

Corn plant tissues were transformed with the plant GUS expression constructs using particle bombardment, listed in Table 17, below. Regulatory elements presented in example  
15 7 were cloned into a base plant expression vector using standard methods known in the art. The resulting plant expression vectors contained a right border region from *Agrobacterium tumefaciens*, a first transgene cassette to test the regulatory or chimeric regulatory element comprised of, a regulatory or chimeric regulatory element, operably linked to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO:  
20 1102), operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS) that possessed a processable intron (GUS-2, SEQ ID NO: 1091), operably linked to the 3' termination region from the rice lipid transfer protein gene (T-Os.LTP-1:1:1, SEQ ID NO: 1089); a second transgene selection cassette used for selection of transformed plant cells that conferred resistance to the herbicide glyphosate (driven by the rice Actin 1 transcriptional regulatory  
25 element group, SEQ ID NO: 1098), and a left border region from *A. tumefaciens*. The

resulting plasmids, pMON117992, pMON117993, pMON117994, pMON117995, pMON117996, pMON117997, pMON117998, pMON117999, pMON130551, pMON140500, pMON140501, pMON140502, pMON140503, pMON140504, pMON140505, pMON140506, pMON140507 and pMON140508 are used to transform corn plant tissue using particle bombardment.

**Table 17. Binary plant transformation constructs and regulatory elements.**

Construct	Regulatory Elements	Reference Cluster
pMON117992	P-SETit.EST CLUS675389-2-1:1:2 (SEQ ID NO: 50) L-SETit.EST CLUS675389-2-1:1:1 (SEQ ID NO: 130)	SETIT-28Jul09-CLUS675389_2
pMON117993	P-SETit.Pro1-1:1:2 (SEQ ID NO: 77) L-SETit.Pro1-1:1:1 (SEQ ID NO: 151)	SETIT-28Jul09-CLUS674121_1
pMON117994	P-SETit.Pro2-1:1:3 (SEQ ID NO: 78) L-SETit.Pro2-1:1:2 (SEQ ID NO: 152)	SETIT-28Jul09-CLUS681682_1
pMON117995	P-SETit.CLUS882664-1-1:1:2 (SEQ ID NO: 41) L-SETit.CLUS882664-1-1:1:1 (SEQ ID NO: 123)	SETIT-28Jul09-CLUS882664_1
pMON117996	EXP-SETit.CLUS19108 (SEQ ID NO: 13) P-SETit.CLUS19108-1:1:2 (SEQ ID NO: 40) L-SETit.CLUS19108-1:1:2 (SEQ ID NO: 122) I-SETit.CLUS19108-1:1:1 (SEQ ID NO: 174) L-SETit.CLUS19108-1:1:1 (SEQ ID NO: 121)	SETIT-28Jul09-CLUS19108_-4
pMON117997	P-SETit.Alc1-1:1:1 (SEQ ID NO: 28) L-SETit.Alc1-1:1:1 (SEQ ID NO: 110)	SETIT-28Jul09-CLUS675196_-2
pMON117998	P-SETit.Alc2-1:1:2 (SEQ ID NO: 30) L-SETit.Alc2-1:1:1 (SEQ ID NO: 111)	SETIT-28Jul09-CLUS675787_1
pMON117999	P-SETit.Dzs-1:1:4 (SEQ ID NO: 47) L-SETit.Dzs-1:1:1 (SEQ ID NO: 128)	SETIT-28Jul09-CLUS719393_1
pMON130551	P-SETit.Alc1-1:1:2 (SEQ ID NO: 29) L-SETit.Alc1-1:1:1 (SEQ ID NO: 110)	SETIT-28Jul09-CLUS675196_-2
pMON140500	P-SETit.Dzs-1:1:5 (SEQ ID NO: 48)	SETIT-28Jul09-CLUS719393_1

	L-SETit.Dzs-1:1:1 (SEQ ID NO: 128)	
pMON140501	P-SETit.Ssp1-1:1:1 (SEQ ID NO: 93) L-SETit.Ssp1-1:1:1 (SEQ ID NO: 164)	SETIT-28Jul09- CLUS1194621_1
pMON140502	P-SETit.Ssp1-1:1:2 (SEQ ID NO: 94) L-SETit.Ssp1-1:1:1 (SEQ ID NO: 164)	SETIT-28Jul09- CLUS1194621_1
pMON140503	EXP-SETit.CLUS120796-1 (SEQ ID NO: 12) P-SETit.CLUS120796-1-1:1:1 (SEQ ID NO: 39) L-SETit.CLUS120796-1-1:1:2 (SEQ ID NO: 120) I-SETit.CLUS120796-1-1:1:1 (SEQ ID NO: 173) L-SETit.CLUS120796-1-1:1:1 (SEQ ID NO: 119)	SETIT-28Jul09-CLUS2300_3
pMON140504	P-SETit.CLUS1164825-1-1:1:1 (SEQ ID NO: 37) L-SETit.CLUS1164825-1-1:1:1 (SEQ ID NO: 118)	SETIT-28Jul09- CLUS1164825_1
pMON140505	EXP-SETit.Ubq5 (SEQ ID NO: 22) P-SETit.Ubq5-1:1:2 (SEQ ID NO: 104) L-SETit.Ubq5-1:1:1 (SEQ ID NO: 170) I-SETit.Ubq5-1:1:2 (SEQ ID NO: 178)	SETIT-28Jul09- CLUS12299_7
pMON140506	P-SETit.FM54-1:1:2 (SEQ ID NO: 52)	SETIT-28Jul09- CLUS1102871_1
pMON140507	P-SETit.FM63-1:1:2 (SEQ ID NO: 53)	SETIT-28Jul09- CLUS1019870_1
pMON140508	P-SETit.CLUS1165324-1:1:1 (SEQ ID NO: 38)	SETIT-28Jul09- CLUS1165324_1

The plant transformation vector, pMON117992 is comprised of the promoter element, P-SETit.EST CLUS675389-2-1:1:2 (SEQ ID NO: 50), operably linked 5' to the leader element, L-SETit.EST CLUS675389-2-1:1:1 (SEQ ID NO:130). The plant transformation

5 vector, pMON117993 is comprised of the promoter element, P-SETit.Pro1-1:1:2 (SEQ ID NO: 77), operably linked 5' to the leader element, L-SETit.Pro1-1:1:1 (SEQ ID NO: 151). The plant transformation vector, pMON117994 is comprised of the promoter element, P-SETit.Pro2-1:1:3 (SEQ ID NO: 78), operably linked 5' to the leader element, L-SETit.Pro2-1:1:2 (SEQ ID NO: 152). The plant transformation vector, pMON117995 is comprised of the

10 promoter element, P-SETit.CLUS882664-1-1:1:2 (SEQ ID NO: 41), operably linked 5' to the leader element, L-SETit.CLUS882664-1-1:1:1 (SEQ ID NO: 123). The plant transformation

vector, pMON117996 is comprised of the transcriptional regulatory element group, EXP-SETit.CLUS19108 (SEQ ID NO: 13), which is further comprised of the promoter element, P-SETit.CLUS19108-1:1:2 (SEQ ID NO: 40), operably linked 5' to the leader element, L-SETit.CLUS19108-1:1:2 (SEQ ID NO: 122), operably linked 5' to the intron element, I-SETit.CLUS19108-1:1:1 (SEQ ID NO: 174), operably linked 5' to the leader element, L-SETit.CLUS19108-1:1:1 (SEQ ID NO: 121). The plant transformation vector, pMON117997 is comprised of the promoter element, P-SETit.Alc1-1:1:1 (SEQ ID NO: 28), operably linked 5' to the leader element, L-SETit.Alc1-1:1:1 (SEQ ID NO: 110). The plant transformation vector, pMON117998 is comprised of the promoter element, P-SETit.Alc2-1:1:2 (SEQ ID NO: 30), operably linked 5' to the leader element, L-SETit.Alc2-1:1:1 (SEQ ID NO: 111). The plant transformation vector, pMON117999 is comprised of the promoter element, P-SETit.Dzs-1:1:4 (SEQ ID NO: 47), operably linked 5' to the leader element, L-SETit.Dzs-1:1:1 (SEQ ID NO: 128). The plant transformation vector, pMON130551 is comprised of the promoter element, P-SETit.Alc1-1:1:2 (SEQ ID NO: 29), operably linked 5' to the leader element, L-SETit.Alc1-1:1:1 (SEQ ID NO: 110). The plant transformation vector, pMON140500 is comprised of the promoter element, P-SETit.Dzs-1:1:5 (SEQ ID NO: 48), operably linked 5' to the leader element, L-SETit.Dzs-1:1:1 (SEQ ID NO: 128). The plant transformation vector, pMON140501 is comprised of the promoter element, P-SETit.Ssp1-1:1:1 (SEQ ID NO: 93), operably linked 5' to the leader element, L-SETit.Ssp1-1:1:1 (SEQ ID NO: 164). The plant transformation vector, pMON140502 is comprised of the promoter element, P-SETit.Ssp1-1:1:2 (SEQ ID NO: 94), operably linked 5' to the leader element, L-SETit.Ssp1-1:1:1 (SEQ ID NO: 164). The plant transformation vector, pMON140503 is comprised of the transcriptional regulatory element group, EXP-SETit.CLUS120796-1 (SEQ ID NO: 12), which is further comprised of the promoter element, P-SETit.CLUS120796-1-1:1:1 (SEQ ID NO: 39), operably linked 5' to the leader element, L-SETit.CLUS120796-1-1:1:2 (SEQ ID NO: 120), operably linked 5' to the intron element, I-SETit.CLUS120796-1-1:1:1 (SEQ ID NO: 173), operably linked 5' to the leader element, L-SETit.CLUS120796-1-1:1:1 (SEQ ID NO: 119). The plant transformation vector, pMON140504 is comprised of the promoter element, P-SETit.CLUS1164825-1-1:1:1 (SEQ ID NO: 37), operably linked 5' to the leader element, L-SETit.CLUS1164825-1-1:1:1 (SEQ ID NO: 118). The plant transformation vector, pMON140505 is comprised of the transcriptional regulatory element group, EXP-SETit.Ubq5 (SEQ ID NO: 22), which is further comprised of the promoter element, P-SETit.Ubq5-1:1:2 (SEQ ID NO: 104), operably linked 5' to the leader element, L-SETit.Ubq5-1:1:1 (SEQ ID NO: 170), operably linked 5' to the

intron element, I-SETit.Ubq5-1:1:2 (SEQ ID NO: 178). The plant transformation vector, pMON140506 is comprised of the promoter element, P-SETit.FM54-1:1:2 (SEQ ID NO: 52).The plant transformation vector, pMON140507 is comprised of the promoter element, P-SETit.FM63-1:1:2 (SEQ ID NO: 53).The plant transformation vector, pMON140508 is comprised of the promoter element, P-SETit.CLUS1165324-1:1:1 (SEQ ID NO: 38).

Corn plant tissues are transformed using particle bombardment methods and LH244 corn seeds as outlined in Example 3 above. The bombarded root and leaf tissues are allowed to incubate in the dark for 24 hours at 26°C. Following this overnight incubation, the tissues are stained in solution for GUS expression overnight at 37 degrees Celsius. After staining overnight, the tissues are soaked in 70% ethanol overnight to remove chlorophyll and reveal the GUS staining. The tissues are then photographed and a rating scale of “0” to “4” reflecting the level of GUS expression is assigned to each construct.

Expression of the GUS transgene demonstrated in each tissue is used to infer the relative potential level and specificity of each element’s capacity to drive transgene expression in stably transformed corn plants. Average GUS expression ratings are provided in Table 18 below.

**Table 18. GUS expression ratings for particle bombardment assay of potential seed promoters.**

Construct	Regulatory Elements	Embryo	Endosperm	Root	Leaf
pMON117992	P-SETit.EST CLUS675389-2-1:1:2 (SEQ ID NO: 50) L-SETit.EST CLUS675389-2-1:1:1 (SEQ ID NO: 130)	1	1	1	0
pMON117993	P-SETit.Pro1-1:1:2 (SEQ ID NO: 77) L-SETit.Pro1-1:1:1 (SEQ ID NO: 151)	1	3	1	0
pMON117994	P-SETit.Pro2-1:1:3 (SEQ ID NO: 78) L-SETit.Pro2-1:1:2 (SEQ ID NO: 152)	1	3	1	0
pMON117995	P-SETit.CLUS882664-1-1:1:2 (SEQ ID NO: 41) L-SETit.CLUS882664-1-1:1:1 (SEQ ID NO: 123)	0	0	1	0



pMON117996	EXP-SETit.CLUS19108 (SEQ ID NO: 13) P-SETit.CLUS19108-1:1:2 (SEQ ID NO: 40) L-SETit.CLUS19108-1:1:2 (SEQ ID NO: 122) I-SETit.CLUS19108-1:1:1 (SEQ ID NO: 174) L-SETit.CLUS19108-1:1:1 (SEQ ID NO: 121)	3	3	2	0
pMON117997	P-SETit.Alc1-1:1:1 (SEQ ID NO: 28) L-SETit.Alc1-1:1:1 (SEQ ID NO: 110)	1	4	1	0
pMON117998	P-SETit.Alc2-1:1:2 (SEQ ID NO: 30) L-SETit.Alc2-1:1:1 (SEQ ID NO: 111)	0	3	1	0
pMON117999	P-SETit.Dzs-1:1:4 (SEQ ID NO: 47) L-SETit.Dzs-1:1:1 (SEQ ID NO: 128)	2	3	2	0
pMON130551	P-SETit.Alc1-1:1:2 (SEQ ID NO: 29) L-SETit.Alc1-1:1:1 (SEQ ID NO: 110)	1	2	1	0
pMON140500	P-SETit.Dzs-1:1:5 (SEQ ID NO: 48) L-SETit.Dzs-1:1:1 (SEQ ID NO: 128)	0	3	3	0
pMON140501	P-SETit.Ssp1-1:1:1 (SEQ ID NO: 93) L-SETit.Ssp1-1:1:1 (SEQ ID NO: 164)	0	2	1	0
pMON140502	P-SETit.Ssp1-1:1:2 (SEQ ID NO: 94) L-SETit.Ssp1-1:1:1 (SEQ ID NO: 164)	0	3	1	0
pMON140503	EXP-SETit.CLUS120796-1 (SEQ ID NO: 12) P-SETit.CLUS120796-1-1:1:1 (SEQ ID NO: 39) L-SETit.CLUS120796-1-1:1:2 (SEQ ID NO: 120) I-SETit.CLUS120796-1-1:1:1 (SEQ ID NO: 173) L-SETit.CLUS120796-1-1:1:1 (SEQ ID NO: 119)	1	0	2	0

pMON140504	P-SETit.CLUS1164825-1-1:1:1 (SEQ ID NO: 37) L-SETit.CLUS1164825-1-1:1:1 (SEQ ID NO: 118)	0	0	1	0
pMON140505	EXP-SETit.Ubq5 (SEQ ID NO: 22) P-SETit.Ubq5-1:1:2 (SEQ ID NO: 104) L-SETit.Ubq5-1:1:1 (SEQ ID NO: 170) I-SETit.Ubq5-1:1:2 (SEQ ID NO: 178)	4	5	4	4
pMON140506	P-SETit.FM54-1:1:2 (SEQ ID NO: 52)	1	0	1	0
pMON140507	P-SETit.FM63-1:1:2 (SEQ ID NO: 53)	1	0	2	0
pMON140508	P-SETit.CLUS1165324-1:1:1 (SEQ ID NO: 38)	0	0	1	0

In all cases, some transgene expression was observed in tissues bombarded with the construct shown above in Table 18. Highest levels of expression in the seed were observed for the tissues bombarded with the constructs, pMON117996 ((EXP-SETit.CLUS19108 (SEQ ID NO: 13) comprised of P-SETit.CLUS19108-1:1:2 (SEQ ID NO: 40) + L-SETit.CLUS19108-1:1:2 (SEQ ID NO: 122) + I-SETit.CLUS19108-1:1:1 (SEQ ID NO: 174) + L-SETit.CLUS19108-1:1:1 (SEQ ID NO: 121)) and pMON140505 ((EXP-SETit.Ubq5 (SEQ ID NO: 22) comprised of P-SETit.Ubq5-1:1:2 (SEQ ID NO: 104) + L-SETit.Ubq5-1:1:1 (SEQ ID NO: 170) + I-SETit.Ubq5-1:1:2 (SEQ ID NO: 178)). Surprisingly, the construct pMON140505 ((EXP-SETit.Ubq5 (SEQ ID NO: 22) comprised of P-SETit.Ubq5-1:1:2 (SEQ ID NO: 104) + L-SETit.Ubq5-1:1:1 (SEQ ID NO: 170) + I-SETit.Ubq5-1:1:2 (SEQ ID NO: 178)) demonstrated high levels of constitutive expression which had not been initially predicted by the EST counts provided in Table 15 of example 7. Some constructs such as, pMON117998 (P-SETit.Alc2-1:1:2 (SEQ ID NO: 30) + L-SETit.Alc2-1:1:1 (SEQ ID NO: 111)); pMON140500 (P-SETit.Dzs-1:1:5 (SEQ ID NO: 48) + L-SETit.Dzs-1:1:1 (SEQ ID NO: 128)); pMON140501 (P-SETit.Ssp1-1:1:1 (SEQ ID NO: 93) + L-SETit.Ssp1-1:1:1 (SEQ ID NO: 164) and pMON140502 (P-SETit.Ssp1-1:1:2 (SEQ ID NO: 94) + L-SETit.Ssp1-1:1:1 (SEQ ID NO: 164)), demonstrated expression in the endosperm and not the embryo. In addition, while the construct, pMON140500 (P-SETit.Dzs-1:1:5 (SEQ ID NO: 48) + L-SETit.Dzs-1:1:1 (SEQ ID NO: 128)) demonstrated expression in only the endosperm, the construct, pMON117999 (P-SETit.Dzs-1:1:4 (SEQ ID NO: 47) + L-SETit.Dzs-1:1:1

(SEQ ID NO: 128)), which is comprised of a longer version of the Dzs promoter, (P-SETit.Dzs-1:1:5 (SEQ ID NO: 48)), demonstrated expression in both endosperm and embryo, suggesting the potential for an embryo enhancer comprising the fragment deleted from P-SETit.Dzs-1:1:4 (SEQ ID NO: 47) to produce P-SETit.Dzs-1:1:5 (SEQ ID NO: 48).

### 5           **Example 9: Regulatory Elements Driving GUS Transgene Expression in Transgenic Corn or Wheat**

Corn or wheat plants are transformed with constructs comprising regulatory elements such as the transcriptional regulatory element groups, provided as SEQ ID NOS: 1 through 22; or the promoters provided as SEQ ID NOS: 23 through 105 and SEQ ID NOS: 353  
10 through 536, operably linked to any of the leaders provided as SEQ ID NOS: 106 through 171 and 537 through 588. The transcriptional regulatory element groups or promoters can be operably linked to a marker transgene such as GUS similar to that as described in example 2 above. In addition, intron elements such as those provided as SEQ ID NOS: 172 through 267 and SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778 can be operably  
15 linked between the expression elements and transgene of interest to improve expression or modulate the expression of the transgene within the transformed plant. The plants are transformed using agrobacterium or particle bombardment methods known in the art. Transformants containing one or two copies of the transgene cassette are selected using methods known in the art. Transformants are then assayed to determine the level of  
20 expression of the marker transgene in various tissues of the plant similar to that as described in example 2. F1 progeny are produced by either outcrossing the transformed event with an untransformed event, or through self-fertilization. The F1 progeny are grown and expression of the marker transgene is determined using progeny possessing one or two copies of the transgene cassette. Chimeric regulatory elements comprised of any of the promoters, leader and introns presented above are selected based upon the level of expression and tissue  
25 specificity of expression to drive transgenes of agronomic or commercial importance.

### **Example 10: Enhancers Derived from the Regulatory Elements**

Enhancers are derived from the promoter elements presented as SEQ ID NOS: 23 through 105 and SEQ ID NOS: 353 through 536. The enhancer element may be comprised  
30 of one or more cis regulatory elements that when operably linked 5' or 3' to a promoter element, or operably linked 5' or 3' to additional enhancer elements that are operably linked to a promoter, can enhance or modulate expression of a transgene, or provide expression of a

transgene in a specific cell type or plant organ or at a particular time point in development or circadian rhythm. Enhancers are made by removing the TATA box or functionally similar elements and any downstream sequence from the promoters that allow transcription to be initiated from the promoters presented as SEQ ID NOS: 23 through 105 and SEQ ID NOS: 5 353 through 536 or fragments thereof, in which the TATA box or functionally similar elements and sequence downstream of the TATA box are removed.

Enhancer elements are derived from the promoter elements presented as SEQ ID NOS: 23 through 105 and SEQ ID NOS: 353 through 536 and cloned using methods known in the art to be operably linked 5' or 3' to a promoter element, or operably linked 5' or 3' to 10 additional enhancer elements that are operably linked to a promoter. Alternatively, enhancer elements are cloned using methods known in the art to be operably linked to one or more copies of the enhancer element which are operably linked 5' or 3' to a promoter element, or operably linked 5' or 3' to additional enhancer elements that are operably linked to a promoter. Enhancer elements can also be cloned using methods known in the art to be 15 operably linked 5' or 3' to a promoter element derived from a different genus organism, or operably linked 5' or 3' to additional enhancer elements derived from other genus organisms or the same genus organism that are operably linked to a promoter derived from either the same or different genus organism, resulting in a chimeric regulatory element. A GUS expression plant transformation vector is constructed using methods known in the art similar 20 to the constructs described in the previous examples in which the resulting plant expression vectors contains a right border region from *A. tumefaciens*, a first transgene cassette to test the regulatory or a chimeric regulatory element comprised of, a regulatory or chimeric regulatory element, operably linked to an intron derived from the HSP70 heat shock protein of *Zea mays* or any of the introns presented as SEQ ID NOS: 172 through 267 and SEQ ID 25 NOS: 317 through 323 and SEQ ID NOS: 589 through 778 or any other intron, operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS) that either possesses a processable intron (GUS-2, SEQ ID NO: 1091) or no intron (GUS-1, SEQ ID NO: 1090), operably linked to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088) or the 3' termination region from the rice lipid transfer protein gene (T- 30 Os.LTP-1:1:1, SEQ ID NO: 1089); a second transgene selection cassette used for selection of transformed plant cells that confers resistance to the herbicide glyphosate (driven by the rice Actin 1 promoter), or alternatively, the antibiotic kanamycin (driven by the rice Actin 1 promoter) and a left border region from *A. tumefaciens*. The resulting plasmids are used to transform corn plants or other genus plants by the methods described above or by other

*Agrobacterium*-mediated or particle bombardment methods known in the art. Alternatively, protoplast cells derived from corn or other genus plants are transformed using methods known in the art to perform transient assays

GUS expression driven by the test regulatory element comprising one or more enhancers is evaluated in stable or transient plant assays to determine the effects of the enhancer element on expression of a transgene. Modifications to one or more enhancer elements or duplication of one or more enhancer elements is performed based upon empirical experimentation and the resulting gene expression regulation that is observed using each regulatory element composition. Altering the relative positions of one or more enhancers in the resulting regulatory or chimeric regulatory element may affect the transcriptional activity or specificity of the regulatory or chimeric regulatory element and is determined empirically to identify the best enhancers for the desired transgene expression profile within the corn plant or other genus plant.

#### **Example 11: Analysis of Intron-Mediated Enhancement of GUS Activity Using Plant**

##### **Derived Protoplasts**

The introduction of a foreign gene into a new plant host does not always result in a high expression of the incoming gene. Furthermore, if dealing with complex traits, it is sometimes necessary to modulate several genes with spatially or temporarily different expression pattern. Introns can principally provide such modulation. However multiple use of the same intron in one plant has shown to exhibit disadvantages. In those cases it is necessary to have a collection of basic control elements for the construction of appropriate recombinant DNA elements. However, the available collection of introns with expression enhancing properties is limited and alternatives are needed.

In plants, the inclusion of some introns in gene constructs leads to increased mRNA and protein accumulation relative to constructs lacking the intron. This effect has been termed “intron mediated enhancement” (IME) of gene expression (Mascarenhas *et al.*, (1990) *Plant Mol. Biol.* 15:913-920). Introns known to stimulate expression in plants have been identified in maize genes (*e.g.* tubA1, Adh1, Sh1, Ubi1 (Jeon *et al.* (2000) *Plant Physiol.* 123:1005-1014; Callis *et al.* (1987) *Genes Dev.* 1:1183-1200; Vasil *et al.* (1989) *Plant Physiol.* 91:1575-1579; Christiansen *et al.* (1992) *Plant Mol. Biol.* 18:675-689) and in rice genes (*e.g.* salt, *tpi*: McElroy *et al.*, *Plant Cell* 2:163-171 (1990); Xu *et al.*, *Plant Physiol.* 106:459-467 (1994)). Similarly, introns from dicotyledonous plant genes like those from petunia (*e.g.* *rbcS*), potato (*e.g.* *st-ls1*) and from *Arabidopsis thaliana* (*e.g.* *ubq3* and *pat1*) have been

found to elevate gene expression rates (Dean *et al.* (1989) *Plant Cell* 1:201-208; Leon *et al.* (1991) *Plant Physiol.* 95:968-972; Norris *et al.* (1993) *Plant Mol Biol* 21:895-906; Rose and Last (1997) *Plant J.* 11:455-464). It has been shown that deletions or mutations within the splice sites of an intron reduce gene expression, indicating that splicing might be needed for  
5 IME (Mascarenhas *et al.* (1990) *Plant Mol Biol.* 15:913-920; Clancy and Hannah (2002) *Plant Physiol.* 130:918-929). However, that splicing per se is not required for a certain IME in dicotyledonous plants has been shown by point mutations within the splice sites of the *pat1* gene from *A. thaliana* (Rose and Beliakoff (2000) *Plant Physiol.* 122:535-542).

Enhancement of gene expression by introns is not a general phenomenon because  
10 some intron insertions into recombinant expression cassettes fail to enhance expression (*e.g.* introns from dicot genes (*rbcS* gene from pea, phaseolin gene from bean and the *stls-1* gene from *Solanum tuberosum*) and introns from maize genes (*adh1* gene the ninth intron, *hsp81* gene the first intron)) (Chee *et al.* (1986) *Gene* 41:47-57; Kuhlemeier *et al.* (1988) *Mol Gen Genet* 212:405-411; Mascarenhas *et al.* (1990) *Plant Mol. Biol.* 15:913-920; Sinibaldi and  
15 Mettler (1992) In WE Cohn, K Moldave, eds, *Progress in Nucleic Acid Research and Molecular Biology*, Vol 42. Academic Press, New York, pp 229-257; Vancanneyt *et al.* 1990 *Mol. Gen. Genet.* 220:245-250). Therefore, not each intron can be employed in order to manipulate the gene expression level of non-endogenous genes or endogenous genes in transgenic plants. What characteristics or specific sequence features must be present in an  
20 intron sequence in order to enhance the expression rate of a given gene is not known in the prior art and therefore from the prior art it is not possible to predict whether a given plant intron, when used heterologously, will cause IME.

An intron is selected based upon experimentation and comparison with an intronless expression vector control to empirically select an intron and configuration within the vector  
25 T-DNA element arrangement for optimal expression of a transgene. For example, in the expression of an herbicide resistance gene, such as CP4 which confers resistance to the herbicide glyphosate, it is desirable to have transgene expression within the reproductive tissues as well as the vegetative tissues, to prevent the loss of yield when applying the herbicide. An intron in this instance would be selected upon its ability when operably linked  
30 to a constitutive promoter, to enhance expression of the herbicide resistance conferring transgene, particularly within the reproductive cells and tissues of the transgenic plant and thus providing both vegetative and reproductive tolerance to the transgenic plant, when sprayed with the herbicide (see for example International Patent Application, WO2007/098042A2).

Introns presented as SEQ ID NOS: 16 through 306 are identified using genomic DNA contigs in comparison to expressed sequence tag clusters or cDNA contigs to identify exon and intron sequences within the genomic DNA. In addition, 5' UTR or leader sequences are also used to define the intron/exon splice junction of one or more introns under conditions when the gene sequence encodes a leader sequence that is interrupted by one or more introns. Introns presented as SEQ ID NOS: 172 through 267 and SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778 are cloned using methods known in the art into a plant transformation vector to be operably linked 3' to a transcriptional regulatory element and leader fragment and operably linked 5' to either a second leader fragment or to coding sequences as depicted in the two transgene cassettes presented in FIG. 15. A first possible transgene cassette (Transgene Cassette Configuration 1 in FIG. 15) is comprised of a promoter or chimeric promoter element [A], operably linked 5' to a leader element [B], operably linked 5' to a test intron element [C], operably linked to a coding region [D], which is operably linked to a 3' UTR element [E]. Alternatively, a second possible transgene cassette (Transgene Cassette Configuration 2 in Figure 15) is comprised of a promoter or chimeric promoter element [F], operably linked 5' to a first leader element or first leader element fragment [G], operably linked 5' to a test intron element [H], operably linked 5' to a second leader element or first leader element second fragment [I], operably linked to a coding region [J], which is operably linked to a 3' UTR element [K].

The first 6 nucleotides on the 5' end and the last 6 nucleotides on the 3' end of the introns presented as SEQ ID NOS: 172 through 267 and SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778 represent nucleotides before and after the intron/exon splice junction, respectively. These short 6 nucleotide sequences can be altered or modified by having additional sequence appended (*i.e.* native or artificial) to facilitate cloning of the intron into a plant transformation vector, so long as the seventh and eighth nucleotides from the 5' end (GT) and the seventh and eighth nucleotide from the 3' end (AG) of SEQ ID NOS: 172 through 267 and SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778 are preserved, thus preserving the intron/exon splice junction of the intron. It is preferable to avoid using the nucleotide sequence TG or G just prior to the seventh and eighth nucleotides from the 5' end (GT) and the nucleotide sequence, A or AT from the seventh and eighth nucleotide from the 3' end (AG) to eliminate the potential of unwanted stop and start codons from being formed during processing of the messenger RNA into the final transcript.

The introns are assayed for the ability to enhance expression in transient assay or stable plant assay. For transient assay of intron mediated enhancement, a base plant vector is

constructed using methods known in the art. The intron is cloned into a base plant vector which comprises an expression cassette comprised of a constitutive promoter such as the Cauliflower mosaic virus promoter, P-CaMV.35S-enh-1:1:9 (SEQ ID NO: 1096), operably linked 5' to a leader element, L-CaMV.35S-1:1:15 (SEQ ID NO: 1111), operably linked 5' to  
5 a test intron element (SEQ ID NOS: 172 through 267 and SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778), operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS) that either possesses a processable intron (GUS-2, SEQ ID NO: 1091) or no intron (GUS-1, SEQ ID NO: 1090), operably linked to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). Protoplast cells derived from  
10 corn or other genus plant tissue is transformed with the base plant vector and assayed for activity. A comparison of activity is made using a control plasmid comprised of the same transgene cassette as the test plasmid, but without the test intron to see if the intron provides an intron mediated enhancement effect.

Two plasmids, for use in co-transformation and normalization of data, are also  
15 constructed using methods known in the art. Each plasmid contains a specific luciferase coding sequence which is driven by a constitutive transcriptional regulatory expression element group. The plant vector, pMON19437 is comprised of a transgene cassette comprised of a constitutive promoter (EXP-CaMV.35S-enh, SEQ ID NO: 1095), operably linked 5' to an intron, (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably linked 5' to a firefly  
20 (*Photinus pyralis*) luciferase coding sequence (LUCIFERASE:1:3, SEQ ID NO: 1109), operably linked 5' to a 3' termination region from the *A. tumefaciens* nopaline synthase gene (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). The plant vector, pMON63934 is comprised of a transgene cassette comprised of a constitutive transcriptional regulatory expression element group, (EXP-CaMV.35S-enh-Lhcb1, SEQ ID NO: 1106), operably linked 5' to a sea pansy  
25 (*Renilla reniformis*) luciferase coding sequence (CR-Ren.hRenilla Lucife-0:0:1, SEQ ID NO: 1110), operably linked 5' to a 3' termination region from the *A. tumefaciens* nopaline synthase gene (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088).

Corn leaf protoplasts or other genus plant protoplasts are transformed using a PEG-based transformation method, similar to those known in the art. Protoplast cells are  
30 transformed with a DNA prep comprised of equimolar quantities of the two luciferase expression plasmids, pMON19437 and pMON63934 and one of the test plasmids and incubated overnight in total darkness. After incubation, the cells are rinsed, resuspended and lysed. Measurements of both GUS and luciferase are conducted using aliquots of each lysis preparation. Essentially, the collected, transformed protoplast cells are lysed in 5X passive



lysis buffer (Promega). After allowing for lysis, aliquots of the lysed preparation are placed into two different small-well trays. One tray is used for GUS measurements. For quantitative analysis of GUS expression, total protein is extracted from lysis preparation. One microgram of total protein is used with the fluorogenic substrate 4-methyleumbelliferyl- $\beta$ -D-glucuronide (MUG) in a total reaction volume of 50 microliters. The reaction product, 4-methyleumbelliferone (4-MU), is maximally fluorescent at high pH, where the hydroxyl group is ionized. Addition of a basic solution of sodium carbonate simultaneously stops the assay and adjusts the pH for quantifying the fluorescent product. Fluorescence is measured with excitation at 365 nm, emission at 445 nm using a Fluoromax-3 with Micromax Reader, with slit width set at excitation 2 nm and emission 3nm. GUS values are expressed as pmol of 4-MU protein per minute per milligram protein ( $\text{pmol 4-MU min}^{-1} \text{mg}^{-1} \text{protein}$ ).

The second tray is used to perform a dual luciferase assay as outlined in Example 5 above. To compare the relative ability of the intron to enhance expression, GUS values are expressed as a ratio of GUS to luciferase activity and compared with those levels imparted by the constitutive promoter and a known intron standard such as that as the intron derived from the HSP70 heat shock protein of *Zea mays*, I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102).

For stable plant assay of the introns presented as SEQ ID NOS: 172 through 267 and SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778, a GUS expression plant transformation vector is constructed using methods known in the art similar to the constructs described in the previous examples in which the resulting plant expression vectors contains a right border region from *A. tumefaciens*, a first transgene cassette to test the intron comprised of a constitutive promoter such as the Cauliflower mosaic virus promoter, P-CaMV.35S-enh-1:1:9 (SEQ ID NO: 1096), operably linked 5' to a leader element, L-CaMV.35S-1:1:15 (SEQ ID NO: 1111), operably linked 5' to a test intron element (SEQ ID NOS: 16 through 306), operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS) that either possesses a processable intron (GUS-2, SEQ ID NO: 1091) or no intron (GUS-1, SEQ ID NO: 1090), operably linked to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088) ; a second transgene selection cassette used for selection of transformed plant cells that confers resistance to the herbicide glyphosate (driven by the rice Actin 1 promoter), or alternatively, the antibiotic kanamycin (driven by the rice Actin 1 promoter) and a left border region from *A. tumefaciens*. The resulting plasmids are used to transform corn plants or other genus plants by the methods described above or by *Agrobacterium*-mediated methods known in the art. Single-copy or low copy

number transformants are selected for comparison to single-copy or low copy number transformed plants, transformed with a plant transformation vector identical to the test vector but without the test intron to determine if the test intron provides an intron mediated enhancement effect.

5 Any of the introns presented as SEQ ID NOS: 172 through 267 and SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778 can be modified in a number of ways, such as deleting fragments within the intron sequence which may reduce expression or duplication of fragments with the intron that may enhance expression. In addition, sequences within the intron that may affect the specificity of expression to either particular cells types or  
10 tissues and organs can be duplicated or altered or deleted to affect expression and patterns of expression of the transgene. In addition, the introns presented as SEQ ID NOS: 172 through 267 and SEQ ID NOS: 317 through 323 and SEQ ID NOS: 589 through 778 can be modified to remove any potential start codons (ATG) that may cause unintentional transcripts from being expressed from improperly spliced introns as different, longer or truncated proteins.  
15 Once the intron has been empirically tested, or it has been altered based upon experimentation, the intron is used to enhance expression of a transgene in stably transformed plants that can be of any genus monocot or dicot plant, so long as the intron provides enhancement of the transgene. The intron can also be used to enhance expression in other organisms, such as algae, fungi or animal cells, so long as the intron provides enhancement or  
20 attenuation or specificity of expression of the transgene to which it is operably linked.

#### **Example 12: Plasmid constructs comprised of transgene cassettes for analysis of Intron-Mediated Enhancement of GUS Transgene Activity**

Intron elements isolated from *Setaria italica* are cloned using methods known in the art into plasmid constructs comprising a constitutive promoter to test the effect of the intron  
25 on expression of a GUS transgene driven by a constitutive promoter.

The intron elements presented as SEQ ID NOS: 179 through 267 were cloned into plasmid constructs comprising a constitutive promoter, P-CaMV.35S-enh-1:1:13 (SEQ ID NO: 1112), operably linked 5' to a leader element, L-CaMV.35S-1:1:15 (SEQ ID NO: 1111), operably linked to a test intron element (SEQ ID NOS: 179 through 267), operably linked 5'  
30 to a GUS coding sequence, (GUS-1, SEQ ID NO: 1090), operably linked 5' to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). The plasmid construct identifier along with each intron annotation is presented in Table 19 below.

**Table 19. Plasmid constructs comprising transgene cassettes to evaluate intron-mediated enhancement of GUS transgene expression.**

<b>Construct</b>	<b>Intron Annotation</b>	<b>Intron SEQ ID NO:</b>
pMON138824	I-SETit.14-3-3A-2-1:1:1	179
pMON138826	I-SETit.14-3-3A-3-1:1:2	180
pMON138816	I-SETit.14-3-3A-4-1:1:2	181
pMON138813	I-SETit.14-3-3A-5-1:1:2	182
pMON138829	I-SETit.14-3-3B-2-1:1:1	183
pMON138830	I-SETit.14-3-3B-3-1:1:2	184
pMON138820	I-SETit.14-3-3B-4-1:1:2	185
pMON138821	I-SETit.14-3-3B-5-1:1:2	186
pMON138831	I-SETit.14-3-3C-1-1:1:1	187
pMON138823	I-SETit.14-3-3C-2-1:1:1	188
pMON138817	I-SETit.14-3-3C-3-1:1:2	189
pMON138832	I-SETit.14-3-3C-4-1:1:2	190
pMON138822	I-SETit.14-3-3C-5-1:1:2	191
pMON138825	I-SETit.14-3-3D-1-1:1:2	192
pMON138828	I-SETit.14-3-3D-2-1:1:1	193
pMON138814	I-SETit.14-3-3D-3-1:1:2	194
pMON138827	I-SETit.14-3-3D-4-1:1:3	195
pMON138843	I-SETit.14-3-3D-5-1:1:2	196
pMON138835	I-SETit.14-3-3E-2-1:1:1	197
pMON138836	I-SETit.14-3-3E-3-1:1:2	198
pMON138845	I-SETit.14-3-3E-4-1:1:2	199
pMON138841	I-SETit.14-3-3E-5-1:1:2	200
pMON138794	I-SETit.40S-7S-1_1-1:1:2	201
pMON138792	I-SETit.40S-7S-1_2-1:1:2	202
pMON138791	I-SETit.40S-7S-1_3-1:1:2	203
pMON138788	I-SETit.40S-7S-1_4-1:1:2	204
pMON138798	I-SETit.40S-7S-2_2-1:1:1	205
pMON138802	I-SETit.40S-7S-2_3-1:1:1	206
pMON138807	I-SETit.40S-7S-2_4-1:1:1	207
pMON138809	I-SETit.40S-7S-3_1-1:1:2	208
pMON138810	I-SETit.40S-7S-3_2-1:1:2	209
pMON138800	I-SETit.40S-7S-3_3-1:1:2	210
pMON138837	I-SETit.60S_L10A1-1-1:1:2	211
pMON138847	I-SETit.60S_L10A1-2-1:1:2	212
pMON138840	I-SETit.60S_L10A1-3-1:1:2	213
pMON140752	I-SETit.60S_L10A1-4-1:1:1	214

pMON140750	I-SETit.60S_L10A1-5-1:1:2	215
pMON140758	I-SETit.ASA2-3-1:1:2	216
pMON140757	I-SETit.ClpD-1-1:1:1	217
pMON138783	I-SETit.DnaJ_1-1:1:2	218
pMON138848	I-SETit.DnaJ3-2-1:1:2	219
pMON138786	I-SETit.eEF1g_1-1:1:2	220
pMON138787	I-SETit.eEF1g_4-1:1:3	221
pMON138842	I-SETit.eIF5A1-1-1:1:1	222
pMON138833	I-SETit.eIF5A1-2-1:1:2	223
pMON138844	I-SETit.eIF5A1-3-1:1:2	224
pMON138834	I-SETit.eIF5A1-4-1:1:2	225
pMON140751	I-SETit.eIF5A1-5-1:1:2	226
pMON138838	I-SETit.eIF5A2-1-1:1:2	227
pMON138839	I-SETit.eIF5A2-2-1:1:3	228
pMON138846	I-SETit.eIF5A2-3-1:1:2	229
pMON138849	I-SETit.eIF5A2-4-1:1:2	230
pMON140756	I-SETit.eIF5A2-5-1:1:2	231
pMON140753	I-SETit.eIF5A3-1-1:1:1	232
pMON140769	I-SETit.eIF5A3-2-1:1:2	233
pMON140754	I-SETit.eIF5A3-3-1:1:2	234
pMON140760	I-SETit.eIF5A3-4-1:1:2	235
pMON140763	I-SETit.eIF5A3-5-1:1:2	236
pMON138808	I-SETit.GAD_1-1:1:2	237
pMON138819	I-SETit.GAD_2-1:1:2	238
pMON138815	I-SETit.GAD_3-1:1:2	239
pMON138818	I-SETit.GAD_4-1:1:2	240
pMON140765	I-SETit.Grf1-3-1:1:1	241
pMON138806	I-SETit.GRP-1-1:1:1	242
pMON140768	I-SETit.LSm8-1-1:1:2	243
pMON140767	I-SETit.LSm8-2-1:1:1	244
pMON140771	I-SETit.LSm8-3-1:1:1	245
pMON140762	I-SETit.LSm8-4-1:1:2	246
pMON138812	I-SETit.PGK3_1-1:1:2	247
pMON138804	I-SETit.PGK3_2-1:1:1	248
pMON138793	I-SETit.PIP1_1_2-1:1:1	249
pMON138797	I-SETit.PIP1-1_1-1:1:1	250
pMON138796	I-SETit.PIP1-1_3-1:1:2	251
pMON138785	I-SETit.PIP1-4_3-1:1:2	252
pMON138784	I-SETit.PIP2-2_2-1:1:2	253
pMON138789	I-SETit.PIP2-2_3-1:1:2	254
pMON138790	I-SETit.PIP2-5_2-1:1:2	255
pMON138795	I-SETit.PIP2-5_3-1:1:2	256

pMON140764	I-SETit.Prx17-2-1:1:1	257
pMON140766	I-SETit.Prx3-1-1:1:2	258
pMON140770	I-SETit.SBD-1-1:1:2	259
pMON140761	I-SETit.SBD-2-1:1:1	260
pMON140759	I-SETit.SBD-3-1:1:2	261
pMON138799	I-SETit.TubA2_1-1:1:1	262
pMON138801	I-SETit.TubA2_2-1:1:1	263
pMON138805	I-SETit.TubA2_3-1:1:1	264
pMON138811	I-SETit.TubA3_1-1:1:1	265
pMON138803	I-SETit.TubA3_2-1:1:1	266
pMON140755	I-SETit.Wx1-1-1:1:2	267

Each plasmid was used as template for PCR amplification of the transgene cassette for use in protoplast assay as described below.

### Example 13: Analysis of Intron-Mediated Enhancement of GUS Activity Using Corn Protoplasts

Protoplast cells, derived from corn leaf tissue, are transformed with transgene cassettes in the form of PCR amplicons to determine the effect of each intron on the expression of the transgene, GUS, driven by a constitutive promoter and compared to introns known to have an enhancement effect on transgene expression.

The constructs described in example 12 above and presented in Table 19 of example 12 were used as template to generate PCR amplicons using methods known in the art comprising a constitutive promoter, P-CaMV.35S-enh-1:1:13 (SEQ ID NO: 1112), operably linked 5' to a leader element, L-CaMV.35S-1:1:15 (SEQ ID NO: 1111), operably linked to a test intron element (SEQ ID NOS: 179 through 267), operably linked 5' to a GUS coding sequence, (GUS-1, SEQ ID NO: 1090), operably linked 5' to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). In addition, amplicons, derived from control plasmids were also generated using the same amplification methods from the control plasmid constructs, pMON19469, pMON65328, pMON25455, pMON8677 and pMON33449. The transgene cassette of pMON19469 is comprised of the transcriptional regulatory element group, EXP-CaMV.35S-enh/I-Zm.DnaK-1:1:1 (SEQ ID NO: 1104) and provides a comparison of the constitutive promoter enhanced using the intron I-Zm.DnaK-1:1:1. The transgene cassette of pMON65328 is comprised of the transcriptional regulatory element group, EXP-CaMV.35S-enh-Lhcb1/I-Os.Act1-1:1:9 (SEQ ID NO: 1105) and provides a comparison of enhancement of the

constitutive promoter using the intron, I-Os.Act1-1:1:9. The transgene cassette of pMON25455 is comprised of the transcriptional regulatory element group, EXP-Os.Act1:1:1 (SEQ ID NO: 1098) and provides a comparison of expression using the native promoter, leader and intron of the rice actin 1 gene. The transgene cassette of pMON8677 is comprised of the promoter, P-CaMV.35S-enh-1:1:9 (SEQ ID NO: 1096) operably linked to the GUS coding sequence and provides a comparison of expression with a constitutive promoter without an intron for enhancement. The transgene cassette of pMON33449 is comprised of a variant of the CaMV 35S promoter (P-E35S:1:52, SEQ ID NO: 1117) and provides an additional comparator lacking an intron.

Protoplast cells, derived from corn leaf tissue are transformed using PEG-based transformation methods known in the art. Briefly, each test and control construct is used to provide template for amplification of the transgene cassette comprising each construct. The resulting amplicon is size fractionated on an agarose gel and the amplicon DNA is excised from the gel. The amplicon DNA is extracted from the gel fragment using methods known in the art and quantified by spectrophotometry. Protoplast cells are transformed using PEG methods known in the art and using either 0.3 or 0.1 pico-moles of PCR amplicon DNA. Two to four replicates are performed for each transformation and the average expression imparted by each construct determined. The mean GUS expression observed for each construct derived amplicon is normalized with respect to expression observed for the amplicon derived from pMON19469 comprised of the intron element, I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102). Tables 20 and 21 below show the normalized average GUS expression imparted amplicons derived from the constructs presented in table 19 of example 12 relative to the average GUS expression imparted to amplicons derived from the control plasmids described above using 0.3 and 0.1 pmol of amplicon, respectively.

**Table 20. Intron-mediated enhancement of GUS expression in corn protoplasts relative to I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102) using 0.3 pmol of amplicon DNA.**

Construct	Intron Annotation	Intron SEQ ID NO:	Mean	Std Deviation
pMON138842	I-SETit.eIF5A1-1-1:1:1	222	3.19777	0.14472
pMON140750	I-SETit.60S_L10A1-5-1:1:2	215	2.73551	0.14472
pMON138806	I-SETit.GRP-1-1:1:1	242	2.24406	0.16711
pMON140768	I-SETit.LSm8-1-1:1:2	243	1.79949	0.14472
pMON138847	I-SETit.60S_L10A1-2-	212	1.76055	0.14472

	1:1:2			
pMON138805	I-SETit.TubA2_3-1:1:1	264	1.6441	0.16711
pMON138826	I-SETit.14-3-3A-3-1:1:2	180	1.59333	0.14472
pMON138844	I-SETit.eIF5A1-3-1:1:2	224	1.51864	0.16711
pMON138845	I-SETit.14-3-3E-4-1:1:2	199	1.47841	0.14472
pMON138785	I-SETit.PIP1-4_3-1:1:2	252	1.45536	0.14472
pMON138812	I-SETit.PGK3_1-1:1:2	247	1.36129	0.14472
pMON138809	I-SETit.40S-7S-3_1-1:1:2	208	1.35665	0.14472
pMON138796	I-SETit.PIP1-1_3-1:1:2	251	1.30268	0.14472
pMON140762	I-SETit.LSm8-4-1:1:2	246	1.28583	0.14472
pMON138839	I-SETit.eIF5A2-2-1:1:3	228	1.26341	0.16711
pMON138836	I-SETit.14-3-3E-3-1:1:2	198	1.23008	0.14472
pMON140767	I-SETit.LSm8-2-1:1:1	244	1.22373	0.14472
pMON138824	I-SETit.14-3-3A-2-1:1:1	179	1.2226	0.16711
pMON140754	I-SETit.eIF5A3-3-1:1:2	234	1.22009	0.14472
pMON138829	I-SETit.14-3-3B-2-1:1:1	183	1.17955	0.14472
pMON138827	I-SETit.14-3-3D-4-1:1:3	195	1.15355	0.14472
pMON138831	I-SETit.14-3-3C-1-1:1:1	187	1.13972	0.20467
pMON138817	I-SETit.14-3-3C-3-1:1:2	189	1.09812	0.14472
pMON138783	I-SETit.DnaJ_1-1:1:2	218	1.08489	0.14472
pMON140769	I-SETit.eIF5A3-2-1:1:2	233	1.0434	0.14472
pMON138792	I-SETit.40S-7S-1_2-1:1:2	202	1.04308	0.16711
pMON140760	I-SETit.eIF5A3-4-1:1:2	235	1.04187	0.14472
pMON140758	I-SETit.ASA2-3-1:1:2	216	1.01034	0.14472
pMON19469	I-Zm.DnaK-1:1:1	1102	1	0.14472
pMON140761	I-SETit.SBD-2-1:1:1	260	0.99105	0.16711
pMON138794	I-SETit.40S-7S-1_1-1:1:2	201	0.98962	0.14472
pMON138789	I-SETit.PIP2-2_3-1:1:2	254	0.96195	0.14472
pMON138791	I-SETit.40S-7S-1_3-1:1:2	203	0.94544	0.14472
pMON138830	I-SETit.14-3-3B-3-1:1:2	184	0.92124	0.16711
pMON140766	I-SETit.Prx3-1-1:1:2	258	0.88915	0.14472
pMON138803	I-SETit.TubA3_2-1:1:1	266	0.83768	0.16711
pMON138832	I-SETit.14-3-3C-4-1:1:2	190	0.80355	0.14472
pMON138848	I-SETit.DnaJ3-2-1:1:2	219	0.7898	0.16711
pMON138835	I-SETit.14-3-3E-2-1:1:1	197	0.78927	0.14472
pMON138834	I-SETit.eIF5A1-4-1:1:2	225	0.77272	0.14472
pMON138821	I-SETit.14-3-3B-5-1:1:2	186	0.671	0.14472
pMON138849	I-SETit.eIF5A2-4-1:1:2	230	0.67063	0.14472
pMON138786	I-SETit.eEF1g_1-1:1:2	220	0.6574	0.16711
pMON140756	I-SETit.eIF5A2-5-1:1:2	231	0.6269	0.16711
pMON65328	I-Os.Act1-1:1:19	1113	0.58395	0.14472
pMON138840	I-SETit.60S_L10A1-3-1:1:2	213	0.57366	0.14472

pMON138822	I-SETit.14-3-3C-5-1:1:2	191	0.55845	0.14472
pMON140757	I-SETit.ClpD-1-1:1:1	217	0.53607	0.14472
pMON140753	I-SETit.eIF5A3-1-1:1:1	232	0.52545	0.14472
pMON138820	I-SETit.14-3-3B-4-1:1:2	185	0.52034	0.16711
pMON138800	I-SETit.40S-7S-3_3-1:1:2	210	0.51862	0.16711
pMON138828	I-SETit.14-3-3D-2-1:1:1	193	0.51592	0.14472
pMON138813	I-SETit.14-3-3A-5-1:1:2	182	0.51379	0.14472
pMON138843	I-SETit.14-3-3D-5-1:1:2	196	0.50543	0.14472
pMON138825	I-SETit.14-3-3D-1-1:1:2	192	0.48895	0.14472
pMON138814	I-SETit.14-3-3D-3-1:1:2	194	0.48519	0.14472
pMON138818	I-SETit.GAD_4-1:1:2	240	0.47986	0.14472
pMON138811	I-SETit.TubA3_1-1:1:1	265	0.47762	0.16711
pMON138823	I-SETit.14-3-3C-2-1:1:1	188	0.41543	0.14472
pMON138837	I-SETit.60S_L10A1-1-1:1:2	211	0.40991	0.14472
pMON138846	I-SETit.eIF5A2-3-1:1:2	229	0.40485	0.14472
pMON140763	I-SETit.eIF5A3-5-1:1:2	236	0.40131	0.14472
pMON138838	I-SETit.eIF5A2-1-1:1:2	227	0.37694	0.14472
pMON138801	I-SETit.TubA2_2-1:1:1	263	0.35386	0.14472
pMON138804	I-SETit.PGK3_2-1:1:1	248	0.35284	0.16711
pMON140765	I-SETit.Grf1-3-1:1:1	241	0.32325	0.16711
pMON140755	I-SETit.Wx1-1-1:1:2	267	0.31895	0.16711
pMON138790	I-SETit.PIP2-5_2-1:1:2	255	0.2957	0.16711
pMON140770	I-SETit.SBD-1-1:1:2	259	0.29309	0.14472
pMON138784	I-SETit.PIP2-2_2-1:1:2	253	0.29174	0.14472
pMON138808	I-SETit.GAD_1-1:1:2	237	0.2874	0.14472
pMON138807	I-SETit.40S-7S-2_4-1:1:1	207	0.27522	0.16711
pMON138819	I-SETit.GAD_2-1:1:2	238	0.27038	0.14472
pMON138833	I-SETit.eIF5A1-2-1:1:2	223	0.25147	0.14472
pMON138795	I-SETit.PIP2-5_3-1:1:2	256	0.24221	0.14472
pMON138802	I-SETit.40S-7S-2_3-1:1:1	206	0.23983	0.16711
pMON25455 EXP-Os.Act1 (SEQ ID NO: 1098)	EXP-Os.Act1		0.21736	0.16711
pMON140759	I-SETit.SBD-3-1:1:2	261	0.2172	0.16711
pMON138787	I-SETit.eEF1g_4-1:1:3	221	0.17568	0.14472
pMON138810	I-SETit.40S-7S-3_2-1:1:2	209	0.16997	0.14472
pMON138816	I-SETit.14-3-3A-4-1:1:2	181	0.16765	0.14472
pMON138815	I-SETit.GAD_3-1:1:2	239	0.16017	0.20467
pMON138788	I-SETit.40S-7S-1_4-1:1:2	204	0.15842	0.16711
pMON140752	I-SETit.60S_L10A1-4-1:1:1	214	0.15302	0.14472
pMON140751	I-SETit.eIF5A1-5-1:1:2	226	0.14712	0.14472



pMON138841	I-SETit.14-3-3E-5-1:1:2	200	0.14157	0.14472
pMON8677	No intron		0.14109	0.14472
pMON138798	I-SETit.40S-7S-2_2-1:1:1	205	0.11752	0.16711
pMON138793	I-SETit.PIP1_1_2-1:1:1	249	0.11305	0.14472
pMON33449	No intron		0.10504	0.14472
pMON140764	I-SETit.Prx17-2-1:1:1	257	0.10398	0.16711
pMON140771	I-SETit.LSm8-3-1:1:1	245	0.09975	0.14472
pMON138797	I-SETit.PIP1-1_1-1:1:1	250	0.03152	0.14472
pMON138799	I-SETit.TubA2_1-1:1:1	262	0.01853	0.14472
No DNA			0.001	0.20467

Using 0.3 pmol of amplicon, most of the test intron elements, derived from *S. italica*, enhanced GUS expression relative to the no intron controls using a similar promoter (pMON8677 and pMON33449). Improved enhancement of GUS expression, relative to the intron element, I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102) was observed for the intron elements, I-SETit.eIF5A1-1-1:1:1 (SEQ ID NO: 222), I-SETit.60S\_L10A1-5-1:1:2 (SEQ ID NO: 215), I-SETit.GRP-1-1:1:1 (SEQ ID NO: 242), I-SETit.LSm8-1-1:1:2 (SEQ ID NO: 243), I-SETit.60S\_L10A1-2-1:1:2 (SEQ ID NO: 212), I-SETit.TubA2\_3-1:1:1 (SEQ ID NO: 264), I-SETit.14-3-3A-3-1:1:2 (SEQ ID NO: 180), I-SETit.eIF5A1-3-1:1:2 (SEQ ID NO: 224), I-SETit.14-3-3E-4-1:1:2 (SEQ ID NO: 199), I-SETit.PIP1-4\_3-1:1:2 (SEQ ID NO: 252), I-SETit.PGK3\_1-1:1:2 (SEQ ID NO: 247), I-SETit.40S-7S-3\_1-1:1:2 (SEQ ID NO: 208), I-SETit.PIP1-1\_3-1:1:2 (SEQ ID NO: 251), I-SETit.LSm8-4-1:1:2 (SEQ ID NO: 246), I-SETit.eIF5A2-2-1:1:3 (SEQ ID NO: 228), I-SETit.14-3-3E-3-1:1:2 (SEQ ID NO: 198), I-SETit.LSm8-2-1:1:1 (SEQ ID NO: 244), I-SETit.14-3-3A-2-1:1:1 (SEQ ID NO: 179), I-SETit.eIF5A3-3-1:1:2 (SEQ ID NO: 234), I-SETit.14-3-3B-2-1:1:1 (SEQ ID NO: 183), I-SETit.14-3-3D-4-1:1:3 (SEQ ID NO: 195), I-SETit.14-3-3C-1-1:1:1 (SEQ ID NO: 187), I-SETit.14-3-3C-3-1:1:2 (SEQ ID NO: 189), I-SETit.DnaJ\_1-1:1:2 (SEQ ID NO: 218), I-SETit.eIF5A3-2-1:1:2 (SEQ ID NO: 233), I-SETit.40S-7S-1\_2-1:1:2 (SEQ ID NO: 202), I-SETit.eIF5A3-4-1:1:2 (SEQ ID NO: 235) and I-SETit.ASA2-3-1:1:2 (SEQ ID NO: 216).

Data shown in Table 21 below.

**Table 21. Intron-mediated enhancement of GUS expression in corn protoplasts relative to I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102) using 0.1 pmol amplicon DNA.**

Construct	Intron Annotation	Intron SEQ ID NO:	Mean	Std Deviation
pMON138842	I-SETit.eIF5A1-1-1:1:1	222	3.19777	0.14472
pMON140750	I-SETit.60S_L10A1-5-1:1:2	215	2.73551	0.14472
pMON138806	I-SETit.GRP-1-1:1:1	242	2.24406	0.16711
pMON140768	I-SETit.LSm8-1-1:1:2	243	1.79949	0.14472
pMON138847	I-SETit.60S_L10A1-2-1:1:2	212	1.76055	0.14472
pMON138805	I-SETit.TubA2_3-1:1:1	264	1.6441	0.16711
pMON138826	I-SETit.14-3-3A-3-1:1:2	180	1.59333	0.14472
pMON138844	I-SETit.eIF5A1-3-1:1:2	224	1.51864	0.16711
pMON138845	I-SETit.14-3-3E-4-1:1:2	199	1.47841	0.14472
pMON138785	I-SETit.PIP1-4_3-1:1:2	252	1.45536	0.14472
pMON138812	I-SETit.PGK3_1-1:1:2	247	1.36129	0.14472
pMON138809	I-SETit.40S-7S-3_1-1:1:2	208	1.35665	0.14472
pMON138796	I-SETit.PIP1-1_3-1:1:2	251	1.30268	0.14472
pMON140762	I-SETit.LSm8-4-1:1:2	246	1.28583	0.14472
pMON138839	I-SETit.eIF5A2-2-1:1:3	228	1.26341	0.16711
pMON138836	I-SETit.14-3-3E-3-1:1:2	198	1.23008	0.14472
pMON140767	I-SETit.LSm8-2-1:1:1	244	1.22373	0.14472
pMON138824	I-SETit.14-3-3A-2-1:1:1	179	1.2226	0.16711
pMON140754	I-SETit.eIF5A3-3-1:1:2	234	1.22009	0.14472
pMON138829	I-SETit.14-3-3B-2-1:1:1	183	1.17955	0.14472
pMON138827	I-SETit.14-3-3D-4-1:1:3	195	1.15355	0.14472
pMON138831	I-SETit.14-3-3C-1-1:1:1	187	1.13972	0.20467
pMON138817	I-SETit.14-3-3C-3-1:1:2	189	1.09812	0.14472
pMON138783	I-SETit.DnaJ_1-1:1:2	218	1.08489	0.14472
pMON140769	I-SETit.eIF5A3-2-1:1:2	233	1.0434	0.14472
pMON138792	I-SETit.40S-7S-1_2-1:1:2	202	1.04308	0.16711
pMON140760	I-SETit.eIF5A3-4-1:1:2	235	1.04187	0.14472
pMON140758	I-SETit.ASA2-3-1:1:2	216	1.01034	0.14472
pMON19469	I-Zm.DnaK-1:1:1	1102	1	0.14472
pMON140761	I-SETit.SBD-2-1:1:1	260	0.99105	0.16711
pMON138794	I-SETit.40S-7S-1_1-1:1:2	201	0.98962	0.14472
pMON138789	I-SETit.PIP2-2_3-1:1:2	254	0.96195	0.14472
pMON138791	I-SETit.40S-7S-1_3-1:1:2	203	0.94544	0.14472
pMON138830	I-SETit.14-3-3B-3-1:1:2	184	0.92124	0.16711
pMON140766	I-SETit.Prx3-1-1:1:2	258	0.88915	0.14472

pMON138803	I-SETit.TubA3_2-1:1:1	266	0.83768	0.16711
pMON138832	I-SETit.14-3-3C-4-1:1:2	190	0.80355	0.14472
pMON138848	I-SETit.DnaJ3-2-1:1:2	219	0.7898	0.16711
pMON138835	I-SETit.14-3-3E-2-1:1:1	197	0.78927	0.14472
pMON138834	I-SETit.eIF5A1-4-1:1:2	225	0.77272	0.14472
pMON138821	I-SETit.14-3-3B-5-1:1:2	186	0.671	0.14472
pMON138849	I-SETit.eIF5A2-4-1:1:2	230	0.67063	0.14472
pMON138786	I-SETit.eEF1g_1-1:1:2	220	0.6574	0.16711
pMON140756	I-SETit.eIF5A2-5-1:1:2	231	0.6269	0.16711
pMON65328	I-Os.Act1-1:1:19	1113	0.58395	0.14472
pMON138840	I-SETit.60S_L10A1-3-1:1:2	213	0.57366	0.14472
pMON138822	I-SETit.14-3-3C-5-1:1:2	191	0.55845	0.14472
pMON140757	I-SETit.ClpD-1-1:1:1	217	0.53607	0.14472
pMON140753	I-SETit.eIF5A3-1-1:1:1	232	0.52545	0.14472
pMON138820	I-SETit.14-3-3B-4-1:1:2	185	0.52034	0.16711
pMON138800	I-SETit.40S-7S-3_3-1:1:2	210	0.51862	0.16711
pMON138828	I-SETit.14-3-3D-2-1:1:1	193	0.51592	0.14472
pMON138813	I-SETit.14-3-3A-5-1:1:2	182	0.51379	0.14472
pMON138843	I-SETit.14-3-3D-5-1:1:2	196	0.50543	0.14472
pMON138825	I-SETit.14-3-3D-1-1:1:2	192	0.48895	0.14472
pMON138814	I-SETit.14-3-3D-3-1:1:2	194	0.48519	0.14472
pMON138818	I-SETit.GAD_4-1:1:2	240	0.47986	0.14472
pMON138811	I-SETit.TubA3_1-1:1:1	265	0.47762	0.16711
pMON138823	I-SETit.14-3-3C-2-1:1:1	188	0.41543	0.14472
pMON138837	I-SETit.60S_L10A1-1-1:1:2	211	0.40991	0.14472
pMON138846	I-SETit.eIF5A2-3-1:1:2	229	0.40485	0.14472
pMON140763	I-SETit.eIF5A3-5-1:1:2	236	0.40131	0.14472
pMON138838	I-SETit.eIF5A2-1-1:1:2	227	0.37694	0.14472
pMON138801	I-SETit.TubA2_2-1:1:1	263	0.35386	0.14472
pMON138804	I-SETit.PGK3_2-1:1:1	248	0.35284	0.16711
pMON140765	I-SETit.Grf1-3-1:1:1	241	0.32325	0.16711
pMON140755	I-SETit.Wx1-1-1:1:2	267	0.31895	0.16711
pMON138790	I-SETit.PIP2-5_2-1:1:2	255	0.2957	0.16711
pMON140770	I-SETit.SBD-1-1:1:2	259	0.29309	0.14472
pMON138784	I-SETit.PIP2-2_2-1:1:2	253	0.29174	0.14472
pMON138808	I-SETit.GAD_1-1:1:2	237	0.2874	0.14472
pMON138807	I-SETit.40S-7S-2_4-1:1:1	207	0.27522	0.16711
pMON138819	I-SETit.GAD_2-1:1:2	238	0.27038	0.14472
pMON138833	I-SETit.eIF5A1-2-1:1:2	223	0.25147	0.14472
pMON138795	I-SETit.PIP2-5_3-1:1:2	256	0.24221	0.14472
pMON138802	I-SETit.40S-7S-2_3-1:1:1	206	0.23983	0.16711

pMON25455 EXP-Os.Act1 (SEQ ID NO: 1098)	EXP-Os.Act1		0.21736	0.16711
pMON140759	I-SETit.SBD-3-1:1:2	261	0.2172	0.16711
pMON138787	I-SETit.eEF1g_4-1:1:3	221	0.17568	0.14472
pMON138810	I-SETit.40S-7S-3_2-1:1:2	209	0.16997	0.14472
pMON138816	I-SETit.14-3-3A-4-1:1:2	181	0.16765	0.14472
pMON138815	I-SETit.GAD_3-1:1:2	239	0.16017	0.20467
pMON138788	I-SETit.40S-7S-1_4-1:1:2	204	0.15842	0.16711
pMON140752	I-SETit.60S_L10A1-4- 1:1:1	214	0.15302	0.14472
pMON140751	I-SETit.eIF5A1-5-1:1:2	226	0.14712	0.14472
pMON138841	I-SETit.14-3-3E-5-1:1:2	200	0.14157	0.14472
pMON8677	No intron		0.14109	0.14472
pMON138798	I-SETit.40S-7S-2_2-1:1:1	205	0.11752	0.16711
pMON138793	I-SETit.PIP1_1_2-1:1:1	249	0.11305	0.14472
pMON33449	No intron		0.10504	0.14472
pMON140764	I-SETit.Prx17-2-1:1:1	257	0.10398	0.16711
pMON140771	I-SETit.LSm8-3-1:1:1	245	0.09975	0.14472
pMON138797	I-SETit.PIP1-1_1-1:1:1	250	0.03152	0.14472
pMON138799	I-SETit.TubA2_1-1:1:1	262	0.01853	0.14472
No DNA			0.001	0.20467

The basic trend in enhancement was similar using 0.1 pmol of amplicon. Most of the test intron elements, derived from *S. italica*, enhanced GUS expression relative to the no intron controls using a similar promoter (pMON8677 and pMON33449). Improved enhancement of GUS expression, relative to the intron element, I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102) was observed using the intron elements, I-SETit.LSm8-1-1:1:2 (SEQ ID NO: 243), I-SETit.60S\_L10A1-5-1:1:2 (SEQ ID NO: 215), I-SETit.eIF5A1-3-1:1:2 (SEQ ID NO: 224), I-SETit.GRP-1-1:1:1 (SEQ ID NO: 242), I-SETit.14-3-3D-2-1:1:1 (SEQ ID NO: 193), I-SETit.14-3-3D-3-1:1:2 (SEQ ID NO: 194), I-SETit.eIF5A3-3-1:1:2 (SEQ ID NO: 234), I-SETit.LSm8-4-1:1:2 (SEQ ID NO: 246), I-SETit.LSm8-2-1:1:1 (SEQ ID NO: 244), I-SETit.eIF5A3-2-1:1:2 (SEQ ID NO: 233), I-SETit.eIF5A1-4-1:1:2 (SEQ ID NO: 225), I-SETit.ASA2-3-1:1:2 (SEQ ID NO: 216), I-SETit.60S\_L10A1-2-1:1:2 (SEQ ID NO: 212), I-SETit.eIF5A2-5-1:1:2 (SEQ ID NO: 231), I-SETit.14-3-3B-3-1:1:2 (SEQ ID NO: 184), I-SETit.14-3-3B-2-1:1:1 (SEQ ID NO: 183), I-SETit.DnaJ3-2-1:1:2 (SEQ ID NO: 219), I-SETit.14-3-3A-3-1:1:2 (SEQ ID NO: 180), I-SETit.eIF5A3-4-1:1:2 (SEQ ID NO: 235), I-SETit.14-3-3C-5-1:1:2 (SEQ ID NO: 191), I-SETit.TubA3\_2-1:1:1 (SEQ ID NO: 266), I-SETit.40S-7S-1\_3-1:1:2 (SEQ ID NO: 203), I-SETit.TubA2\_3-1:1:1 (SEQ ID NO: 264), I-

SETit.14-3-3C-3-1:1:2 (SEQ ID NO: 189), I-SETit.Prx3-1-1:1:2 (SEQ ID NO: 258) and I-SETit.DnaJ\_1-1:1:2 (SEQ ID NO: 218).

#### **Example 14: Analysis of Intron-Mediated Enhancement of GUS Activity Using Wheat Protoplasts**

5 Protoplast cells, derived from wheat leaf tissue, are transformed with transgene cassettes in the form of PCR amplicons to determine the effect of each intron on the expression of the transgene, GUS, driven by a constitutive promoter and compared to introns known to have an enhancement effect on transgene expression.

The constructs described in example 12 above and presented in Table 19 of example  
10 12 were used as template to generate PCR amplicons using methods known in the art comprising a constitutive promoter, P-CaMV.35S-enh-1:1:13 (SEQ ID NO: 1112), operably linked 5' to a leader element, L-CaMV.35S-1:1:15 (SEQ ID NO: 1111), operably linked to a test intron element (SEQ ID NOS: 179 through 267), operably linked 5' to a GUS coding sequence, (GUS-1, SEQ ID NO: 1090), operably linked 5' to the Nopaline synthase 3'  
15 termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). In addition, amplicons, derived from control plasmids were also generated using the same amplification methods from the control plasmid constructs, pMON19469, pMON65328, pMON25455, pMON8677 and pMON33449. The transgene cassette of pMON19469 is comprised of the transcriptional regulatory element group, EXP-CaMV.35S-enh/I-  
20 Zm.DnaK-1:1:1 (SEQ ID NO: 1104) and provides a comparison of the constitutive promoter enhanced using the intron I-Zm.DnaK-1:1:1. The transgene cassette of pMON65328 is comprised of the transcriptional regulatory element group, EXP-CaMV.35S-enh-Lhcb1/I-Os.Act1-1:1:9 (SEQ ID NO: 1105) and provides a comparison of enhancement of the constitutive promoter using the intron, I-Os.Act1-1:1:9. The transgene cassette of  
25 pMON25455 is comprised of the transcriptional regulatory element group, EXP-Os.Act1:1:1 (SEQ ID NO: 1098) and provides a comparison of expression using the native promoter, leader and intron of the rice actin 1 gene. The transgene cassette of pMON8677 is comprised of the promoter, P-CaMV.35S-enh-1:1:9 (SEQ ID NO: 1096) operably linked to the GUS coding sequence and provides a comparison of expression with a constitutive promoter  
30 without an intron for enhancement. The transgene cassette of pMON33449 is comprised of a variant of the CaMV 35S promoter (P-E35S:1:52, SEQ ID NO: 1117) and provides an additional comparator lacking an intron.

Protoplast cells, derived from wheat leaf tissue are transformed using PEG-based transformation methods known in the art. Briefly, each test and control construct is used to provide template for amplification of the transgene cassette comprising each construct. The resulting amplicon is size fractionated on an agarose gel and the amplicon DNA is excised from the gel. The amplicon DNA is extracted from the gel fragment using methods known in the art and quantified by spectrophotometry. Protoplast cells are transformed using PEG methods known in the art and using 0.1 pico-moles of PCR amplicon DNA. Two to four replicates are performed for each transformation and the average expression imparted by each construct determined. The mean GUS expression observed for each construct derived amplicon is normalized with respect to expression observed for the amplicon derived from pMON19469 comprised of the intron element, I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102). Tables 22 below show the normalized average GUS expression imparted amplicons derived from the constructs presented in Table 19 of Example 12 relative to the average GUS expression imparted to amplicons derived from the control plasmids described above using 0.1 pmol of amplicon.

**Table 22. Intron-mediated enhancement of GUS expression in wheat protoplasts relative to I-Zm.DnaK-1:1:1 (SEQ ID NO: 1102).**

Construct	Intron Annotation	Intron SEQ ID NO:	Mean	Std Deviation
pMON140768	I-SETit.LSm8-1-1:1:2	243	2.26	0.51
pMON140750	I-SETit.60S_L10A1-5-1:1:2	215	2.14	0.62
pMON65328	I-Os.Act1-1:1:19	1113	2.06	0.96
pMON138844	I-SETit.eIF5A1-3-1:1:2	224	1.92	0.29
pMON138806	I-SETit.GRP-1-1:1:1	242	1.81	0.64
pMON138828	I-SETit.14-3-3D-2-1:1:1	193	1.75	0.71
pMON138814	I-SETit.14-3-3D-3-1:1:2	194	1.71	0.26
pMON140754	I-SETit.eIF5A3-3-1:1:2	234	1.66	0.61
pMON140762	I-SETit.LSm8-4-1:1:2	246	1.6	0.52
pMON140767	I-SETit.LSm8-2-1:1:1	244	1.55	0.26
pMON140769	I-SETit.eIF5A3-2-1:1:2	233	1.53	0.52
pMON138834	I-SETit.eIF5A1-4-1:1:2	225	1.44	0.67
pMON140758	I-SETit.ASA2-3-1:1:2	216	1.37	0.25
pMON138847	I-SETit.60S_L10A1-2-1:1:2	212	1.34	1.03
pMON140756	I-SETit.eIF5A2-5-1:1:2	231	1.33	0.53
pMON138830	I-SETit.14-3-3B-3-1:1:2	184	1.2	0.23
pMON138829	I-SETit.14-3-3B-2-1:1:1	183	1.18	0.41

pMON138848	I-SETit.DnaJ3-2-1:1:2	219	1.17	0.1
pMON138826	I-SETit.14-3-3A-3-1:1:2	180	1.12	0.23
pMON140760	I-SETit.eIF5A3-4-1:1:2	235	1.12	0.49
pMON138822	I-SETit.14-3-3C-5-1:1:2	191	1.1	0.33
pMON138803	I-SETit.TubA3_2-1:1:1	266	1.08	0.19
pMON138791	I-SETit.40S-7S-1_3-1:1:2	203	1.06	0.13
pMON138805	I-SETit.TubA2_3-1:1:1	264	1.03	0.34
pMON138817	I-SETit.14-3-3C-3-1:1:2	189	1.03	0.16
pMON140766	I-SETit.Prx3-1-1:1:2	258	1.03	0.45
pMON138783	I-SETit.DnaJ_1-1:1:2	218	1.02	0.43
pMON19469	I-Zm.DnaK-1:1:1	1102	1	0.96
pMON138846	I-SETit.eIF5A2-3-1:1:2	229	0.99	0.62
pMON138819	I-SETit.GAD_2-1:1:2	238	0.98	0.4
pMON140753	I-SETit.eIF5A3-1-1:1:1	232	0.98	0.16
pMON138835	I-SETit.14-3-3E-2-1:1:1	197	0.97	0.17
pMON138836	I-SETit.14-3-3E-3-1:1:2	198	0.95	0.36
pMON138837	I-SETit.60S_L10A1-1-1:1:2	211	0.93	0.57
pMON138813	I-SETit.14-3-3A-5-1:1:2	182	0.92	0.32
pMON138849	I-SETit.eIF5A2-4-1:1:2	230	0.9	0.3
pMON138809	I-SETit.40S-7S-3_1-1:1:2	208	0.88	0.41
pMON140770	I-SETit.SBD-1-1:1:2	259	0.84	0.5
pMON138811	I-SETit.TubA3_1-1:1:1	265	0.83	0.12
pMON138833	I-SETit.eIF5A1-2-1:1:2	223	0.83	0.56
pMON140761	I-SETit.SBD-2-1:1:1	260	0.82	0.18
pMON138832	I-SETit.14-3-3C-4-1:1:2	190	0.77	0.44
pMON140757	I-SETit.ClpD-1-1:1:1	217	0.77	0.52
pMON140759	I-SETit.SBD-3-1:1:2	261	0.76	0.36
pMON138812	I-SETit.PGK3_1-1:1:2	247	0.75	0.45
pMON140763	I-SETit.eIF5A3-5-1:1:2	236	0.75	0.22
pMON138824	I-SETit.14-3-3A-2-1:1:1	179	0.74	0.39
pMON138839	I-SETit.eIF5A2-2-1:1:3	228	0.73	0.12
pMON33449	No intron		0.68	0.47
pMON138841	I-SETit.14-3-3E-5-1:1:2	200	0.67	0.35
pMON138827	I-SETit.14-3-3D-4-1:1:3	195	0.63	0.45
pMON138815	I-SETit.GAD_3-1:1:2	239	0.6	0.15
pMON25455 EXP-Os.Act1 (SEQ ID NO: 1098)	EXP-Os.Act1		0.57	0.49
pMON138804	I-SETit.PGK3_2-1:1:1	248	0.56	0.42
pMON138823	I-SETit.14-3-3C-2-1:1:1	188	0.54	0.21
pMON140752	I-SETit.60S_L10A1-4-1:1:1	214	0.52	0.46
pMON140755	I-SETit.Wx1-1-1:1:2	267	0.52	0.41

pMON138816	I-SETit.14-3-3A-4-1:1:2	181	0.51	0.2
pMON138808	I-SETit.GAD_1-1:1:2	237	0.5	0.42
pMON138820	I-SETit.14-3-3B-4-1:1:2	185	0.5	0.35
pMON138831	I-SETit.14-3-3C-1-1:1:1	187	0.49	0.42
pMON138838	I-SETit.eIF5A2-1-1:1:2	227	0.49	0.39
pMON138792	I-SETit.40S-7S-1_2-1:1:2	202	0.47	0.49
pMON138799	I-SETit.TubA2_1-1:1:1	262	0.47	0.15
pMON140765	I-SETit.Grf1-3-1:1:1	241	0.47	0.38
pMON138843	I-SETit.14-3-3D-5-1:1:2	196	0.46	0.49
pMON140771	I-SETit.LSm8-3-1:1:1	245	0.43	0.25
pMON138789	I-SETit.PIP2-2_3-1:1:2	254	0.42	0.26
pMON138794	I-SETit.40S-7S-1_1-1:1:2	201	0.4	0.28
pMON138788	I-SETit.40S-7S-1_4-1:1:2	204	0.39	0.32
pMON138840	I-SETit.60S_L10A1-3-1:1:2	213	0.39	0.35
pMON140751	I-SETit.eIF5A1-5-1:1:2	226	0.39	0.48
pMON138785	I-SETit.PIP1-4_3-1:1:2	252	0.38	0.33
pMON8677	No intron		0.38	0.36
pMON138821	I-SETit.14-3-3B-5-1:1:2	186	0.37	0.23
pMON138825	I-SETit.14-3-3D-1-1:1:2	192	0.37	0.31
pMON138797	I-SETit.PIP1-1_1-1:1:1	250	0.33	0.39
pMON140764	I-SETit.Prx17-2-1:1:1	257	0.33	0.41
pMON138786	I-SETit.eEF1g_1-1:1:2	220	0.32	0.29
pMON138818	I-SETit.GAD_4-1:1:2	240	0.32	0.09
pMON138802	I-SETit.40S-7S-2_3-1:1:1	206	0.3	0.22
pMON138801	I-SETit.TubA2_2-1:1:1	263	0.29	0.2
pMON138793	I-SETit.PIP1_1_2-1:1:1	249	0.24	0.33
pMON138807	I-SETit.40S-7S-2_4-1:1:1	207	0.24	0.3
pMON138800	I-SETit.40S-7S-3_3-1:1:2	210	0.22	0.11
pMON138795	I-SETit.PIP2-5_3-1:1:2	256	0.21	0.19
pMON138790	I-SETit.PIP2-5_2-1:1:2	255	0.19	0.18
pMON138796	I-SETit.PIP1-1_3-1:1:2	251	0.18	0.19
pMON138810	I-SETit.40S-7S-3_2-1:1:2	209	0.17	0.16
pMON138787	I-SETit.eEF1g_4-1:1:3	221	0.13	0.17
pMON138784	I-SETit.PIP2-2_2-1:1:2	253	0.1	0.15
pMON138798	I-SETit.40S-7S-2_2-1:1:1	205	0.09	0.09
No DNA			0	0
pMON138842	I-SETit.eIF5A1-1-1:1:1	222		
pMON138845	I-SETit.14-3-3E-4-1:1:2	199		

As was observed in corn protoplast cells, many of the test intron elements provided enhancement of transgene expression relative to the intronless controls. Improved enhancement of GUS expression, relative to the intron element, I-Zm.DnaK-1:1:1 (SEQ ID



NO: 1102) was observed using the intron elements, I-SETit.LSm8-1-1:1:2 (SEQ ID NO: 243), I-SETit.60S\_L10A1-5-1:1:2 (SEQ ID NO: 215), I-SETit.eIF5A1-3-1:1:2 (SEQ ID NO: 224), I-SETit.GRP-1-1:1:1 (SEQ ID NO: 242), I-SETit.14-3-3D-2-1:1:1 (SEQ ID NO: 193), I-SETit.14-3-3D-3-1:1:2 (SEQ ID NO: 194), I-SETit.eIF5A3-3-1:1:2 (SEQ ID NO: 234), I-SETit.LSm8-4-1:1:2 (SEQ ID NO: 246), I-SETit.LSm8-2-1:1:1 (SEQ ID NO: 244), I-SETit.eIF5A3-2-1:1:2 (SEQ ID NO: 233), I-SETit.eIF5A1-4-1:1:2 (SEQ ID NO: 225), I-SETit.ASA2-3-1:1:2 (SEQ ID NO: 216), I-SETit.60S\_L10A1-2-1:1:2 (SEQ ID NO: 212), I-SETit.eIF5A2-5-1:1:2 (SEQ ID NO: 231), I-SETit.14-3-3B-3-1:1:2 (SEQ ID NO: 184), I-SETit.14-3-3B-2-1:1:1 (SEQ ID NO: 183), I-SETit.DnaJ3-2-1:1:2 (SEQ ID NO: 219), I-SETit.14-3-3A-3-1:1:2 (SEQ ID NO: 180), I-SETit.eIF5A3-4-1:1:2 (SEQ ID NO: 235), I-SETit.14-3-3C-5-1:1:2 (SEQ ID NO: 191), I-SETit.TubA3\_2-1:1:1 (SEQ ID NO: 266), I-SETit.40S-7S-1\_3-1:1:2 (SEQ ID NO: 203), I-SETit.TubA2\_3-1:1:1 (SEQ ID NO: 264), I-SETit.14-3-3C-3-1:1:2 (SEQ ID NO: 189), I-SETit.Prx3-1-1:1:2 (SEQ ID NO: 258) and I-SETit.DnaJ\_1-1:1:2 (SEQ ID NO: 218).

**15 Example 15. Assay of 3' transcription termination molecule or 3' UTRs in protoplasts.**

3' transcription termination molecules or 3' UTRs are isolated from Foxtail millet (*Setaria italica* (L.) Beauv) and cloned into plant base vectors using methods known in the art to test the effectiveness of the 3' UTR in terminating transcription as well as enhancing expression of a transgene.

20 3' UTRs useful in providing expression of a transgene in plants are identified based upon the expression of expressed sequence tags (ESTs) in cDNA libraries made from messenger RNA isolated from seed, flower and other tissues derived from Foxtail millet (*Setaria italica* (L.) Beauv). Libraries of cDNA are made from tissues isolated from *S. italica* using methods known to those skilled in the art from flower tissue, seed, leaf and root. The resulting cDNAs are sequenced using various sequencing methods known in the art. The resulting ESTs are assembled into clusters using bioinformatics software such as `clc_ref_assemble_complete` version 2.01.37139 (CLC bio USA, Cambridge, Massachusetts 02142). Transcript abundance of each cluster is determined by counting the number of cDNA reads for each cluster.

30 The identified 3' UTRs may be comprised of sequence derived from cDNA sequence as well as sequence derived from genomic DNA. The cDNA sequence is used to design primers, which are then used with GenomeWalker<sup>TM</sup> (Clontech Laboratories, Inc, Mountain View, CA) libraries constructed following the manufacturer's protocol to clone the 3' region

of the corresponding genomic DNA sequence to provide a longer termination sequence. Analysis of relative transcript abundance either by direct counts or normalized counts of observed sequence reads for each tissue library can be used to infer properties about patterns of expression. For example, some 3' UTRs may be found in transcripts seen in higher abundance in root tissue as opposed to leaf. This is suggestive that the transcript is highly expressed in root and that the properties of root expression may be attributable to the transcriptional regulation of the promoter, the lead, the introns or the 3' UTR. Empirical testing of 3' UTRs identified by the properties of expression within specific organs, tissues or cell types can result in the identification of 3' UTRs that enhance expression in those specific organs, tissues or cell types.

3' UTR sequences isolated from *S. italica* are presented as SEQ ID NOS: 268 through 276 and SEQ ID NOS: 779 through 924. Table 23 below presents 3' UTRs identified as being useful for control of expression and enhancement of root expression or constitutive expression.

**Table 23. Transcription termination or 3' UTR elements derived from *S. italica*.**

Annotation	SEQ ID NO:	Description
T-SETit.Act1-1:1:1	268	Actin 1
T-SETit.Act8-1:1:1	269	Actin 8
T-SETit.Ams1-1:1:1	270	S-adenosylmethionine synthetase 1
T-SETit.Ctpt-1:1:2	271	Triose phosphate/phosphate translocator, chloroplast precursor
T-SETit.Fba-1:1:1	272	Fructose-bisphosphate aldolase
T-SETit.Fnr-1:1:1	273	Ferredoxin-NADP+ reductase
T-SETit.Mes2-1:1:1	274	Methionine synthase 2
T-SETit.Ntr-1:1:1	275	Nitrite transporter
T-SETit.Sus2-1:1:1	276	Sucrose synthase 2
T-SETit.36384-1:1:1	779	Cluster 36384
T-SETit.37025-1:1:1	780	Cluster 37025
T-SETit.37470-1:1:1	781	Cluster 37470
T-SETit.Ams2-1:1:1	782	S-adenosylmethionine synthetase 2
T-SETit.Atps-1:1:1	783	ATP synthase subunit gamma
T-SETit.Cab-1:1:1	784	Chlorophyll a/b binding protein
T-SETit.Cab1-1:1:1	785	Chlorophyll a/b binding protein
T-SETit.Ctpt-1:1:1	786	Triose phosphate/phosphate translocator, chloroplast precursor
T-SETit.DnaK-1:1:1	787	Heat shock protein

T-SETit.Fba-1:1:2	788	Fructose-bisphosphate aldolase
T-SETit.Fba-1:1:3	789	Fructose-bisphosphate aldolase
T-SETit.Fba-1:1:4	790	Fructose-bisphosphate aldolase
T-SETit.Gapdh-1:1:1	791	Glyceraldehyde-3-phosphate dehydrogenase
T-SETit.MES2_nno-1:1:1	792	Methionine synthase 2
T-SETit.Oep-1:1:1	793	33kDa oxygen evolving protein
T-SETit.Pea-1:1:1	794	Proton-exporting ATPase
T-SETit.Pod-1:1:1	795	pyruvate orthophosphate dikinase
T-SETit.Ppc-1:1:1	796	Phosphoenolpyruvate carboxylase
T-SETit.Psi-K-1:1:1	797	Photosystem I reaction center subunit
T-SETit.Psi-L-1:1:1	798	Photosystem I reaction center subunit
T-SETit.Rfe-s-1:1:1	799	Rieske Fe-S
T-SETit.TubA-1:1:1	800	Tubulin A
T-contig00388	801	Root 3' UTR
T-contig05482	802	Root 3' UTR
T-contig08555	803	Root 3' UTR
T-contig08556	804	Root 3' UTR
T-contig09485	805	Root 3' UTR
T-contig13749	806	Root 3' UTR
T-contig16157	807	Root 3' UTR
T-contig18936	808	Root 3' UTR
T-contig18994	809	Root 3' UTR
T-contig21387	810	Root 3' UTR
T-contig23385	811	Root 3' UTR
T-contig24188	812	Root 3' UTR
T-contig24188	813	Root 3' UTR
T-contig24832	814	Root 3' UTR
T-contig24890	815	Root 3' UTR
T-contig24916	816	Root 3' UTR
T-contig25097	817	Root 3' UTR
T-contig25509	818	Root 3' UTR
T-contig25584	819	Root 3' UTR
T-contig26532	820	Root 3' UTR
T-contig28013	821	Root 3' UTR
T-contig29922	822	Root 3' UTR
T-contig34261	823	Root 3' UTR
T-contig34311	824	Root 3' UTR
T-contig34749	825	Root 3' UTR
T-contig35408	826	Root 3' UTR
T-contig35550	827	Root 3' UTR
T-contig35785	828	Root 3' UTR

T-contig35943	829	Root 3' UTR
T-contig36050	830	Root 3' UTR
T-contig36266	831	Root 3' UTR
T-contig36378	832	Root 3' UTR
T-contig36502	833	Root 3' UTR
T-contig36728	834	Root 3' UTR
T-contig36811	835	Root 3' UTR
T-contig36883	836	Root 3' UTR
T-contig37316	837	Root 3' UTR
T-contig37476	838	Root 3' UTR
T-contig37510	839	Root 3' UTR
T-contig37704	840	Root 3' UTR
T-contig37883	841	Root 3' UTR
T-contig37920	842	Root 3' UTR
T-contig37959	843	Root 3' UTR
T-contig37976	844	Root 3' UTR
T-contig38003	845	Root 3' UTR
T-SETIT-28JUL09-CLUS3016_12	846	Root 3' UTR
T-10SETIT-28JUL09-CLUS1332_4	847	Constitutive 3' UTR
T-17SETIT-28JUL09-CLUS1910_15	848	Constitutive 3' UTR
T-7SETIT-28JUL09-CLUS2844_11	849	Constitutive 3' UTR
T-contig00006	850	Constitutive 3' UTR
T-contig00142	851	Constitutive 3' UTR
T-contig00191	852	Constitutive 3' UTR
T-contig00205	853	Constitutive 3' UTR
T-contig00242	854	Constitutive 3' UTR
T-contig00263	855	Constitutive 3' UTR
T-contig01883	856	Constitutive 3' UTR
T-contig02157	857	Constitutive 3' UTR
T-contig02856	858	Constitutive 3' UTR
T-contig02883	859	Constitutive 3' UTR
T-contig04253	860	Constitutive 3' UTR
T-contig05397	861	Constitutive 3' UTR
T-contig05720	862	Constitutive 3' UTR
T-contig10626	863	Constitutive 3' UTR
T-contig10874	864	Constitutive 3' UTR
T-contig11193	865	Constitutive 3' UTR
T-contig14970	866	Constitutive 3' UTR
T-contig26892	867	Constitutive 3' UTR
T-contig32186	868	Constitutive 3' UTR
T-contig32187	869	Constitutive 3' UTR
T-contig33439	870	Constitutive 3' UTR

T-contig33682	871	Constitutive 3' UTR
T-contig34270	872	Constitutive 3' UTR
T-contig34378	873	Constitutive 3' UTR
T-contig35132	874	Constitutive 3' UTR
T-contig35270	875	Constitutive 3' UTR
T-contig35388	876	Constitutive 3' UTR
T-contig35412	877	Constitutive 3' UTR
T-contig35488	878	Constitutive 3' UTR
T-contig35982	879	Constitutive 3' UTR
T-contig36384	880	Constitutive 3' UTR
T-contig36588	881	Constitutive 3' UTR
T-contig36702	882	Constitutive 3' UTR
T-contig36980	883	Constitutive 3' UTR
T-contig36992	884	Constitutive 3' UTR
T-contig36993	885	Constitutive 3' UTR
T-contig37025	886	Constitutive 3' UTR
T-contig37162	887	Constitutive 3' UTR
T-contig37351	888	Constitutive 3' UTR
T-contig37386	889	Constitutive 3' UTR
T-contig37448	890	Constitutive 3' UTR
T-contig37456	891	Constitutive 3' UTR
T-contig37638	892	Constitutive 3' UTR
T-contig37732	893	Constitutive 3' UTR
T-contig37897	894	Constitutive 3' UTR
T-contig37927	895	Constitutive 3' UTR
T-contig37962	896	Constitutive 3' UTR
T-contig37980	897	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS11107_1	898	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS11705_1	899	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS11899_1	900	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS12698_2	901	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS13580_1	902	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS1404_1	903	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS14743_1	904	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS181186_1	905	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS19095_1	906	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS1910_13	907	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS1910_14	908	Constitutive 3' UTR
T-SETIT28JUL09CLUS1910_16	909	Constitutive 3' UTR
T-SETIT28JUL09CLUS1910_17	910	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS1910_18	911	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS1910_19	912	Constitutive 3' UTR

T-SETIT-28JUL09-CLUS2157_4	913	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS2166_1	914	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS243_3	915	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS3485_1	916	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS364_1	917	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS36567_1	918	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS42130_1	919	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS52844_1	920	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS7004_1	921	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS83_23	922	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS937_1	923	Constitutive 3' UTR
T-SETIT-28JUL09-CLUS95524_1	924	Constitutive 3' UTR

The 3' UTRs of the present invention, presented as SEQ ID NOS: 268 through 276 and SEQ ID NOS: 779 through 924 are tested in transient protoplast assays. A constitutive or other type promoter is used to drive expression of a transgene such as GUS. Plant vectors are constructed using methods known in the art in which a transgene cassette is used to test the properties of the 3' UTR as well as provide transcript that can be analyzed to understand the effectiveness of the 3' UTR in controlling expression of the transgene and processing of the resulting transcript.

For transient assay of the test 3' UTR effectiveness, a base plant vector is constructed using methods known in the art. The 3' UTR is cloned into a base plant vector which comprises a first transgene cassette to test the 3' UTR comprised of, a constitutive promoter such as the Cauliflower mosaic virus promoter, P-CaMV.35S-enh-1:1:9 (SEQ ID NO: 1096), operably linked 5' to a leader element, L-Ta.Lhcb1-1:1:1 (SEQ ID NO: 1114), operably linked to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS) that either possessed a processable intron (GUS-2, SEQ ID NO: 1091) or no intron (GUS-1, SEQ ID NO: 1090), operably linked to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088) or the 3' termination region from the rice lipid transfer protein gene (T-Os.LTP-1:1:1, SEQ ID NO: 1089); a second transgene selection cassette used for selection of transformed plant cells that conferred resistance to the herbicide glyphosate (driven by the rice Actin 1 transcriptional regulatory element group, SEQ ID NO: 1098), or alternatively, the antibiotic kanamycin (driven by the rice Actin 1 transcriptional regulatory element group, SEQ ID NO: 1098) and a left border region from *A. tumefaciens*.

Several experimental observations are made to characterize the 3' UTR in protoplast assay. For example, the level of expression is determined using GUS staining as described in previous examples to assess the amount of protein expressed and is normalized using methods known in the art to draw a comparison in protein expression levels of the test 3' UTR relative to the T-AGRtu.nos control. Total RNA is extracted and probed on Northern blots with probes specific to the GUS coding sequence to assess the size or sizes of transcripts produced in the protoplast cells to determine the effectiveness of the 3' UTR in terminating transcription. Alternatively, the sequence 3' of the 3' UTR can be used to prime amplification reactions from reverse transcribed RNA to detect transcripts in which read-through has occurred beyond the 3' UTR. Total RNA probed on Northern blots can also reveal the relative abundance of transcript when compared to the T-AGRtu.nos control.

**Example 16. Assay of transcriptional termination sequences or 3' UTRs in stable plants.**

The 3' UTRs, presented as SEQ ID NOS: 268 through 276 and SEQ ID NOS: 779 through 924 are tested in stably transformed corn plants. For stable plant assay of the 3' UTR, a GUS expression plant transformation vector is constructed using methods known in the art similar to the constructs described in the previous examples. The 3' UTR is cloned into a base plant vector which comprises a first transgene cassette to test the 3' UTR comprised of, a constitutive promoter such as the Cauliflower mosaic virus promoter, P-CaMV.35S-enh-1:1:9 (SEQ ID NO: 1096), operably linked 5' to a leader element, L-Ta.Lhcb1-1:1:1 (SEQ ID NO: 1114), or alternatively, a root promoter such as Lipid transfer protein promoter, P-Os.Rcc3-1:1:24 (SEQ ID NO: 1093), operably linked 5' to the leader, L-Os.Rcc3-1:1:2 (SEQ ID NO: 1094), operably linked to an intron derived from the HSP70 heat shock protein of *Zea mays* (I-Zm.DnaK-1:1:1, SEQ ID NO: 1102), operably linked to a coding sequence for  $\beta$ -glucuronidase (GUS) that either possessed a processable intron (GUS-2, SEQ ID NO: 1091) or no intron (GUS-1, SEQ ID NO: 1090), operably linked to a test 3' termination region; a second transgene selection cassette used for selection of transformed plant cells that conferred resistance to the herbicide glyphosate (driven by the rice Actin 1 transcriptional regulatory element group, SEQ ID NO: 1098), or alternatively, the antibiotic kanamycin (driven by the rice Actin 1 transcriptional regulatory element group, SEQ ID NO: 1098) and a left border region from *A. tumefaciens*.

The resulting plasmids are used to transform corn plants. Single-copy or low copy number transformants are selected for comparison to single-copy or low copy number

transformed plants, transformed with a plant transformation vector identical to the test vector but comprising a well characterized 3' UTR, such as the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088) operably linked to the GUS transgene.

5           Corn plants are transformed as described above or by *Agrobacterium*-mediated transformation methods known in the art. Tissue is harvested at different time points of development and from different organs and assayed for GUS activity to assess the amount of protein expressed in each tissue at different time windows of development and is compared to expression of GUS in control transformed plants. Total RNA is extracted from the different  
10 tissues of interest and probed on Northern blots with probes specific to the GUS coding sequence to assess the size or sizes of transcripts produced within each of the selected tissues to determine the effectiveness of the 3' UTR in terminating transcription and the relative abundance of message in each selected tissue. Alternatively, the sequence 3' of the 3' UTR can be used to prime amplification reactions from reverse transcribed RNA to detect  
15 transcripts in which read-through has occurred beyond the 3' UTR or assess the amount of transcript expressed in each tissue. The most useful and effective 3' UTRs are selected for use in transgene cassettes in which genes of agronomic importance are expressed.

#### **Example 17: Analysis of 3' UTR-Mediated Enhancement of GUS Activity In Transgenic Corn Plants.**

20           Plant binary vectors are constructed using methods known in the art similar to those described in the previous example and used to transform corn plants to test the effectiveness of selected 3' UTRs.

Plant transformation vectors are used to transform corn plants to test the effectiveness in controlling expression of the GUS transgene, when driven by either a constitutive promoter, (P-FMV.35S-enh-1:1:1 (SEQ ID NO: 1115) + L-Ta.Lhcb1-1:1:1 (SEQ ID NO: 1114)) or an enhanced root promoter, ((E-CaMV.35S-enh-1:1:1 (SEQ ID NO: 1116) + P-Os.Rcc3-1:1:24 (SEQ ID NO: 1093) + L-Os.Rcc3-1:1:2 (SEQ ID NO: 1094)). The resulting  
25 plant expression vectors contains a right border region from *Agrobacterium tumefaciens*, a first transgene cassette to test the 3' UTR comprised of a either a constitutive promoter, (P-FMV.35S-enh-1:1:1 (SEQ ID NO: 1115) + L-Ta.Lhcb1-1:1:1 (SEQ ID NO: 1114)) or an  
30 enhanced root promoter, ((E-CaMV.35S-enh-1:1:1 (SEQ ID NO: 1116) + P-Os.Rcc3-1:1:24 (SEQ ID NO: 1093) + L-Os.Rcc3-1:1:2 (SEQ ID NO: 1094)), operably linked 5' to a coding sequence for  $\beta$ -glucuronidase (GUS) that possesses a processable intron (GUS-2, SEQ ID



NO: 1091) operably linked to the test 3' UTR; a second transgene selection cassette used for selection of transformed plant cells that confers resistance to the herbicide glyphosate (driven by the rice Actin 1 promoter), and a left border region from *A. tumefaciens*. Table 24 below shows the test constructs and the corresponding promoter and 3' UTR.

5 **Table 24. Transcriptional termination or 3' UTR test constructs in which GUS is driven either by a constitutive promoter or an enhanced root promoter.**

<b>Test 3' UTR</b>	<b>Constitutive Constructs P-FMV.35S-enh-1:1:1 (SEQ ID NO: 1115) L-Ta.Lhcb1-1:1:1 (SEQ ID NO: 1114)</b>	<b>Enhanced Root Constructs E-CaMV.35S-enh-1:1:1 (SEQ ID NO: 1116) P-Os.Rcc3-1:1:24 (SEQ ID NO: 1093) L-Os.Rcc3-1:1:2 (SEQ ID NO: 1094)</b>
T-SETit.Ntr-1:1:1 (SEQ ID NO: 275)	pMON126964	pMON126965
T-SETit.Sus2-1:1:1 (SEQ ID NO: 276)	pMON126966	pMON126967
T-SETit.Ctpt-1:1:2 (SEQ ID NO: 271)	pMON126968	pMON126969
T-SETit.Fnr-1:1:1 (SEQ ID NO: 273)	pMON126974	pMON126975
T-SETit.Mes2-1:1:1 (SEQ ID NO: 274)	pMON126976	pMON126977
T-SETit.Fba-1:1:1 (SEQ ID NO: 272)	pMON126978	pMON126979
T-SETit.Act8-1:1:1 (SEQ ID NO: 269)	pMON127033	
T-SETit.Act1-1:1:1 (SEQ ID NO: 268)	pMON127035	
T-SETit.Ams1-1:1:1 (SEQ ID NO: 270)	pMON127041	

Corn plants are transformed using *Agrobacterium*-mediated methods known in the art and as described in the previous examples. Transformed corn plants are assayed for GUS activity as described in the previous examples. Tables 25 and 26 show the average level of GUS expression observed in various tissues isolated from the transformed plants in which the constitutive promoter, ((P-FMV.35S-enh-1:1:1 (SEQ ID NO: 1115) + L-Ta.Lhcb1-1:1:1 (SEQ ID NO: 1114)), drove expression of the GUS transgene. Tables 27 and 28 show the average level of GUS expression observed in tissues isolated from the transformed plants in which the enhanced root promoter, ((E-CaMV.35S-enh-1:1:1 (SEQ ID NO: 1116) + P-Os.Rcc3-1:1:24 (SEQ ID NO: 1093) + L-Os.Rcc3-1:1:2 (SEQ ID NO: 1094)), drove

expression of the GUS transgene. Enhancement of expression imparted by the 3' UTR is inferred by the relative comparison amongst the constructs transformed comprising each UTR and specific promoter.

**Table 25. Average R<sub>0</sub> GUS expression of a constitutive promoter driving GUS with different 3' UTRs.**

Construct	3' UTR Annotation	V3 Root	V7 Root	VT Root	V3 Leaf	V7 Leaf	VT Leaf
pMON126964	T-SETit.Ntr-1:1:1 (SEQ ID NO: 275)	45.54	136.70	103.51	112.24	151.17	304.15
pMON126966	T-SETit.Sus2-1:1:1 (SEQ ID NO: 276)	12.01	nd	123.25	33.30	140.51	100.88
pMON126968	T-SETit.Ctpt-1:1:2 (SEQ ID NO: 271)	9.76	nd	108.38	231.03	417.04	157.53
pMON126974	T-SETit.Fnr-1:1:1 (SEQ ID NO: 273)	7.74	nd	53.30	163.74	357.60	456.63
pMON126976	T-SETit.Mes2-1:1:1 (SEQ ID NO: 274)	nd	nd	454.44	235.62	268.63	235.73
pMON126978	T-SETit.Fba-1:1:1 (SEQ ID NO: 272)	nd	158.85	45.89	60.97	250.87	121.41
pMON127033	T-SETit.Act8-1:1:1 (SEQ ID NO: 269)	nd	nd	203.77	129.06	240.13	460.74
pMON127035	T-SETit.Act1-1:1:1 (SEQ ID NO: 268)	117.02	174.21	557.50	412.92	511.88	531.34
pMON127041	T-SETit.Ams1-1:1:1 (SEQ ID NO: 270)	842.04	564.84	246.90	986.33	225.68	279.58

The average expression of the GUS gene was effected by different 3' UTRs. GUS expression driven by the constitutive promoter appeared to be affected by the use of different 3' UTRs, particularly with respect to leaf expression. An enhancement of leaf expression at V3 and V7 stage could be seen when the 3' UTR, T-SETit.Ctpt-1:1:2 (SEQ ID NO: 271) was used in combination with the constitutive promoter. Enhancement was provided for all 3 leaf stages when combining the constitutive promoter with the 3' UTRs, T-SETit.Mes2-1:1:1 (SEQ ID NO: 274), T-SETit.Act1-1:1:1 (SEQ ID NO: 268) and T-SETit.Ams1-1:1:1 (SEQ ID NO: 270). Enhancement of expression in the root using the constitutive promoter was observed through out all 3 stages using the 3' UTR, T-SETit.Ams1-1:1:1 (SEQ ID NO: 270) and at VT stage using the 3' UTRs, T-SETit.Mes2-1:1:1 (SEQ ID NO: 274), T-SETit.Act8-1:1:1 (SEQ ID NO: 269) and T-SETit.Act1-1:1:1 (SEQ ID NO: 268).

**Table 26. Average R<sub>0</sub> GUS expression of a constitutive promoter driving GUS with different 3' UTRs.**

Construct	3' UTR Annotation	VT Anther	VT Silk	21 DAP Embryo	21 DAP Endosperm
pMON126964	T-SETit.Ntr-1:1:1 (SEQ ID NO: 275)	140.38	96.86	36.85	139.47
pMON126966	T-SETit.Sus2-1:1:1 (SEQ ID NO: 276)	166.05	110.71	275.98	223.52
pMON126968	T-SETit.Ctpt-1:1:2 (SEQ ID NO: 271)	219.71	101.88	72.49	235.36
pMON126974	T-SETit.Fnr-1:1:1 (SEQ ID NO: 273)	350.21	419.08	107.48	243.22
pMON126976	T-SETit.Mes2-1:1:1 (SEQ ID NO: 274)	401.38	370.21	174.26	366.92
pMON126978	T-SETit.Fba-1:1:1 (SEQ ID NO: 272)	41.62	59.81	115.59	168.81
pMON127033	T-SETit.Act8-1:1:1 (SEQ ID NO: 269)	324.04	435.63	133.10	262.52
pMON127035	T-SETit.Act1-1:1:1 (SEQ ID NO: 268)	408.61	707.00	119.39	419.52
pMON127041	T-SETit.Ams1-1:1:1 (SEQ ID NO: 270)	346.60	246.92	134.79	251.23

Enhancement of GUS expression in the anther and pollen when using a constitutive promoter could be observed for the 3' UTRs, T-SETit.Fnr-1:1:1 (SEQ ID NO: 273), T-SETit.Mes2-1:1:1 (SEQ ID NO: 274), T-SETit.Act8-1:1:1 (SEQ ID NO: 269), T-SETit.Act1-1:1:1 (SEQ ID NO: 268) and T-SETit.Ams1-1:1:1 (SEQ ID NO: 270). Enhancement of GUS expression in the endosperm when using a constitutive promoter could be observed for the 3' UTR, T-SETit.Sus2-1:1:1 (SEQ ID NO: 276). Enhancement of GUS expression in the embryo when using a constitutive promoter could be observed in most of the 3' UTRs relative to the lowest expressor, T-SETit.Ntr-1:1:1 (SEQ ID NO: 275). The greatest amount of enhancement in embryo was observed using the 3' UTRs, T-SETit.Mes2-1:1:1 (SEQ ID NO: 274) and T-SETit.Act1-1:1:1 (SEQ ID NO: 268).

Readthrough was assessed using PCR methods known in the art. Those 3' UTRs demonstrating some readthrough in assay were T-SETit.Ntr-1:1:1 (SEQ ID NO: 275), T-SETit.Sus2-1:1:1 (SEQ ID NO: 276), T-SETit.Ctpt-1:1:2 (SEQ ID NO: 271), T-SETit.Mes2-1:1:1 (SEQ ID NO: 274) and T-SETit.Act1-1:1:1 (SEQ ID NO: 268). Those 3' UTRs in which readthrough was not observed were T-SETit.Fnr-1:1:1 (SEQ ID NO: 273), T-

SETit.Fba-1:1:1 (SEQ ID NO: 272), T-SETit.Act8-1:1:1 (SEQ ID NO: 269) and T-SETit.Ams1-1:1:1 (SEQ ID NO: 270).

**Table 27. Average R<sub>0</sub> GUS expression of an enhanced root promoter driving GUS with different 3' UTRs.**

Construct	3' UTR Annotation	V3 Root	V7 Root	VT Root	V3 Leaf	V7 Leaf	VT Leaf
pMON126965	T-SETit.Ntr-1:1:1 (SEQ ID NO: 275)	7.86	11.39	8.34	0.00	5.60	8.89
pMON126967	T-SETit.Sus2-1:1:1 (SEQ ID NO: 276)	67.45	44.80	8.98	0.00	0.00	0.00
pMON126969	T-SETit.Ctpt-1:1:2 (SEQ ID NO: 271)	35.07	28.96	6.28	7.44	5.07	0.00
pMON126975	T-SETit.Fnr-1:1:1 (SEQ ID NO: 273)	45.53	12.99	10.67	81.88	0.00	0.00
pMON126977	T-SETit.Mes2-1:1:1 (SEQ ID NO: 274)	16.64	50.03	23.55	0.00	20.47	0.00
pMON126979	T-SETit.Fba-1:1:1 (SEQ ID NO: 272)	30.95	19.79	14.22	0.00	7.47	0.00

5 Using an enhanced root promoter, enhancement in root at V3 and V7 stage was observed using the 3' UTR, T-SETit.Sus2-1:1:1 (SEQ ID NO: 276). Slight enhancement of root expression could also be observed when using the 3' UTRs, T-SETit.Ctpt-1:1:2 (SEQ ID NO: 271) (V3 and V7 stage), T-SETit.Fnr-1:1:1 (SEQ ID NO: 273) (V3 stage) and T-SETit.Fba-1:1:1 (SEQ ID NO: 272) (V3 stage). An enhancement of leaf expression was  
10 observed when using the enhanced root promoter in combination with T-SETit.Fnr-1:1:1 (SEQ ID NO: 273).

**Table 28. Average R<sub>0</sub> GUS expression of an enhanced root promoter driving GUS with different 3' UTRs.**

Construct	3' UTR Annotation	VT Anther	VT Silk	21 DAP Embryo	21 DAP Endosperm
pMON126965	T-SETit.Ntr-1:1:1 (SEQ ID NO: 275)	6.59	5.16	0.00	0.00
pMON126967	T-SETit.Sus2-1:1:1 (SEQ ID NO: 276)	12.24	0.00	115.97	21.68
pMON126969	T-SETit.Ctpt-1:1:2 (SEQ ID NO: 271)	0.00	0.00	7.20	10.77
pMON126975	T-SETit.Fnr-1:1:1 (SEQ ID NO: 273)	5.71	12.57	7.60	0.00
pMON126977	T-SETit.Mes2-1:1:1 (SEQ ID NO: 274)	7.28	0.00	0.00	5.18
pMON126979	T-SETit.Fba-1:1:1 (SEQ ID NO: 272)	11.25	0.00	0.00	8.38

Enhancement of expression in the embryo was observed for the 3' UTR, T-SETit.Sus2-1:1:1 (SEQ ID NO: 276) when combined with the enhanced root promoter. The 3' UTR elements presented above each had an effect on expression of GUS when combined in operable linkage with either a constitutive promoter or an enhanced root promoter.

#### 5 **Example 18. Identification and Assay of Chloroplast Transit Peptides (CTPs).**

It is well known that the cells of eukaryotic organisms, and more particularly plant cells, contain distinct sub-cellular compartments, or organelles, delimited by characteristic membrane systems and performing specialized functions within the cell. In photosynthetic leaf cells of higher plants the most conspicuous organelles are the chloroplasts, which exist in  
10 a semi-autonomous fashion within the cell, containing their own genetic system and protein synthesis machinery, but relying upon a close cooperation with the nucleo-cytoplasmic system in their development and biosynthetic activities.

Most chloroplast proteins are coded for in the nuclear DNA and are the products of protein synthesis on cytoplasmic ribosomes, many as soluble higher molecular weight precursors. These precursors are then translocated through either one or both of the plastid  
15 envelope membranes, processed, and assembled into their final organellar compartment or holoenzyme complex. *In vitro* reconstitution experiments using isolated chloroplasts, have demonstrated that the uptake and processing of over one hundred nuclear-encoded, cytoplasmically synthesized precursors by chloroplasts occurs by an energy-dependent, post-  
20 translational mechanism.

The most extensively characterized of these nuclear-encoded chloroplast proteins is the small subunit of ribulose-1,5-bisphosphate (RuBP) carboxylase. This polypeptide is synthesized on free cytoplasmic ribosomes as a precursor of 20,000 daltons containing an amino terminal extension or transit peptide of approximately 5-6,000 daltons. During or  
25 immediately after import of the precursor into the chloroplast, the transit peptide is proteolytically removed in two steps by a soluble protease, yielding a mature small subunit polypeptide of 15,000 daltons. This polypeptide is then assembled with an endogenous large subunit into the functional RuBP carboxylase holoenzyme.

These different properties of the transit peptides are at the basis of the recombinant  
30 DNAs, more particularly recombinant vectors including a DNA sequence coding for a determined protein or polypeptide, particularly a foreign protein, sought to be introduced and processed in chloroplasts, as well as the processes for the introduction of such foreign

polypeptide or protein into the chloroplasts, for instance in the thylacoid membranes or, preferably, in the stroma thereof.

Chloroplast transit peptides (CTPs) are isolated from *S. italica* based upon an analysis of EST cluster sequences and homology to known plastid targeted molecules. Clusters of EST sequences are used to deduce the coding sequence of chloroplast targeted protein molecules. A fragment derived from the 5' end of the coding sequence of the deduced chloroplast targeted molecule is cloned using methods known in the art to produce a chimeric molecule in which a coding sequence encoding a non-chloroplast targeted molecule is fused in frame at the 5' end with a DNA fragment encoding the putative transit peptide sequence and cloned into a plant expression vector to determine the ability and efficiency of the putative CTP to cause importation of the chimeric molecule into the chloroplast and subsequent processing of the transit peptide sequence into the mature, processed protein.

Translated *S. italica* EST sequence clusters are compared with DNA sequences encoding known plastid targeted molecules from monocots such as corn, sorghum and rice to determine the completeness of the cluster coding sequence and deduce the potential N-terminal amino acid sequence that will be useful as a transit peptide coding sequence. In some instances, the 5' most portion of the *S. italica* EST cluster is absent. In those conditions, a degenerative oligo is designed based upon an alignment of sorghum and rice coding sequences, encoding homologs to the *S. italica* protein coding sequence to facilitate amplification of the unknown 5' sequence.

Sequences encoding plastid targeted proteins useful in isolating and cloning *S. italica* CTPs are presented as SEQ ID NOS: 1034 through 1060. Protein sequences representing plastid targeted peptides useful in identification of *S. italica* CTPs are presented as 1061 through 1087. Nucleotide sequences encoding transit peptides shown in Table 29 below are presented as SEQ ID NOS: 277 through 284, 289 through 293, 296, 301 through 304 and 307 through 316. Protein sequences of the encoded transit peptides are presented as SEQ ID NOS: 324 through 350.

Constructs for use in cloning the transit peptides and sequences encoding the transit peptide as well as the transit peptide sequence IDs are presented in Table 29 below.

**Table 29. Plasmid constructs for use in cloning chloroplast transit peptides and associated protein and nucleotide coding sequences.**

<b>Construct</b>	<b>Annotation</b>	<b>Protein SEQ ID NO:</b>	<b>Nucleotide SEQ ID NO:</b>	<b>Description</b>
pMON136303	GOI-TS-APX	325	277	Ascorbate Peroxidase
pMON139282	GOI-TS-APX:1:2	325	278	Ascorbate Peroxidase
pMON139283	GOI-TS-APX2:1:1	326	279	Ascorbate Peroxidase
pMON139281	GOI-TS-CNT:1:2	332	280	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase
pMON139291	GOI-TS-DHDPS:1:2	334	281	Dihydrodipicolinate synthase precursor, chloroplastic
pMON139288	GOI-TS-Fe-SD:1:1	335	282	Iron-superoxidedismutases, chloroplastic
pMON139287	GOI-TS-PPR:1:1	340	283	Pentatricopeptide repeat-containing protein, putative
pMON139276	TS-SETit.APG6-1:1:1	324	284	Casein lytic proteinase B3 heat shock protein-like
pMON139284	TS-SETit.APX3-1:1:1	327	289	Ascorbate Peroxidase
pMON139278	TS-SETit.ASA2-1:1:1	328	290	Anthranilate Synthase alpha 2 subunit
pMON139285	TS-SETit.CC10-1:1:1	329	291	Chloroplast Chaperonin 10 Kd subunit
pMON136299	TS-SETit.CHoR1-1:1:1	330	292	Calcium homeostasis regulator
pMON139280	TS-SETit.ClpD-1:1:1	331	293	ATP-dependent Clp protease ATP-binding subunit
pMON136296	TS-SETit.CR88-1:1:1	333	296	Heat-shock protein putative
pMON139290	TS-SETit.G-tyrA-1:1:1	336	301	GTP-binding protein tyrA
pMON136301	TS-SETit.HDh-1:1:1	337	302	Haloacid dehalogenase-like hydrolase
pMON139289	TS-SETit.IMP-1:1:1	338	303	Inositol-1-monophosphatase, putative, chloroplastic
pMON136297	TS-SETit.MDH-1:1:1	339	304	Putative NAD-malate dehydrogenase
pMON136302	TS-SETit.PSPR-3-	341	307	Plastid-specific 30S

	1:1:1			ribosomal protein 3
pMON136291	TS-SETit.RbcS_1-1:1:1	342	308	Small subunit RUBISCO
pMON136292	TS-SETit.RbcS_2-1:1:1	343	309	Small subunit RUBISCO
pMON136293	TS-SETit.RbcS_3-1:1:1	344	310	Small subunit RUBISCO
pMON136294	TS-SETit.RbcS_4-1:1:1	345	311	Small subunit RUBISCO
pMON139277	TS-SETit.ShkG-1:1:1	346	312	5-enolpyruvylshikimate-3-phosphate synthase precursor
pMON139286	TS-SETit.SRP43-1:1:1	347	313	Signal recognition particle 43 kDa protein, chloroplastic
pMON139279	TS-SETit.TDh-1:1:1	348	314	Threonine dehydratase biosynthetic, chloroplast precursor
pMON136304	TS-SETit.ThR-1:1:1	349	315	Thioredoxin
pMON136287	TS-SETit.Wx1-1:1:1	350	316	Putative granule bound starch synthase

The transit peptide encoding sequences presented as SEQ ID NOS: 325, 326, 332, 334, 335 and 340 are derived and cloned from DNA sequences comprising a processable intron. The plasmids construct pMON136303 is comprised of the transit peptide encoding sequence, GOI-TS-APX, presented as SEQ ID NO: 277, which is further comprised of the element, TS-SETit.APX.ex1-1:1:1 (SEQ ID NO: 286), operably linked 5' to the intron element, I-SETit.APX-1:1:1 (SEQ ID NO: 318), operably linked 5' to the element, TS-SETit.APX.ex2-1:1:2 (SEQ ID NO: 287). The plasmids construct pMON139282 is comprised of the transit peptide encoding sequence, GOI-TS-APX:1:2, presented as SEQ ID NO: 278, which is further comprised of the element, TS-SETit.APX.ex1-1:1:1 (SEQ ID NO: 286), operably linked 5' to the intron element, I-SETit.APX-1:1:2 (SEQ ID NO: 319), operably linked 5' to the element, TS-SETit.APX.ex2-1:1:2 (SEQ ID NO: 287). The plasmids construct pMON139283 is comprised of the transit peptide encoding sequence, GOI-TS-APX2:1:1, presented as SEQ ID NO: 279, which is further comprised of the element, TS-SETit.APX.2.ex1-1:1:1 (SEQ ID NO: 285), operably linked 5' to the intron element, I-SETit.APX.2-1:1:1 (SEQ ID NO: 317), operably linked 5' to the element, TS-SETit.APX.2.ex2-1:1:1 (SEQ ID NO: 288). The plasmids construct pMON139281 is comprised of the transit peptide encoding sequence, GOI-TS-CNT:1:2, presented as SEQ ID



NO: 280, which is further comprised of the element, TS-SETit.CNT.ex1-1:1:1 (SEQ ID NO: 294), operably linked 5' to the intron element, I-SETit.CNT.1-1:1:1 (SEQ ID NO: 320), operably linked 5' to the element, TS-SETit.CNT.ex2-1:1:2 (SEQ ID NO: 295). The plasmids construct pMON139291 is comprised of the transit peptide encoding sequence, GOI-TS-DHDPS:1:2, presented as SEQ ID NO: 281, which is further comprised of the element, TS-SETit.DHDPS.Ex1-1:1:1 (SEQ ID NO: 297), operably linked 5' to the intron element, I-SETit.DHDPS\_1-1:1:1 (SEQ ID NO: 321), operably linked 5' to the element, TS-SETit.DHDPS.Ex2-1:1:1 (SEQ ID NO: 298). The plasmids construct pMON139288 is comprised of the transit peptide encoding sequence, GOI-TS-Fe-SD:1:1, presented as SEQ ID NO: 282, which is further comprised of the element, TS-SETit.Fe-SD.ex1-1:1:1 (SEQ ID NO: 299), operably linked 5' to the intron element, I-SETit.Fe-SD-1:1:1 (SEQ ID NO: 322), operably linked 5' to the element, TS-SETit.Fe-SD.ex2-1:1:1 (SEQ ID NO: 300). The plasmids construct pMON139287 is comprised of the transit peptide encoding sequence, GOI-TS-PPR:1:1, presented as SEQ ID NO: 283, which is further comprised of the element, TS-SETit.PPR.ex1-1:1:1 (SEQ ID NO: 305), operably linked 5' to the intron element, I-SETit.PPR-1:1:2 (SEQ ID NO: 323), operably linked 5' to the element, TS-SETit.PPR.ex2-1:1:2 (SEQ ID NO: 306).

Isolated coding sequences encoding *S. italica* CTPs are tested using plant vectors designed for use in either transient protoplast or stable transformation plant assays. DNA fragments encoding the CTP are cloned in frame with a GUS coding sequence using methods known in the art. A plant transformation vector is constructed using methods known in the art and is comprised in a similar manner as the plant vectors described in the previous examples. The expression cassette used to test the CTP is comprised of a constitutive promoter such as the Cauliflower mosaic virus promoter, P-CaMV.35S-enh-1:1:9 (SEQ ID NO: 1096), operably linked 5' to a leader element, L-Ta.Lhcb1-1:1:1 (SEQ ID NO: 1097), operably linked 5' to a coding sequence for GFP in which the test CTP is fused in frame at the 5' end of the GFP coding sequence to enable translation of a chimeric CTP-GFP molecule, operably linked to the Nopaline synthase 3' termination region from *A. tumefaciens* (T-AGRtu.nos-1:1:13, SEQ ID NO: 1088). For stable plant transformation, a second transgene selection cassette used for selection of transformed plant cells that confers resistance to the herbicide glyphosate (driven by the rice Actin 1 promoter) is cloned adjacent to the CTP test transgene cassette. The two transgene cassettes are flanked at the by a right border and left border region from *Agrobacterium tumefaciens* to allow for stable integration of both transgene cassettes in the plant cell genome.

For transient assay testing of the CTP, protoplast cells are transformed with the plant plasmid construct comprising the CTP test transgene cassette. Transformed protoplast cells are observed using microscopy and fluorescence to determine the relative amount of GFP protein present in the chloroplast and in the cytosol. An effective CTP will cause most GFP  
5 fluorescence to appear in the chloroplast stroma or thylacoid, depending upon the type of CTP chosen. Protein is isolated and electrophoresed on polyacrylamide gels and stained using standard methods and compared with a non-plastid targeted GFP protein standard to determine if proper processing of the transit peptide has occurred.

For stable plant transformation, corn plants are transformed as described above by  
10 using *Agrobacterium*-mediated transformation methods known in the art. Tissues are harvested from the developing transformants and viewed microscopically with fluorescence to determine the relative amounts of GFP protein in the chloroplast and cytosol. Protein is isolated and electrophoresed on polyacrylamide gels and stained using standard methods and compared with a non-plastid targeted GFP protein standard to determine if proper processing  
15 of the transit peptide has occurred.

Having illustrated and described the principles of the present invention, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all modifications that are within the spirit and scope of the claims. All publications and published patent documents cited herein  
20 are hereby incorporated by reference to the same extent as if each individual publication or patent application is specifically and individually indicated to be incorporated by reference.

## CLAIMS

Claim 1. A DNA molecule comprising a DNA sequence selected from the group consisting of:

- a) a sequence with at least 85 percent sequence identity to any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924;
- b) a sequence comprising any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924; and
- c) a fragment of any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924, wherein the fragment has gene-regulatory activity, or wherein the encoded peptide functions to localize an operably linked polypeptide within a cell;

wherein said sequence is operably linked to a heterologous transcribable polynucleotide molecule.

Claim 2. The DNA molecule of claim 1, wherein said sequence has at least 90 percent sequence identity to a DNA sequence of any of SEQ ID NOs: 1-323 or SEQ ID NOS: 352-924.

Claim 3. The DNA molecule of claim 1, wherein said sequence has at least 95 percent sequence identity to a DNA sequence selected from the group consisting of SEQ ID NOs: 1-323 and SEQ ID NOs: 352-924.

Claim 4. The DNA molecule of claim 1, wherein the DNA sequence comprises a regulatory element.

Claim 5. The DNA molecule of claim 1, wherein the heterologous transcribable polynucleotide molecule comprises a gene of agronomic interest.

Claim 6. The DNA molecule of claim 1, wherein the heterologous transcribable polynucleotide molecule comprises a gene capable of providing herbicide resistance in plants.

Claim 7. The DNA molecule of claim 1, wherein the transcribable polynucleotide molecule comprises a gene capable of providing plant pest control in plants.

Claim 8. A transgenic plant cell comprising a DNA construct comprising a sequence selected from the group consisting selected from the group consisting of:

- a) a sequence with at least 85 percent sequence identity to any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924;

- b) a sequence comprising any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924; and
  - c) a fragment of any of SEQ ID NOs: 1-323 or SEQ ID NOs: 352-924, wherein the fragment has gene-regulatory activity, or wherein the encoded peptide functions to localize an operably linked polypeptide within a cell;
- wherein said sequence is operably linked to a heterologous transcribable polynucleotide molecule.

Claim 9. The transgenic plant cell of claim 8, wherein said transgenic plant cell is a monocotyledonous plant cell.

Claim 10. The transgenic plant cell of claim 8, wherein said transgenic plant cell is a dicotyledonous plant cell.

Claim 11. A transgenic plant, or part thereof, comprising the DNA molecule of claim 1.

Claim 12. A progeny plant of the transgenic plant of claim 11, or a part thereof, wherein the progeny plant or part thereof comprises said DNA molecule.

Claim 13. A transgenic seed, wherein the seed comprises the DNA molecule of claim 1.

Claim 14. A DNA molecule comprising a DNA sequence encoding a chloroplast transit peptide, wherein the protein sequence of the encoded transit peptide is selected from the group consisting of:

- a) a transit peptide protein sequence of any of SEQ ID NOs: 324-350; and
- b) a transit peptide protein sequence with at least 95 percent sequence identity to any of SEQ ID NOs: 324-350;

wherein the chloroplast transit peptide-encoding DNA molecule is operably linked to a heterologous transcribable polynucleotide molecule.

Claim 15. The DNA molecule of claim 14 comprising a DNA sequence encoding a chloroplast transit peptide, wherein the DNA sequence comprises the sequence of any of SEQ ID NOs: 277-284, 289-293, 296, 301-304 or 307-316.

Claim 16. A transgenic plant cell comprising the DNA molecule of claim 14.

Claim 17. The transgenic plant cell of claim 16, wherein said transgenic plant cell is a monocotyledonous plant cell.

Claim 18. The transgenic plant cell of claim 16, wherein said transgenic plant cell is a dicotyledonous plant cell.

Claim 19. A transgenic plant, or part thereof, comprising the DNA molecule of claim 14.

Claim 20. A progeny plant, or part thereof, of the transgenic plant of claim 19, wherein the progeny plant or part thereof comprises said DNA molecule.

Claim 21. A transgenic seed comprising the DNA molecule of claim 14.

P-SETit.Act8-1:1:5  
P-SETit.Act8-1:1:6  
-----  
TGTGCCATACCCGAGTGGCCATTATATAAAATGTAAACTTGATTGACGAAATAATTTCAGGC  
-----  
P-SETit.Act8-1:1:5  
P-SETit.Act8-1:1:6  
-----  
ACAAGCTGGAAAAATAATTAATAATTTTTTTCGATCAATCAGGGCATCAGGTACAAAA  
-----  
P-SETit.Act8-1:1:5  
P-SETit.Act8-1:1:6  
-----  
GCTGGACAGTGTGATCGAAAAAGGCAACACCTGGAAATGGGGTGTTCGTTGGTGGTGAG  
-----  
P-SETit.Act8-1:1:5  
P-SETit.Act8-1:1:6  
-----  
CGCAGCAGGCCAAAAGCCGACCCAAAGCAACATCCAGCGAAGAACCAGTTGGACTTGGAC  
-----  
P-SETit.Act8-1:1:5  
P-SETit.Act8-1:1:6  
-----  
CGACCGATTGGGGGCGGCACGTA CTGATGCTGCGGGGCCACTAGGCAGATCGGACGG  
-----  
P-SETit.Act8-1:1:5  
P-SETit.Act8-1:1:6  
-----  
CGGGTGCCTCCGGTCGCCCATCGTGC GGTC CAGGTTGCTCAGCATCTCCAGCTCA  
-----  
P-SETit.Act8-1:1:5  
P-SETit.Act8-1:1:6  
-----  
TTCAGCCTCAGCGGCTTTGGGGGCTCTATTTATTTCTCTCTCTTTTTCGAC  
-----  
P-SETit.Act8-1:1:5  
P-SETit.Act8-1:1:6  
-----  
TCGCTCTCATTTTCTCCATTTCTTTTCTTTGGTCCCGGACCACAGGTACTTCCAGTGG  
-----  
P-SETit.Act8-1:1:5  
P-SETit.Act8-1:1:6  
-----  
AAGGAATATAATTGTAGTTTTCTATTGTGTAATAAGCATGTCTCTGATACACAATAGAC  
-----  
ATGAAATACCTTGGTGCCAGGGTCTC ACTCTCTCTGGTTATGTTGATTCTTGATCC  
ATGAAATACCTTGGTGCCAGGGTCTC ACTCTCTCTGGTTATGTTGATTCTTGATCC  
\*\*\*\*\*  
-----ATTGCTCTGATACACAATAGAC  
\*\*\*\*\*

FIG. 1a

P-SETit. Act8-1:1:5  
AAACTCAAGATGCTGAACTGAAAATCATAAAAATTTTCATCCTTTACAAAATTACCCTAAAGGA  
P-SETit. Act8-1:1:6  
AAACTCAAGATGCTGAACTGAAAATCATAAAAATTTTCATCCTTTACAAAATTACCCTAAAGGA  
\*\*\*\*\*  
P-SETit. Act8-1:1:5  
GTTGTTTTCAATTTGGCTTCAATAAAAGTTCTTCGCCCTTCAACGGTGTGGTTTGTGGAA  
P-SETit. Act8-1:1:6  
GTTGTTTTCAATTTGGCTTCAATAAAAGTTCTTCGCCCTTCAACGGTGTGGTTTGTGGAA  
\*\*\*\*\*  
P-SETit. Act8-1:1:5  
GTTAGAAGCATTGGCTTCGTGGCATGGACCTACTTGCAAATAATTTCCCTCCCAATAAAA  
P-SETit. Act8-1:1:6  
GTTAGAAGCATTGGCTTCGTGGCATGGACCTACTTGCAAATAATTTCCCTCCCAATAAAA  
\*\*\*\*\*  
P-SETit. Act8-1:1:5  
GTTTTTTTTGCAAATAATGCCCTCTTTTGTAGTCCATCGCCCTTTTCTAGCCCCGTGACAACC  
P-SETit. Act8-1:1:6  
GTTTTTTTTGCAAATAATGCCCTCTTTTGTAGTCCATCGCCCTTTTCTAGCCCCGTGACAACC  
\*\*\*\*\*  
P-SETit. Act8-1:1:5  
GGAGTTCAGGAGATGCATAACIGTCTTTGAGATCCCCCTTTTATTTATTTTATTTGATTG  
P-SETit. Act8-1:1:6  
GGAGTTCAGGAGATGCATAACIGTCTTTGAGATCCCCCTTTTATTTATTTTATTTGATTG  
\*\*\*\*\*  
P-SETit. Act8-1:1:5  
CAAACATAATTTTAGTTGGCAGTAGCCAGGATGGGCTAAATAATTCITGAGATGTTGGCCT  
P-SETit. Act8-1:1:6  
CAAACATAATTTTAGTTGGCAGTAGCCAGGATGGGCTAAATAATTCITGAGATGTTGGCCT  
\*\*\*\*\*  
P-SETit. Act8-1:1:5  
AAATAATTTTCAGAAAATGAAGTGTCCCTCCACGTCTCCATGACAAAAAAAATACTCCAT  
P-SETit. Act8-1:1:6  
AAATAATTTTCAGAAAATGAAGTGTCCCTCCACGTCTCCATGACAAAAAAAATACTCCAT  
\*\*\*\*\*  
P-SETit. Act8-1:1:5  
GTTGGACTACAGTCTAAAAAAGAACAGAGCTGTAAACATGATGATTAATAAAAAAAGAAGAA  
P-SETit. Act8-1:1:6  
GTTGGACTACAGTCTAAAAAAGAACAGAGCTGTAAACATGATGATTAATAAAAAAAGAAGAA  
\*\*\*\*\*  
P-SETit. Act8-1:1:5  
CATTGAGACCTCTGTTTTCATGCTGAAATATACCTTGTTTTTCATTTCTTTTACCG  
P-SETit. Act8-1:1:6  
CATTGAGACCTCTGTTTTCATGCTGAAATATACCTTGTTTTTCATTTCTTTTACCG  
\*\*\*\*\*

**FIG. 1b**

P-SETit.Act8-1:1:5 TGGTCAAAAAGGAAATGTTAGATACTAACTGATAAAGTAACTGAAAAATAGATGCATATCCTTAAA  
P-SETit.Act8-1:1:6 TGGTCAAAAAGGAAATGTTAGATACTAACTGATAAAGTAACTGAAAAATAGATGCATATCCTTAAA  
\*\*\*  
P-SETit.Act8-1:1:5 AAAGATCTCCATTTGGAAAAAATATGATAAACACGAAAATTCGTAGACGCCGAAACATCTGA  
P-SETit.Act8-1:1:6 AAAGATCTCCATTTGGAAAAAATATGATAAACACGAAAATTCGTAGACGCCGAAACATCTGA  
\*\*\*  
P-SETit.Act8-1:1:5 AATATTATTCAATTTTGGGGGAAAAAATACACGCCCTCGTATTTCAAACCCGTATCTGT  
P-SETit.Act8-1:1:6 AATATTATTCAATTTTGGGGGAAAAAATACACGCCCTCGTATTTCAAACCCGTATCTGT  
\*\*\*  
P-SETit.Act8-1:1:5 GTCACGGACCACGCATCAATCGAGTCCACGTACCACCCATCCCCTTCCCCTCCCCTTCCCC  
P-SETit.Act8-1:1:6 GTCACGGACCACGCATCAATCGAGTCCACGTACCACCCATCCCCTTCCCCTCCCCTTCCCC  
\*\*\*  
P-SETit.Act8-1:1:5 CCCGCTCTTGTATAAACTCCACCACCCAGTCCCTCGGGG  
P-SETit.Act8-1:1:6 CCCGCTCTTGTATAAACTCCACCACCCAGTCCCTCGGGG  
\*\*\*

FIG. 1c



P-SETit.Alc1-1:1:1	CAATCATTGCCACTGAAATCCCTGAGTATAACATAGTTTCTCACGAAGAAGGCCAAAAGTTG
P-SETit.Alc1-1:1:2	-----
P-SETit.Alc1-1:1:1	AGCCCAAAATTTTCGATAAAAGTCGTTACATCTTATTTTCGTACTTATATCATCTTTACAC
P-SETit.Alc1-1:1:2	-----
P-SETit.Alc1-1:1:1	ACATGATCAATATTTTTCTAGCAAAAAGTCATGCATGCTTCCATATACAGTGAAGATA
P-SETit.Alc1-1:1:2	-----
P-SETit.Alc1-1:1:1	TGGGCATGGATAAAATCCTAGGAACGCCAAAAAAAATTGACCTTGCTATGGCCACGACT
P-SETit.Alc1-1:1:2	-----
P-SETit.Alc1-1:1:1	TCTTGATTCTACCAATTTGACGCA TGAAACCTCTCTCAAGAATGGACATAAAATTTTGGC
P-SETit.Alc1-1:1:2	-----
P-SETit.Alc1-1:1:1	AAAAAAGTTCAACCCAAAGTATAATAACATAACACACGTCATCACAATAGTCTACGTTGCTA
P-SETit.Alc1-1:1:2	-----
P-SETit.Alc1-1:1:1	GCAATGATGGGAACAATACATGCTCATAAACCAAAACATCATTTGTTAAAAATTTTGGTT
P-SETit.Alc1-1:1:2	-----
P-SETit.Alc1-1:1:1	ACAIGTTTGATTCACATGTTGCCGTAAAAATGGTGTGATGAGCTTAGACGAAGGACTCAT
P-SETit.Alc1-1:1:2	-----
P-SETit.Alc1-1:1:1	TGGGCTTCCGTTAATCTTGTGCAAAACATCTCAAAAGTCATTAATCAAAATTCATAAAGTGA
P-SETit.Alc1-1:1:2	-----
P-SETit.Alc1-1:1:1	ACGATAACAACACTTTAAACCGGCGTAATAGTTTAAATAGAGGTAGCGTATAGGGTCGGAAT
P-SETit.Alc1-1:1:2	-----

FIG. 2a

P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
ATCATCTTCTTTGTAGCCATAGGCATGTGCGACCCCAAGAATGAAATGGAAATATGATACC  
-----  
P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
GTTAAGTTGAGCCAGTGTCTCATGTGTGTGCACCGATGAAATTTGGTTCTGAGTAT  
-----  
P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
ATATGGAAAACAAGAGTCTAGGTTTAAATCCGATCCAATTGTTACCACACATCCAGAAA  
-----  
P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
CACTATCTCAACTTTTCATAGAAATATTATGCAAAAGCAGGAAGAAAACCTCCTATGG  
-----  
P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
ATCGATATAGATGGCCCTACAATAATGTATCTGTAGACAAGGTAGAAATACATTCAAATATG  
-----  
P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
TCAAAAAGTAGAATCTTGGGAAACTACAATGTGCACAAGCAGCGGTCCCTCCACCTTTGAGC  
-----  
P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
GACTTTCACCTTTAGAGAGATAGCTCTAACATATGCATGCGGTAGATCTGGTGCAATATAT  
-----  
P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
CCACACCGATTGCAAAACGGATTCCACGCAACCACCAACGCAACACCTTGTTCGGTGACT  
-----  
P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
TACAATACTGACTTAATCTAGGTTCTTGTGTATTGGCTCATTACTTACTTGATAACTTCT  
-----  
P-SETit.Alc1-1:1:1  
P-SETit.Alc1-1:1:2  
TCTAGAACTAGTGCCTTACCCTCTATCATCCATGAGGATTGGCTCATGGAACAAATCCTAT  
-----CATCCATGAGGATTGGCTCATGGAACAAATCCTAT  
\*\*\*\*\*

FIG. 2b



P-SETit.Cys1:1:2	GGTGGGTTAATCTTTATGGGTAGTTTAGTGTGGCTTGCAACTTTATCATGAAAAATAGGT
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	TGTATAGTCACCTTGTGGCTTGCAACTTTATCATGAAGGAAAGTTGTATAGTCAIGTGT
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	CAAAAGTTTGCATCTCTAAACCCCTGCATGTACTAAATCGTAGACACTGAAGCTCCAAAAG
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	TGGAATTTTGGATTTCTCTGAAGAACCCTTCGGAGTTTGAGGAGATTTTGGAGTTGATCC
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	CCTGTGAAGCCGGACCTTCGGACAACACTAACCTTTTACAGATCAAGGCAAGCCCCGGT
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	GCATCTATACCTACCTACTTTGGAGTTATTATTCATGTGTTCAATTGTTGGTTATTTTC
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	CGTATGAATGCATTAAGTCTAGGAGTTGATTGAAACCTATTCTTGTGCATGATCCTACCT
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	GATTTAGAGGACATCTTTGCCACTTGTTCACTAATTAGTAACTATCCTATGCTTAGCAAT
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	GCTTAGATACTTCAAGCAACTTGGATTGCTTAGCTCTAAGGAAAATTTTGGTCAACATG
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	TTAGTTAAGGAAAAATAGCCTGGAAAATTGGAAAATGCAGACAGAAGCTAGAGATGGTAGTT
P-SETit.Cys1:1:3	-----
P-SETit.Cys1:1:2	CTGTTTGCTAAGTTAATTGGTTAAGGCCCGATCGTTGTCTTAAACCTTTGATCAAGTGAG
P-SETit.Cys1:1:3	-----

FIG. 3a

P-SETit.Cys-1:1:2  
P-SETit.Cys-1:1:3  
-----  
TTCTACCTGGTTGCTTACTGGGTATGGACCAGTAAAGCCCGGTAGTTAGTTGGCTCTT  
-----  
TGATCGGGAATAATTTCGTACCCGIGCTTGACGTGCTGGAGAATGGTAGGGGCATAGCCCTG  
-----  
AAACTCACATGGAGATCAGGCCAGACGTGGGGTCCCATGTGGGTGCGTCCCCTGGGTTCCG  
-----  
GGTAGTCATGTTCCCAATTTTTGGGTACGGCTAATAGAAAAGGTCATTATATGCAACCTAG  
-----  
ACAGTTGTACATAGCTGGTGGTTGGGTATCTCCTGCAGGATGTAATTTGATCCGGATCGC  
-----  
CGCAATTTCTCGGATATGAATGCACCTTGATCACCGTTAAGCATCATACTGTAATCCAGTTG  
-----  
AATATAATGTTTTTCCCTAGAAATGAATATGCTGTATGACTTAGTTCACCTGAGTGCTCAAA  
-----  
ATGTTTTATTTCATCTAGACTGGTTAGGTAACAACATAAACAATAAAGAAATAAAGTTA  
-----  
AGGTTCCACTTATAGTAAGCCCTTCCGCAAAAATGTGAGTCCGCCAGTATACTAAACAGC  
-----  
TATCATTCAGTTTTTGGAAAAAAAACACTATCTATAATTGGTTAATCGGGTCAGTCTTGCT  
-----GCT  
\*\*\*  
AAGTACCCCTCGGACTCAGGGGAATCCTTCGTTGCTATTTTCAGAACTACAGCAAGAGTCCG  
AAGTACCCCTCGGACTCAGGGGAATCCTTCGTTGCTATTTTCAGAACTACAGCAAGAGTCCG  
\*\*\*\*\*  
P-SETit.Cys-1:1:2  
P-SETit.Cys-1:1:3

FIG. 3b

P-SETit.Cys-1:1:2 CCGAGGGAGAAGCCCGAAGAAATTAGGGTATTTTACGAGTCTATAATACCCTAGAAAT  
 P-SETit.Cys-1:1:3 CCGAGGGAGAAGCCCGAAGAAATTAGGGTATTTTACGAGTCTATAATACCCTAGAAAT  
 \*\*\*\*\*

P-SETit.Cys-1:1:2 AAAACTCAGATGTTTAACTTTCACCCGGACTAGTTTATGTTTTAAACTCTTAGAACTGTT  
 P-SETit.Cys-1:1:3 AAAACTCAGATGTTTAACTTTCACCCGGACTAGTTTATGTTTTAAACTCTTAGAACTGTT  
 \*\*\*\*\*

P-SETit.Cys-1:1:2 CTGACCTGCATGTTAAGTTTGTTCAAAATTAAGTTGTAATAATTGACACCTCGATATG  
 P-SETit.Cys-1:1:3 CTGACCTGCATGTTAAGTTTGTTCAAAATTAAGTTGTAATAATTGACACCTCGATATG  
 \*\*\*\*\*

P-SETit.Cys-1:1:2 TATGTA AAAATGTAATAATTGTTGATACCTTCCCATCGTGGAAAGTGATCCTGATGTAIGG  
 P-SETit.Cys-1:1:3 TATGTA AAAATGTAATAATTGTTGATACCTTCCCATCGTGGAAAGTGATCCTGATGTAIGG  
 \*\*\*\*\*

P-SETit.Cys-1:1:2 CTATGGATCACGTCGTGGATGTTTCGAGGAGTCTTAAGGACACTCGACGGAATACCCGGAC  
 P-SETit.Cys-1:1:3 CTATGGATCACGTCGTGGATGTTTCGAGGAGTCTTAAGGACACTCGACGGAATACCCGGAC  
 \*\*\*\*\*

P-SETit.Cys-1:1:2 TTAGACTGTTTTAAGTGCCTTCGGATAAATTGTTGATAGCGGATTAGACCGCGCTTA  
 P-SETit.Cys-1:1:3 TTAGACTGTTTTAAGTGCCTTCGGATAAATTGTTGATAGCGGATTAGACCGCGCTTA  
 \*\*\*\*\*

P-SETit.Cys-1:1:2 AACCAGTTTAAAGTTGGGCGGTTCCGCCACACTATCCCTTATTACTGATTTTGGTTTGGTT  
 P-SETit.Cys-1:1:3 AACCAGTTTAAAGTTGGGCGGTTCCGCCACACTATCCCTTATTACTGATTTTGGTTTGGTT  
 \*\*\*\*\*

P-SETit.Cys-1:1:2 TGGGTCCATCCGAGATGAAGAAGAGGCAAGCACATGTACTGGAATAATAATCCCTGGATCG  
 P-SETit.Cys-1:1:3 TGGGTCCATCCGAGATGAAGAAGAGGCAAGCACATGTACTGGAATAATAATCCCTGGATCG  
 \*\*\*\*\*

P-SETit.Cys-1:1:2 AAGGTATGAGCATCACACATTTAAATGCTAGTATTAAGAAGAGGCAAGCACATGTACTGCG  
 P-SETit.Cys-1:1:3 AAGGTATGAGCATCACACATTTAAATGCTAGTATTAAGAAGAGGCAAGCACATGTACTGCG  
 \*\*\*\*\*

FIG. 3c

P-SETit.Cys-1:1:2 AATAATATCCGAGATGCAAGTTTACAACAATAAAGTCTGGCATTGCATCACCTGCTCAACC  
P-SETit.Cys-1:1:3 AATAATATCCGAGATGCAAGTTTACAACAATAAAGTCTGGCATTGCATCACCTGCTCAACC  
\* \* \* \* \*

P-SETit.Cys-1:1:2 CATGTCCAATCACATGATAAATCCTACCAAACTTAGCACGAGCTGAATTAATAATTTTCCTT  
P-SETit.Cys-1:1:3 CATGTCCAATCACATGATAAATCCTACCAAACTTAGCACGAGCTGAATTAATAATTTTCCTT  
\* \* \* \* \*

P-SETit.Cys-1:1:2 AATCCGTTTTATATATATGTACAAAATGCCACCATTGCCACATAAAATGCATAAAGTTCTTCCCA  
P-SETit.Cys-1:1:3 AATCCGTTTTATATATATGTACAAAATGCCACCATTGCCACATAAAATGCATAAAGTTCTTCCCA  
\* \* \* \* \*

P-SETit.Cys-1:1:2 GATAAATTCATAGGCCACCATTGCTATTTTATAAAGAAATCCTTCAGAATTTAAGCTGAAA  
P-SETit.Cys-1:1:3 GATAAATTCATAGGCCACCATTGCTATTTTATAAAGAAATCCTTCAGAATTTAAGCTGAAA  
\* \* \* \* \*

P-SETit.Cys-1:1:2 GGAACCTTGACTAAAGGAGGAACAACAAGGATAGAGTCTAGTGCTCCATCCCATGCTACAG  
P-SETit.Cys-1:1:3 GGAACCTTGACTAAAGGAGGAACAACAAGGATAGAGTCTAGTGCTCCATCCCATGCTACAG  
\* \* \* \* \*

P-SETit.Cys-1:1:2 ATTTTAGTTCAGTTTTCAACCAACAAGAATTAATAATTTTCTAGCAAAATGAAAAATTC  
P-SETit.Cys-1:1:3 ATTTTAGTTCAGTTTTCAACCAACAAGAATTAATAATTTTCTAGCAAAATGAAAAATTC  
\* \* \* \* \*

P-SETit.Cys-1:1:2 AAGGGTCATTTCTTAAGGTTTGAAGTTATACCATTTTGCATATATATCATAAAGTATACA  
P-SETit.Cys-1:1:3 AAGGGTCATTTCTTAAGGTTTGAAGTTATACCATTTTGCATATATATCATAAAGTATACA  
\* \* \* \* \*

P-SETit.Cys-1:1:2 AACTTTAGCCATATAATTTTGTGCTGGCCACACCTTGGTACTTTATAAAGACCCCTGCACA  
P-SETit.Cys-1:1:3 AACTTTAGCCATATAATTTTGTGCTGGCCACACCTTGGTACTTTATAAAGACCCCTGCACA  
\* \* \* \* \*

FIG. 3d

P-SETit.Cys-1:1:2 ATTTAAGATAAAAATAATTCGGCAAATAAAGGGGAGAAAACAAGGAAACGAGTCAGGT  
P-SETit.Cys-1:1:3 ATTTAAGATAAAAATAATTCGGCAAATAAAGGGGAGAAAACAAGGAAACGAGTCAGGT  
\* \* \* \* \*  
P-SETit.Cys-1:1:2 GCCCATCCCATGTTCCATCGAGATGAAGAAGGGGCAAGCCACATGTACTTGAATATAT  
P-SETit.Cys-1:1:3 GCCCATCCCATGTTCCATCGAGATGAAGAAGGGGCAAGCCACATGTACTTGAATATAT  
\* \* \* \* \*  
P-SETit.Cys-1:1:2 TGGATCGAAGGTATTATCAGCATCAAATAATTGAAATACGAGTAGTATTAATAAATTTGAG  
P-SETit.Cys-1:1:3 TGGATCGAAGGTATTATCAGCATCAAATAATTGAAATACGAGTAGTATTAATAAATTTGAG  
\* \* \* \* \*  
P-SETit.Cys-1:1:2 CAGCGGGAAGGTTCCTTATGGCCCTCAGTGATAGCTAATCTAACTTTACAATATCTCTGAC  
P-SETit.Cys-1:1:3 CAGCGGGAAGGTTCCTTATGGCCCTCAGTGATAGCTAATCTAACTTTACAATATCTCTGAC  
\* \* \* \* \*  
P-SETit.Cys-1:1:2 ATTGCATCACATGCTCATCCCCATGTCACATGATAATAATCCTACCAAGTCAGCACGAGC  
P-SETit.Cys-1:1:3 ATTGCATCACATGCTCATCCCCATGTCACATGATAATAATCCTACCAAGTCAGCACGAGC  
\* \* \* \* \*  
P-SETit.Cys-1:1:2 CACATGAAATTCCTTAATCCCTTTTTTATATATATAATAATGCAAAAAGATTGGATCAGAG  
P-SETit.Cys-1:1:3 CACATGAAATTCCTTAATCCCTTTTTTATATATATAATAATGCAAAAAGATTGGATCAGAG  
\* \* \* \* \*  
P-SETit.Cys-1:1:2 CAAGTAGCTAAGTATCCTGCAATCCCTCTTCAATGTCATGAATCATATCCCCCGATGAACCA  
P-SETit.Cys-1:1:3 CAAGTAGCTAAGTATCCTGCAATCCCTCTTCAATGTCATGAATCATATCCCCCGATGAACCA  
\* \* \* \* \*  
P-SETit.Cys-1:1:2 CATAACCTCCCTTTTATTCCTTCAAAAACAAGTTAAAAAATTTTGTTCAGAAAAATGAATTT  
P-SETit.Cys-1:1:3 CATAACCTCCCTTTTATTCCTTCAAAAACAAGTTAAAAAATTTTGTTCAGAAAAATGAATTT  
\* \* \* \* \*

FIG. 3e



P-SETit.Cys-1:1:2 TTAACCTCGGTTTTCTTAAGGTTTGATTTTTTTGCGTCTATATAAGTTCTTTACAGAT  
P-SETit.Cys-1:1:3 TTAACCTCGGTTTTCTTAAGGTTTGATTTTTTTGCGTCTATATAAGTTCTTTACAGAT  
\*\*\*\*\*  
P-SETit.Cys-1:1:2 AACGTACCTTTGCTAGTTTATATGAATCCTGCAGAGTTTCAAGGTGAAATTTAAGGAAC  
P-SETit.Cys-1:1:3 AACGTACCTTTGCTAGTTTATATGAATCCTGCAGAGTTTCAAGGTGAAATTTAAGGAAC  
\*\*\*\*\*  
P-SETit.Cys-1:1:2 TTGGAAGTGGGAGAAACTGAGGATCGAGTCCAGTGCCTTTATCGGTTTATCATCCCATACT  
P-SETit.Cys-1:1:3 TTGGAAGTGGGAGAAACTGAGGATCGAGTCCAGTGCCTTTATCGGTTTATCATCCCATACT  
\*\*\*\*\*  
P-SETit.Cys-1:1:2 AATGATTTCTTTTTTGTTCAGTTGCAATGTTGCAACCCTGGGATGACGAAATCGAATATGCAAAT  
P-SETit.Cys-1:1:3 AATGATTTCTTTTTTGTTCAGTTGCAATGTTGCAACCCTGGGATGACGAAATCGAATATGCAAAT  
\*\*\*\*\*  
P-SETit.Cys-1:1:2 CACATGTACAGGGTTTACTGGCATCTTTTTTGGATCGAAGGTACGAGCACCGCATTTGAA  
P-SETit.Cys-1:1:3 CACATGTACAGGGTTTACTGGCATCTTTTTTGGATCGAAGGTACGAGCACCGCATTTGAA  
\*\*\*\*\*  
P-SETit.Cys-1:1:2 TATGAGCACTGACGTTAAGCTTCGTTAGTGGGTGAGGTTTTGCACTGTGTAATAATTTCTCT  
P-SETit.Cys-1:1:3 TATGAGCACTGACGTTAAGCTTCGTTAGTGGGTGAGGTTTTGCACTGTGTAATAATTTCTCT  
\*\*\*\*\*  
P-SETit.Cys-1:1:2 ACTCGTGATTCAGTGATCTGCATAAATGTCAACGGCCGTATAGGAGCAACTACTATTAA  
P-SETit.Cys-1:1:3 ACTCGTGATTCAGTGATCTGCATAAATGTCAACGGCCGTATAGGAGCAACTACTATTAA  
\*\*\*\*\*  
P-SETit.Cys-1:1:2 GCATCCATTCCGCCCATCTATTTCCGATCAACGGCTCACT  
P-SETit.Cys-1:1:3 GCATCCATTCCGCCCATCTATTTCCGATCAACGGCTCACT  
\*\*\*\*\*

FIG. 3f

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
TTTTGGGACGGAGCAGCTTTAGAGCTGGGATGACGGCCCTCTCTTCGTGTGCTCGAGGCG  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
TTGCGTCACCCCTAGGTTTCCCTAGGCCAGAGTAAGATAGTTTTGCCTGCGGCAACGCC  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
TTTAGTATTGGAAACCTTGGCTGCTTTCCTCAAGGCTTTCGTCCAAAATGAAGAATATT  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
TTTCTTCGATGCCCTCGCGAACGGGGCCTCGACGCTGAAGAAGAAAAGACACGATTCCC  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
CCAGGATCCACCCAGCCTCCTTTGAACCTCTTTGTACTCCGAAGGAGATTCGGCACCAAG  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
AATAAAATCACCACGATCTTTAAACCTTAAATGGAATTGATCTACAAAAATGAAGCGCAGG  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
AAAAGTTATGTAACGAAGAGAAAGCATGAAGAAAAATCAATACAGAGGAGGACAAGCGTA  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
GAGTCTTAATTTCTTTGGTAAACTTGAAACATTTCCGACCAATCCGGGCAAGGGATAGGCC  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
CTTTGGTATCAGTCCAGTCCGAGTCCAGAATTGCCCAGCTACAGCGGACAGGCCACACTC  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
CAAGCATGCGGTACTCCTCGATAATATCGCAAGTGTATACTTGCAAGCAAGAGACGAA  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
-----  
GCATCGAGATGAAGGCATCACCCCTCGGCACATCGGGAGGGTCAATCTTGTAATCATCAT  
-----

FIG. 4a

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
GAAGAGCCTCCATCTTTTAGTGACGAGGCAGTTGGGCTCACCAGTCTCTTCAGGCTCCA  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
CTTCATGATAGAGCTAGTGTGATGCCAATCGATAGGCCACTTGTTGCCGATATGCAGCCA  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
CGGGGTTCAAGACTCCAGTCCGTTACATGAAGTTGAGACATCCAAACTGAGCTTGTTTCC  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
CGACCACTCCATTAGCACTTCTCCAAACAAGTACTTCAGCTGGTGATGCACCTTGTGAG  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
CATTAGTGAAACTGACTGCCGAGGACTTGATTTTTGTTGTTTGCAAATCCACATATACG  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
TCGAAAGATGAACGAGAGCCGTGGGTGTCAACTGATGCAGGTAGATATCATACAGACGGA  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
GTATATCTATAACAATTGGGTCCAACTCAAAAACGAAAGACCAACATAAAGGAGATCACGGA  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
AGATGACGACCTTGTGTCTTCGGGTTTCGGAATAACCTCAGAATTGGGTGCACGGCTAG  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
CAAAGACCATCTTGTTCGCTTCAGTAAAGTCGATGTCATTGGGATGAAGCAAGCTTCAGC  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
CAAAAACAACAGTCTTTGGCATAAATCTGATCACCATCGTCACCCGAAAGGCGGTTGCATT  
-----  
P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
TTGGGGCCATTGAAATACAATGAAGAACACAAATTAAGCAACCAAAAATGAAGTCGTCGGAAG  
-----

FIG. 4b

P-SETit.Dzs-1:1:4 AATACCAAACCCAGTGAAGGGGGCTTTAGACCTCCATCAAAGAAAAGCGATAGCGGTAAAA  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 GTAAATGGCACAGAGCCACACAGGGTTATTTATAGGTAGAGCGAAAGAGAAACAGTAACA  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 AGGGTCAAAGTCAAAAAACCATTAATAAATTTAAATGAAGGATTTCGAATAAGGAAAAA  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 TATATTAATCAAAGGGCAAGGAAGCAAAAATCATGCATGGGCCGATCTTTGGGGAGTTCCCGA  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 AGGATGGTTGCACCCATGTGTTTTAGGATCGTAAGTTCAGCCCATCAGAGGAGAAGATGGC  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 CCTCGCTCACGAGGGGCTACTATTGGGGAACACCACCTTGGGAATCTCCTTCGAAAGGAGA  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 AGTCCTTTTGACACTAACCATGACAGCTAGTTTGCTTTGTGGCGAGCTCTAAGTGCAGGG  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 AAGAAGAAGCGGTACGAAGAGCTCTTAGGCCGTATGAGGGCTCCTTCGGAACAAAACAAT  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 GGCTGACTGGCCCGGGTCTTTGGGAGGGCCGGCATCTGACTCGTCCCTCCAAGGTT  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 TGTCTCAGAGAAAGGCATGGAGTGGCTCAGCAAAGAGACCTCGACTTCCGAAGATGA  
P-SETit.Dzs-1:1:5 -----  
P-SETit.Dzs-1:1:4 ACATGCGTTGAAAGACCATGCTGTCCACCACATGGATTTGAAAGGACCCTACGAGGAGCTA  
P-SETit.Dzs-1:1:5 -----

**FIG. 4c**

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
GCTGGAGGAGTCCATACAAAAGATTTCACCTTGAAGAAAACCCAACTCTGTAGATCATAAAG  
-----

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
CCAACGTCCTTGGAAAAAACCGAGAAATGATACATGGCCCTCATAGAGGTGAATAATTCGA  
-----

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
AATCACTATCAATTTCCTCCAAGACATGATTGCAGAAATGAGGCTAACAGAAATCCACATTGA  
-----

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
GAGGGATGGAGGATCGCCCAAAAGAAAGCCACCAAGAAAACTCATTTGGAAGAGAAGGCGT  
-----

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
GTGAGCAACTGACTGGCAAAGAGAAAACCTGGATTGGATCATGCAATGGATCTCCAAAACCTA  
-----

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
GACCAAAGTCCAGATCAGAGAGGCCACCCATGGTATGGTAGCCTAGAGGAAGGATCACCA  
-----

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
TGATCGCCGAGGAAITGGAGAGCACGACACCGGGAGGAGGGGACATCGCCAGTCACCAT  
-----

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
ACTCGCATTTTTAGCGTCTGCTCATCTCCACCTACCCCTCCCCTAGTCCCCTATCTAGT  
-----

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
TGCAGTGCCCACTAGATGGTGCCACCACGCTAACCTAAACGTCAAGTCTCCTTCC  
-----

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
CCGGCACCGCCCTATCTAGTTGCAATGCCACCACCTAGATGGTGCCACCACGCTAACC  
\*\*\*\*\*  
-----AAACGTCAAGTCTCCTTCC  
\*\*\*\*\*

P-SETit.Dzs-1:1:4  
P-SETit.Dzs-1:1:5  
CCGGCACCGCCCTATCTAGTTGCAATGCCACCACCTAGATGGTGCCACCACGCTAACC  
\*\*\*\*\*

FIG. 4d

P-SETit.Dzs-1:1:4 TAAACGTCAAGTCTCTCTGCCCCAGCACCGCCCATCAGCAATCGTTGCCCTGCCCAACAG  
P-SETit.Dzs-1:1:5 TAAACGTCAAGTCTCTCTCTGCCCCAGCACCGCCCATCAGCAATCGTTGCCCTGCCCAACAG  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 CACGACACGATGGCACTTATGGAATCCTCTTAATACCAACACACTACTCCAATCCAAAAATT  
P-SETit.Dzs-1:1:5 CACGACACGATGGCACTTATGGAATCCTCTTAATACCAACACACTACTCCAATCCAAAAATT  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 TAACATTGCAGTTTGATTTTGCTCTCGACCTAAAGAAATCACAATAATTAATTTAGGTGTC  
P-SETit.Dzs-1:1:5 TAACATTGCAGTTTGATTTTGCTCTCGACCTAAAGAAATCACAATAATTAATTTAGGTGTC  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 TGGAAATCTATGAAGTGGAGAGAGTAATAATAAATCATGTATATATATGTCCAGTTGTTGT  
P-SETit.Dzs-1:1:5 TGGAAATCTATGAAGTGGAGAGAGTAATAATAAATCATGTATATATATGTCCAGTTGTTGT  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 TTGGGAAACCTGTCTGTGCAAAAAGCAGGGTCCTTTGCCCATGTACTTTTACTATCAG  
P-SETit.Dzs-1:1:5 TTGGGAAACCTGTCTGTGCAAAAAGCAGGGTCCTTTGCCCATGTACTTTTACTATCAG  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 TCAACAAGGCAGCATTCCTCTATAAACCACGAAAGTAAAGGAACCAAGACCATCACAGA  
P-SETit.Dzs-1:1:5 TCAACAAGGCAGCATTCCTCTATAAACCACGAAAGTAAAGGAACCAAGACCATCACAGA  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 CTCCGTGCATGAGTTATGACATGTAAGGAAATCAGCCCTGGACAAATCAATTGCAGAA  
P-SETit.Dzs-1:1:5 CTCCGTGCATGAGTTATGACATGTAAGGAAATCAGCCCTGGACAAATCAATTGCAGAA  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 GTCCACAGAAGACTCCAATGTGTGAGTAATGACATGTAAGCCAAATCAGCCTGGACAAAAT  
P-SETit.Dzs-1:1:5 GTCCACAGAAGACTCCAATGTGTGAGTAATGACATGTAAGCCAAATCAGCCTGGACAAAAT  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 TCCGTTGCAGAAGACCACAGTAGACTCCATGTATGAGTTAAGAGTATATGACATGTAGAG  
P-SETit.Dzs-1:1:5 TCCGTTGCAGAAGACCACAGTAGACTCCATGTATGAGTTAAGAGTATATGACATGTAGAG  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 CATAACAGCCCTGGACAAAATCCCTTGCAGAAGTTCTCGATCCACCTCAGCTAGTCAC  
P-SETit.Dzs-1:1:5 CATAACAGCCCTGGACAAAATCCCTTGCAGAAGTTCTCGATCCACCTCAGCTAGTCAC  
\* \* \* \* \*  
P-SETit.Dzs-1:1:4 GATGCAATCACCTTACTTTAGCAAAAAGCAAAAACAACCTGAGATATGCAGTCACATCCTTA  
P-SETit.Dzs-1:1:5 GATGCAATCACCTTACTTTAGCAAAAAGCAAAAACAACCTGAGATATGCAGTCACATCCTTA  
\* \* \* \* \*

FIG. 4e

P-SETit.Dzs-1:1:4 ATAAACTCGTCATACTACCCCTCACCCCTATTATCAAAAAGTAAGAACAACAAAATACTTTTC  
P-SETit.Dzs-1:1:5 ATAAACTCGTCATACTACCCCTCACCCCTATTATCAAAAAGTAAGAACAACAAAATACTTTTC  
\*\*\*\*\*  
P-SETit.Dzs-1:1:4 CCATGTACCCCATGTCTGAATATCATGGAGGCGATCATGTGGTCCAAAAACAGTTAGGGA  
P-SETit.Dzs-1:1:5 CCATGTACCCCATGTCTGAATATCATGGAGGCGATCATGTGGTCCAAAAACAGTTAGGGA  
\*\*\*\*\*  
P-SETit.Dzs-1:1:4 AATCTCCAAATTTGACTAGATGCCATGTACCCCTCCAGCTTATCTACCCCTCCAACTTTCAG  
P-SETit.Dzs-1:1:5 AATCTCCAAATTTGACTAGATGCCATGTACCCCTCCAGCTTATCTACCCCTCCAACTTTCAG  
\*\*\*\*\*  
P-SETit.Dzs-1:1:4 TTCTGCAAAATGACAAAAACCTTATTGATCTGCACCGTGTITGTAGCATATAAGCACATATA  
P-SETit.Dzs-1:1:5 TTCTGCAAAATGACAAAAACCTTATTGATCTGCACCGTGTITGTAGCATATAAGCACATATA  
\*\*\*\*\*  
P-SETit.Dzs-1:1:4 AATAGGACCTAGATGAATGCTCAGCCCA  
P-SETit.Dzs-1:1:5 AATAGGACCTAGATGAATGCTCAGCCCA  
\*\*\*\*\*

FIG. 4f

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
GGATGATGGATTGCTTTTGAGAAATGACTTCTTAAAGCGTCAAGCTTTTCTGACTGTGTCA  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
GGTCAGCTTCAGGTTGAAAACCTTATGCTTGTGCTCTCAGTAGTGTATATAACCTTTGGACCA  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
ACGTTCCGGGCAGAGAACTCACATACATCAAGACA TCTGGCAGAGT TTTGGATGGTTGAA  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
CCAGAAAAATGCAATTTGCAAACTTGCAGGTGAGCTGCTTCTTGAITGACTAACTTTATTACT  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
TTGTGGTTCGCCACCTATCAAAATTGGATCAITGGGGTTTTGTGTGGACATAAATAATGTTA  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
AACTTGATAAAATTGTGTACAGGATGATATGAACTGTGCAGAAAAAATATGTACAATAACC  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
TCTGCCAGTGGTTACTCGATCATTTGGAAGAAGACATAGATTCATTAGCAAAAAAATACG  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
AGAGAAAACAGTAGACATAATCAAAC TGCAAT TGACCGTCTGAAGCTTGTTCCTCAACGC  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
ATTTTGAACGTAATTCATACACAATGGCTGTGGACATTCGAAAAATACAGAACAAGGTT  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
GAATGGGGAATCGATTTAGCATCTGAGCATGAAAGGTATGTGCAAGTTGTCAGTTGATCT  
-----

P-SETit.Gst-1:1:1  
P-SETit.Gst-1:1:2  
TACTGGTCTTCACCTGATTTACCTTCTTTGATTGGTTAACTGTAACACGTTACTGTGTA  
-----

**FIG. 5a**



P-SETit.Gst-1:1:1	AGGTATTTGACTGAGGTGATATAAAGAAGCCAGTTATTGTTTATAACTATCCCAAGAA
P-SETit.Gst-1:1:2	-----
P-SETit.Gst-1:1:1	ATAAAGCATTTTATATGAGGCTCAATGATGATCAGAAGACTGTTTCTGCTATGGATGTT
P-SETit.Gst-1:1:2	-----
P-SETit.Gst-1:1:1	CTTGCGCCACAGGTATAGATCGAATTGTGAAAATGCTATGATGCTGTCCATTGTTCACT
P-SETit.Gst-1:1:2	-----
P-SETit.Gst-1:1:1	GAAGTGCCCCACACCATTCTACCCCTCAATCAGCATGGATAAAGCAGCCAGATTCAATGTC
P-SETit.Gst-1:1:2	-----
P-SETit.Gst-1:1:1	TGCTCTTAGTAGAATAAATACTATTTCTTTCTGCCTTTTAGAATTTAGATAATCTCTGGATT
P-SETit.Gst-1:1:2	-----
P-SETit.Gst-1:1:1	CTTTTTTCGCCTTTTACCAAATTACAAAGAACAATAAAGCTGCAATAAATAAAGATA
P-SETit.Gst-1:1:2	-----
P-SETit.Gst-1:1:1	ACAATAATTACATTTTCTGAAAATAGCTGTATGCTTGCAACTACATGCTGGATAATTTTGCT
P-SETit.Gst-1:1:2	-----
P-SETit.Gst-1:1:1	TTGCCCTGCTTTGTATATAGTTTGAAGTGAGCTTTGTGGCCGATGATTTCTACCTAACCA
P-SETit.Gst-1:1:2	-----
P-SETit.Gst-1:1:1	TAAATTAATTTCCCTGGTAGGTTGGTGAATTGACTGGTGAAGCCAGAGGAGCATCTAGA
P-SETit.Gst-1:1:2	-----
P-SETit.Gst-1:1:1	TGTCCTGAAACAGAGGTACCGACTTGCTCAATAATAATTTGTTTCTATCAGGATCAATAT
P-SETit.Gst-1:1:2	-----CAGGAT-----T

\*\*\*\*\*  
\*

FIG. 5b

P-SETit.Gst-1:1:1  
 TAGGTTTCATTTGCTCGTGGTAGCAAGCAGTTTGTCCATCATTTGGTTCACGA  
 P-SETit.Gst-1:1:2  
 TAGGTTTCATTTGCTCGTGGTAGCAAGCAGTTTGTCCATCATTTGGTTCACGA  
 \*\*\*\*\*  
 P-SETit.Gst-1:1:1  
 ATATTTGCTGAACATTGTCCAATAATTGTTCTACAGAAATTTGGATGCGGGATTGCCCAT  
 P-SETit.Gst-1:1:2  
 ATATTTGCTGAACATTGTCCAATAATTGTTCTACAGAAATTTGGATGCGGGATTGCCCAT  
 \*\*\*\*\*  
 P-SETit.Gst-1:1:1  
 CACCCCTGATGCGAGCTAGTCTGCTTTCATGGCGAGCGGTGACCTCGACGTGCAGGGTGC  
 P-SETit.Gst-1:1:2  
 CACCCCTGATGCGAGCTAGTCTGCTTTCATGGCGAGCGGTGACCTCGACGTGCAGGGTGC  
 \*\*\*\*\*  
 P-SETit.Gst-1:1:1  
 CAGGTAGAGTGCCCATCACCCCTGACGGAGCTAGTCTGCTTACGCAAGCAGCGACGTC  
 P-SETit.Gst-1:1:2  
 CAGGTAGAGTGCCCATCACCCCTGACGGAGCTAGTCTGCTTACGCAAGCAGCGACGTC  
 \*\*\*\*\*  
 P-SETit.Gst-1:1:1  
 ACAAGTGTAGTAAAAGGCCTCGTTGACCCACGCCAAATCCACCGCTTCCCTTAGCACCTT  
 P-SETit.Gst-1:1:2  
 ACAAGTGTAGTAAAAGGCCTCGTTGACCCACGCCAAATCCACCGCTTCCCTTAGCACCTT  
 \*\*\*\*\*  
 P-SETit.Gst-1:1:1  
 CTTCCCCGAGCACCCGGCAGGCAGCAGCGAGATCTAGGCAGCAGCCATCGCTCCTTCTC  
 P-SETit.Gst-1:1:2  
 CTTCCCCGAGCACCCGGCAGGCAGCAGCGAGATCTAGGCAGCAGCCATCGCTCCTTCTC  
 \*\*\*\*\*  
 P-SETit.Gst-1:1:1  
 CCCCCTGCTCCTGTATAAATAGCAGCAGGGGCAACCAATTCATCAACTCGTATCA  
 P-SETit.Gst-1:1:2  
 CCCCCTGCTCCTGTATAAATAGCAGCAGGGGCAACCAATTCATCAACTCGTATCA  
 \*\*\*\*\*  
 P-SETit.Gst-1:1:1  
 T  
 P-SETit.Gst-1:1:2  
 T  
 \*

FIG. 5c

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
TCCACCGATCATCACACAGCCAGTAGTGGGGTGGCCAAAGCAATCAGGCACCCGGCA  
-----

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
ATGCGAGCTGATGCGTGATGATGGTGCTACCAACAAGTACTATAAAATTTCTGATTTG  
-----

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
AAAGGGATTGGCCTCGATATTTATTAGCTCCCGGCTTTTGTACGACACGTTAGCATG  
-----

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
CGTGCCCTCTAGAAGCTAGTCCGGGTATTACCGCTAGAAAAGTCCCGAAAATGAAGCATTT  
-----

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
ACCACCCGTAAGCTCATTTTCTTTATGATGAGTAGACACCGTACCAACATTGAGGACC  
-----

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
GATTGGTTGGCTCCCAAATCTGCCCTGCCAAACTAGGGCAAGTTCATAAAATTTTGACAT  
-----

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
TCGCTTGGTTGGCAATCAATTAATCCATTCTAAAATTTCTGCCTAGGTTTGTATATAA  
-----

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
CATGCCCTATATTTGGTCTACTCAAAATTTGGTAGGTAAATTTGAACACCCAACAAAT  
-----

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
CAGGCTATTTAICTTAICTCTTTCTCAATTTTCATTACACAGCAAGGCAGTAATTA  
-----

P-SETit. Ifr-1:1:2  
P-SETit. Ifr-1:1:3  
AGGACCGTATATACAATGGATGTAAGAAATAAAATGTATAAGTAGAAAATATTTGGCATGC  
-----

FIG. 6a

P-SETit.Ifr-1:1:2 CTCGTGCTGGTGCAATGTCGATAATGCTCTCAATTAGAAAGTTGGAGACAGGTTATGCTTAGG  
 P-SETit.Ifr-1:1:3 -----

P-SETit.Ifr-1:1:2 ATAGTCCCAACCTATGATAATCTGTGTCTATACTGCCACATAAGTAAGACATCACCTTA  
 P-SETit.Ifr-1:1:3 -----

P-SETit.Ifr-1:1:2 GAAATTACATTCACAAACCTATAAATTTCTTAGTGGATCCTTAATTAATTCAATCATCTC  
 P-SETit.Ifr-1:1:3 -----

P-SETit.Ifr-1:1:2 TCCCTCAATTCCTCATCAATATGAAGACACCATCTTCTTCCAATGCAAAATTAACACT  
 P-SETit.Ifr-1:1:3 -----

P-SETit.Ifr-1:1:2 GTCTAGGATCTAGGTTCAGGTGTGATACTGGGTC TTGCAATGAGATCCAGTTTCTTGTTTC  
 P-SETit.Ifr-1:1:3 -----

P-SETit.Ifr-1:1:2 TTCCAATTCTCTCATTTAAATATAATAATCACATAAGCAAAGATCCTATGTAGCTGCAC  
 P-SETit.Ifr-1:1:3 -----

P-SETit.Ifr-1:1:2 AATTAATGCTATGGAAACTATCCTAATCGGAGGGTTGGACTGCTCCTGCCTATGGCGGC  
 P-SETit.Ifr-1:1:3 -----CCTGCCTATGGCGGC  
 \*\*\*\*\*

P-SETit.Ifr-1:1:2 TTATCCCCCATTGCTAACCCTGAAAATCGAAAAGGAGTGCAATGACAGGGCAAACACTAG  
 P-SETit.Ifr-1:1:3 TTATCCCCCATTGCTAACCCTGAAAATCGAAAAGGAGTGCAATGACAGGGCAAACACTAG  
 \*\*\*\*\*

FIG. 6b

P-SETit.Ifr-1:1:2 TGTGCGCTGCATCAATAAATCGTCCATGATTATATAGAGGTAGCATGACTTTTTTAGGCCGT  
P-SETit.Ifr-1:1:3 TGTGCGCTGCATCAATAAATCGTCCATGATTATATAGAGGTAGCATGACTTTTTTAGGCCGT  
\*\*\*\*\*  
P-SETit.Ifr-1:1:2 CGTGTCCCTAATCAATCAGAAAAAGAAAGCCAAACCTAATCGCTATGGCCCGCAACCCCGAT  
P-SETit.Ifr-1:1:3 CGTGTCCCTAATCAATCAGAAAAAGAAAGCCAAACCTAATCGCTATGGCCCGCAACCCCGAT  
\*\*\*\*\*  
P-SETit.Ifr-1:1:2 GCGACTATGCGGAGTATATGGAACCCCGTTGCTACTCCCCCTATATATCGTGGAGTCTGA  
P-SETit.Ifr-1:1:3 GCGACTATGCGGAGTATATGGAACCCCGTTGCTACTCCCCCTATATATCGTGGAGTCTGA  
\*\*\*\*\*  
P-SETit.Ifr-1:1:2 TGGCAATCCAACGGCAGACG  
P-SETit.Ifr-1:1:3 TGGCAATCCAACGGCAGACG  
\*\*\*\*\*

FIG. 6c

P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
ACTATGGCGGCGTGGTGAGACCCCGCACGTGGTGTTCITCAAGGCGATGAGAGTGAG  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
GTGAGTCCAACGGCGGATGTGGCGAGGTCCCGCGCATTTGTATTATCGCGGTGGCGACT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
GCGAGCAGCCTAAGAGAGGTCCGGATTCGGTGGTATCACTCTGTCAGATAGAGTGTGGT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
GTTGCAGCGGCACTACAGCGGAGGTCTCGTATGGCGGTGGCCATCTCGGTGCAGTGCAG  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
TCTGTTTCTATCGGTTCCCTGTCGATGCCGGACCACGCCCTAGACGTTTCGGCGGAGTTATG  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
AGCGTGGATGTTGGTGGTGCCCGCGTGCGGAGCAGAGCCGGTGAGACCCGTTGATGTCC  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
CAATCCAATCGGTAGGGTGTGATAGTTTTGGTGTGAGTGGCTATCTGCATTGATGTCCCT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
AATCCGACCAGGCGAGTTTGTATTGTTTTTCTTTTCTTTTCCCTTTTATAATTTTTTCAGTC  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
TGAAATTCITTTTTTAAATAAATCGGCAGCTCTCCAATAATGGTTCGTTCAAAAAAAGAA  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
TTTTTAAGAAAAGATAGATAAAAAGATAAACCAAAATTCGAAATTTGATTCAGATACCCCT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
CGAAATGGAAGTTGGCCGTCCACTTAAAAATGAACCGCATGATTCCTCAATAATTCAGT  
-----

FIG. 7a

P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
GCCGTCCTCCTGTTACTCTTTAAATTCATTACGTGACATCGGCCCGTTGATAAGCCGT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
GCTCCTTGTCGCTAATTCCTATTACGTGCTGATAGAGGCGAGTCAATCAAACGTTGCACT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
TTGTAACCCCTTAATCTTTGCTAAGAGTCACATGACCCCGTACATCTCGATCGATCCTCTT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
TTAGTTGGCGCGGGTTTTACCAATGTGAAGCAATGTGCCGGCATGCTGCTGTCGCAACT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
AGCTCTTCATCAAGCTATCGACAGATGAGATGCGCTCCCATTTGGAACCCGAAAATGGGTGGA  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
AATTCGTTGATTCTGTGCTGCCGACCGTGGAAAGTGAACCCACCCTTCCCCCTCGCCG  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
CAGGCATGTAGTCCGCGTGGATTTTGAACACCACAGCTGACATATGCCCTCCATGTCCATT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
CCTGCCGTGGGAATCTCTGCACCCTGGTCTGCCCTAGTCCACAGGCTGCGGCCGCCACCATC  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
TAAATGCATGTTTGAATATCAGTAGTTAAATTTTATCACATGTCACATCGGATATTCGGA  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
TGCTAAATTAGTAGGACTAAACATAGCTAATTATAAAATTAATTGCAGAACTTCGGGGCTA  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
ATTCATGAGACCAATTTATTGAGGCTAATTAATCTATCATTAGCAAAATGGTTACTGTAGC  
-----

FIG. 7b

P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
-----  
ACCAATATGTCAAAATCATGGACTAAATTAGCCCTTAAATAGATTTATCTCACAAAATTAGACTC  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
-----  
CATCTGTGTAATTAGTTTATATAATTAGATTATATTTAAATACTTCTAATTAGTATCCAAAAT  
-----  
P-SETit.Nrt2-1:1:2  
P-SETit.Nrt2-1:1:3  
-----  
ATCCGATGTGACTGTGAAGTTTAAACACAATGTAGCCAAACACCCCGACACCCCGGTCCG  
-----  
CGGCCCGCCCGCCCGTTGGATGCCGCCCTCGCCACGCCATGCGACGCAACGCCCTGCAC  
CGGCCCGCCCGCCCGTTGGATGCCGCCCTCGCCACGCCATGCGACGCAACGCCCTGCAC  
\*\*\*\*\*  
TGCATTTTCGTGGCCCTTGCGTAGGGGCACCGGAAGAGCCGCTGGTTTCGTTTCGTTCCTCC  
TGCATTTTCGTGGCCCTTGCGTAGGGGCACCGGAAGAGCCGCTGGTTTCGTTTCGTTCCTCC  
\*\*\*\*\*  
CTGGCCCGGTCAGCTGATTTCCGAGCAGGGTCGAATCGATCGCGCGGTCGCCGCGATCC  
CTGGCCCGGTCAGCTGATTTCCGAGCAGGGTCGAATCGATCGCGCGGTCGCCGCGATCC  
\*\*\*\*\*  
CACCAAGCCGGAGCCCTTCGGGGCCCTCTAAAAGCAAGTCAGGCTCGCGAAGTGACCCGA  
CACCAAGCCGGAGCCCTTCGGGGCCCTCTAAAAGCAAGTCAGGCTCGCGAAGTGACCCGA  
\*\*\*\*\*

FIG. 7c



P-SETit.Nrt2-1:1:2 AAAGATACATTACTACCAGCTGCAGCGCTGCTGTGGCCCTGCCCTCGTCAAGATCTGAC  
P-SETit.Nrt2-1:1:3 AAAGATACATTACTACCAGCTGCAGCGCTGCTGTGGCCCTGCCCTCGTCAAGATCTGAC  
\*\*\*\*\*

P-SETit.Nrt2-1:1:2 TTTTGAATCGCATAAATTAGCAGCTTGTTTAATAAGCTTAAACGATTGATCCTACTCCTTG  
P-SETit.Nrt2-1:1:3 TTTTGAATCGCATAAATTAGCAGCTTGTTTAATAAGCTTAAACGATTGATCCTACTCCTTG  
\*\*\*\*\*

CCGACA  
CCGACA  
\*\*\*\*\*

FIG. 7d

P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
CCATCTCCTTGAAAGAGAACTATGTTAAGTTTCAACAAGAGACAACAATGCCCTTGT  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
GAACTGACAAAAGAGATAACAGGAAAAGACAAGGATTCGAGGAAGCAAGGCAAGTGA  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
GCAGTGAATTGCAGTACTACCGAACAAAGACTGATTTTAGTACAGTACCCTAATTATTTG  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
GAATAATGAATAAGAGAGAGATAATGTTGGACGGAATAAAAAATTGTGAACGGAAAAATAACTA  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
GATATGTTTTGGGAGGCACCTAACAAAGACTTTAATTTGGAAAGAGAGATACAAACATATAAA  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
CATGTTAGTGGATAGATAAATATGGTGCTTATGTTAGAAAAGTACATTTATTTTATGGT  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
TATTTTCATAGATCTGGAACATTAATACAAAACTAATCCTATGGATACAAAACTGCATGA  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
AAAGTAAATCATGAAATTTATTTCTAATATTGGGCATCATATTTAAGTCACGTTGCAGAAATTT  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
GAACTTAAAACTCGATTCGTACGTGGAGAAAACAAAAAAGATCAAATCTCATTAGAGGTAT  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
ATTGGACCATTTAAAGTAGTTTGGGGGAATAAAATCGAGCCTAAGTTTATTTAGGGGTTA  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
AGAGACAGATAAAGTAGATTGGTGTAGGTAGTAAATAATGGACATTTTCCTAGTTGTATCT  
-----  
P-SETit.Ppc-1:1:3  
P-SETit.Ppc-1:1:4  
-----  
GGTATGTACTATTTGGCTTAAACATATATAAGTCCCAAGTAGTGCAAATTGGGATTCAAA  
-----

FIG. 8a

P-SETit.Ppc-1:1:3 TACTAAATTCCTTTACGTAGAGATGAGGAAGGAGAATTACAAAAGCAGCAATTGCCACAGC  
P-SETit.Ppc-1:1:4 -----

P-SETit.Ppc-1:1:3 CTTGCTTCAGTTTTGCTCGATGCAAGCAGGGTGGAGTGGTGGACAGGCATGGACTAGCGG  
P-SETit.Ppc-1:1:4 -----

P-SETit.Ppc-1:1:3 CACGGAAGACAACATCATGTCATGTGCTTCCAGAGTTGCCAACGGCAAAGAGCAGCAATG  
P-SETit.Ppc-1:1:4 CACGGAAGACAACATCATGTCATGTGCTTCCAGAGTTGCCAACGGCAAAGAGCAGCAATG  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 TGTGTAGTTCGGCCATGGGCTCGGTACAGGTGGTTCGTCCTCCAGAACAGGCAGGAGA  
P-SETit.Ppc-1:1:4 TGTGTAGTTCGGCCATGGGCTCGGTACAGGTGGTTCGTCCTCCAGAACAGGCAGGAGA  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 GGGGTAGCTGGCTGAGCATCCATCCGTGATGAGCCATAACCCGTTCGCAAACGGGCAGC  
P-SETit.Ppc-1:1:4 GGGGTAGCTGGCTGAGCATCCATCCGTGATGAGCCATAACCCGTTCGCAAACGGGCAGC  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 TGGAGAGATAATCGGAATAATGTGATCCTCCACGGCGTTGCCACAGATGTCACGGATCATG  
P-SETit.Ppc-1:1:4 TGGAGAGATAATCGGAATAATGTGATCCTCCACGGCGTTGCCACAGATGTCACGGATCATG  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 CATGCAATGTCGTCATTTAGCAGATGTTGAGTCTGATACACCCCTTCACACCAAGCAAATT  
P-SETit.Ppc-1:1:4 CATGCAATGTCGTCATTTAGCAGATGTTGAGTCTGATACACCCCTTCACACCAAGCAAATT  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 ATAACCAAGATAAGGATTCGAGGAAGCAGAAGGAGGCAAGTGAGCAGTGACAAAGTACGTC  
P-SETit.Ppc-1:1:4 ATAACCAAGATAAGGATTCGAGGAAGCAGAAGGAGGCAAGTGAGCAGTGACAAAGTACGTC  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 CACAACACAATCCAGACGAAAAACGAACACATCAATGCCCTCGGCGCCAAATCGTAGTAATG  
P-SETit.Ppc-1:1:4 CACAACACAATCCAGACGAAAAACGAACACATCAATGCCCTCGGCGCCAAATCGTAGTAATG  
\*\*\*\*\*

FIG. 8b

P-SETit.Ppc-1:1:3 CTGCAACAACAAGTCCCTGTGAAGACGGTCTCTATTAATAAGCCACACATGGAGCCGAT  
P-SETit.Ppc-1:1:4 CTGCAACAACAAGTCCCTGTGAAGACGGTCTCTATTAATAAGCCACACATGGAGCCGAT  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 ATATAATCCGAAAGATAATGTAATAATAGAGAAGCGTCAATGTTTTTCAAAAAATAAAAAA  
P-SETit.Ppc-1:1:4 ATATAATCCGAAAGATAATGTAATAATAGAGAAGCGTCAATGTTTTTCAAAAAATAAAAAA  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 AAAAAATAAActGGGAACAAGCCCAATCGCAAGGCCGGAGCGAGCTTGCTTTTCAGAGTCA  
P-SETit.Ppc-1:1:4 AAAAAATAAActGGGAACAAGCCCAATCGCAAGGCCGGAGCGAGCTTGCTTTTCAGAGTCA  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 GTGAGCCACCCGGGGGAGCTGACGGGTAGGGGAAGAGGAACAACATCGTGCAATGTC  
P-SETit.Ppc-1:1:4 GTGAGCCACCCGGGGGAGCTGACGGGTAGGGGAAGAGGAACAACATCGTGCAATGTC  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 TTCAAGAGTTGCCAACGGCAAAAGGAGCAGCAGTGTGTAGTTCGGCTATGGGCTCAGTC  
P-SETit.Ppc-1:1:4 TTCAAGAGTTGCCAACGGCAAAAGGAGCAGCAGTGTGTAGTTCGGCTATGGGCTCAGTC  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 ACAGGTGGGTTTCGTCTCCAGAACCCAGCAGGAGAGGGGTTTGTGGCTGGCTGTGCATC  
P-SETit.Ppc-1:1:4 ACAGGTGGGTTTCGTCTCCAGAACCCAGCAGGAGAGGGGTTTGTGGCTGGCTGTGCATC  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 CATCCGTGATGAGCCTAAACCGTTGCGCAAAATGGACACCTGGAGCGATAATGTGAATATG  
P-SETit.Ppc-1:1:4 CATCCGTGATGAGCCTAAACCGTTGCGCAAAATGGACACCTGGAGCGATAATGTGAATATG  
\*\*\*\*\*

P-SETit.Ppc-1:1:3 TGATCCTCCACGGCGTTGCCACAAAATGTCACATGATCCTCATCCATGTTGTCCATATAGCACATG  
P-SETit.Ppc-1:1:4 TGATCCTCCACGGCGTTGCCACAAAATGTCACATGATCCTCATCCATGTTGTCCATATAGCACATG  
\*\*\*\*\*

FIG. 8c

P-SETit.Ppc-1:1:3 CTTGAGTCCAAGTCCCTCCACACCAAGCAAAATTATAGCCCAAGACAAGGATTC AAGGAAGC  
P-SETit.Ppc-1:1:4 CTTGAGTCCAAGTCCCTCCACACCAAGCAAAATTATAGCCCAAGACAAGGATTC AAGGAAGC  
\*\*\*\*\*  
P-SETit.Ppc-1:1:3 AAGAAGCGGAGTGAGCAGTGACAAAAGTATGTCCACAGCACAAATCCGTACGAAAAACGAGCGG  
P-SETit.Ppc-1:1:4 AAGAAGCGGAGTGAGCAGTGACAAAAGTATGTCCACAGCACAAATCCGTACGAAAAACGAGCGG  
\*\*\*\*\*  
P-SETit.Ppc-1:1:3 CATCAGTGCCCTCAGCGCCAATCGTAGTGTTCGCGATCAAAATAAGTCCGTGAAGCCGG  
P-SETit.Ppc-1:1:4 CATCAGTGCCCTCAGCGCCAATCGTAGTGTTCGCGATCAAAATAAGTCCGTGAAGCCGG  
\*\*\*\*\*  
P-SETit.Ppc-1:1:3 TCTCTATTAATTGCCACGATGAAGCCGATGTGTGGTCCGAAAGATAATATGCAATAAT  
P-SETit.Ppc-1:1:4 TCTCTATTAATTGCCACGATGAAGCCGATGTGTGGTCCGAAAGATAATATGCAATAAT  
\*\*\*\*\*  
P-SETit.Ppc-1:1:3 AGAGAAGCGTTATGTTTTCCCTGAAAAAAACCITGGGAACAAGCCTGTCGCAAGCCGGGA  
P-SETit.Ppc-1:1:4 AGAGAAGCGTTATGTTTTCCCTGAAAAAAACCITGGGAACAAGCCTGTCGCAAGCCGGGA  
\*\*\*\*\*  
P-SETit.Ppc-1:1:3 GCCTCGCTTCCGCTTGCTTTTACAGTCAGTGAGCCACCGGGGGAGCTGACGGGTA  
P-SETit.Ppc-1:1:4 GCCTCGCTTCCGCTTGCTTTTACAGTCAGTGAGCCACCGGGGGAGCTGACGGGTA  
\*\*\*\*\*  
P-SETit.Ppc-1:1:3 GGGGAAGAGGAAGACAGCACCGTGCAATGTGCTTCCAGAGTTGCCAACGGCAAAATGAGCAG  
P-SETit.Ppc-1:1:4 GGGGAAGAGGAAGACAGCACCGTGCAATGTGCTTCCAGAGTTGCCAACGGCAAAATGAGCAG  
\*\*\*\*\*  
P-SETit.Ppc-1:1:3 CAGTGTGTAGTTGGCCATGGGCTCGGTACAGGTGGGTTTCGTCTCCAGAACCCAGC  
P-SETit.Ppc-1:1:4 CAGTGTGTAGTTGGCCATGGGCTCGGTACAGGTGGGTTTCGTCTCCAGAACCCAGC  
\*\*\*\*\*

FIG. 8d

P-SETit.Ppc-1:1:3  
 P-SETit.Ppc-1:1:4  
 AGGAGAGGGGTATGTGGCTGGCTGTGCATCCATCCGTGATGAGCCATAAACCGTTGCGCA  
 AGGAGAGGGGTATGTGGCTGGCTGTGCATCCATCCGTGATGAGCCATAAACCGTTGCGCA  
 \*\*\*\*\*

P-SETit.Ppc-1:1:3  
 P-SETit.Ppc-1:1:4  
 AACGGACACCTAGAGCGATAATGTAATGTGATCCTCCACGGCGTTGCCACAAAATGTCAC  
 AACGGACACCTAGAGCGATAATGTAATGTGATCCTCCACGGCGTTGCCACAAAATGTCAC  
 \*\*\*\*\*

P-SETit.Ppc-1:1:3  
 P-SETit.Ppc-1:1:4  
 TGATCATGCATGTGTCCATAATAGCACATGCTTGTAGTCCAAAGTCCCTCCACACCAAGCA  
 TGATCATGCATGTGTCCATAATAGCACATGCTTGTAGTCCAAAGTCCCTCCACACCAAGCA  
 \*\*\*\*\*

P-SETit.Ppc-1:1:3  
 P-SETit.Ppc-1:1:4  
 AATTAATTATAGCCAAGAGAAGGATTCGAGGAAGAAGGCGATTGAGCAGTGACAAGT  
 AATTAATTATAGCCAAGAGAAGGATTCGAGGAAGAAGGCGATTGAGCAGTGACAAGT  
 \*\*\*\*\*

P-SETit.Ppc-1:1:3  
 P-SETit.Ppc-1:1:4  
 ACGTCGACAGCACAAACCAGACGAAACCAGGCAACGAGCACATCGTCCGACGGCTGC  
 ACGTCGACAGCACAAACCAGACGAAACCAGGCAACGAGCACATCGTCCGACGGCTGC  
 \*\*\*\*\*

P-SETit.Ppc-1:1:3  
 P-SETit.Ppc-1:1:4  
 CCTTCGGCCCTCCCTTGCATTCGTGATTCGCCGGCGGTACATGCCCTCCGCTTGAAAA  
 CCTTCGGCCCTCCCTTGCATTCGTGATTCGCCGGCGGTACATGCCCTCCGCTTGAAAA  
 \*\*\*\*\*

P-SETit.Ppc-1:1:3  
 P-SETit.Ppc-1:1:4  
 AAAAAAAGCCCTTTCCATCCGCCATGAGGCGTGCAATCCCGTGACACACTCGCCGA  
 AAAAAAAGCCCTTTCCATCCGCCATGAGGCGTGCAATCCCGTGACACACTCGCCGA  
 \*\*\*\*\*

P-SETit.Ppc-1:1:3  
 P-SETit.Ppc-1:1:4  
 CTCCCATCCGTTAATTTGTTTTTTTAGAAGCCCATCCGTTAATTTAAACCCACCCGCG  
 CTCCCATCCGTTAATTTGTTTTTTTAGAAGCCCATCCGTTAATTTAAACCCACCCGCG  
 \*\*\*\*\*

P-SETit.Ppc-1:1:3  
 P-SETit.Ppc-1:1:4  
 CGCCTCGCTCGACCTCGTGCCA  
 CGCCTCGCTCGACCTCGTGCCA  
 \*\*\*\*\*

FIG. 8e







P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4  
-----GACTTACTAAGAAATCATAGACAAATTAATTTTCAGCTGATGCATGACAAAAATAAATAA  
P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4  
-----CCTACCTACTAAAACTTTGGCCCTTTCTTCAGAAAATGTTGGTAAGAGTAAAT  
TGCCTTGCCTACTATAAACTTTGGCCCTTTCTTCAGAAAATGTTGGTAAGAGTAAAT  
\*\*\*\*\*  
P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4  
TACCAGAGTAGACCCGGGAAAGGGGAGGAGGAGAACATAAGACAGCTATGAATCAGGAGCC  
TACCAGAGTAGACCCGGGAAAGGGGAGGAGGAGAACATAAGACAGCTATGAATCAGGAGCC  
\*\*\*\*\*  
P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4  
TAGTCAAATGAGCAGGTAACCTTGTCCAIGTCACTTTTTTTTGGTCTCCTCCAGGGTT  
TAGTCAAATGAGCAGGTAACCTTGTCCAIGTCACTTTTTTTTGGTCTCCTCCAGGGTT  
\*\*\*\*\*  
P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4  
CAGCAGTTGCTTGGGATGCTAAAAAGTTTGTGACCTCTTTTGAGGCAATGAACATGCTATC  
CAGCAGTTGCTTGGGATGCTAAAAAGTTTGTGACCTCTTTTGAGGCAATGAACATGCTATC  
\*\*\*\*\*  
P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4  
AGTATAGTTGCTAGGGATATAAAATTTTACTCCTCTACCATAATGCAAGAAGCCACGAACC  
AGTATAGTTGCTAGGGATATAAAATTTTACTCCTCTACCATAATGCAAGAAGCCACGAACC  
\*\*\*\*\*  
P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4  
TATCTGCTCTTTGCAATGCATTCATGACAAACTACAGATGTGCATGCATGCCCTCTGAAGT  
TATCTGCTCTTTGCAATGCATTCATGACAAACTACAGATGTGCATGCATGCCCTCTGAAGT  
\*\*\*\*\*  
P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4  
CTTCTGACAGTAACCTACCCTAAAAAAAATCTTCTGACAGTAACCTGCCCTAAAAAAAAGTA  
CTTCTGACAGTAACCTACCCTAAAAAAAATCTTCTGACAGTAACCTGCCCTAAAAAAAAGTA  
\*\*\*\*\*  
P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4  
TGCTGACAGTAACAAAGCACGGCTGTTGTTTTGTTCCCTTGAAGGAACCGGCCACTGATACG  
TGCTGACAGTAACAAAGCACGGCTGTTGTTTTGTTCCCTTGAAGGAACCGGCCACTGATACG  
\*\*\*\*\*

FIG. 9c

P-SETit.Prx3-1:1:3 TGCTGCACGGGAAGAACCAAGCTTTCTCCCGACCTGTTTGAGTTTGGAAAGCTTTCAAAACAA  
 P-SETit.Prx3-1:1:4 TGCTGCACGGGAAGAACCAAGCTTTCTCCCGACCTGTTTGAGTTTGGAAAGCTTTCAAAACAA  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

ACAACATCCAAGCTAAGTCCTTCCAGGAACCTGCTGCTCTCGTGACAAGAATGTAGG  
 ACAACATCCAAGCTAAGTCCTTCCAGGAACCTGCTGCTCTCGTGACAAGAATGTAGG  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

CCATGCGAGCACCTAACACACACGGATCATCACGCGCCGTGTGGCTCAGAGTTGGTACACCA  
 CCATGCGAGCACCTAACACACACGGATCATCACGCGCCGTGTGGCTCAGAGTTGGTACACCA  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

CATCATAICGCCCTGGATTGGTCTGTGTACTTGTGTCCCTGGGAAAATCAGTCACTTATT  
 CATCATAICGCCCTGGATTGGTCTGTGTACTTGTGTCCCTGGGAAAATCAGTCACTTATT  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

TTTAGACCCCTGGATTGAACACACAGAATAATGCAATGCAACCATGTTAAGCATGTTGTGCA  
 TTTAGACCCCTGGATTGAACACACAGAATAATGCAATGCAACCATGTTAAGCATGTTGTGCA  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

CAGCCGTTTGACAAGCCATTCCCCATCTTTCATGTAGTATCCTCACCTAGCTAATGTAA  
 CAGCCGTTTGACAAGCCATTCCCCATCTTTCATGTAGTATCCTCACCTAGCTAATGTAA  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

CACGGCAAAACTACCCTAATTTGGCAGTAGAACTCTGATGTATGGAACCATGACGAATAA  
 CACGGCAAAACTACCCTAATTTGGCAGTAGAACTCTGATGTATGGAACCATGACGAATAA  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

ATGGAANAATTTAGACGCTACTACTACTCATCTTGAAATCCCAGAAGCTTGTGTACCTTT  
 ATGGAANAATTTAGACGCTACTACTACTCATCTTGAAATCCCAGAAGCTTGTGTACCTTT  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

FIG. 9d

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

AAAACCAAAGAAACCAGGGGCATAGTCTGCATATGGTCGTCATTAATAATAGTAAATTAAG  
 AAAACCAAAGAAACCAGGGGCATAGTCTGCATATGGTCGTCATTAATAATAGTAAATTAAG  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

TTCAAAACGTAAACAACCCTTGTGTATAACAANAAGAGCTGGACTAACTATGAAGCAAAAAC  
 TTCAAAACGTAAACAACCCTTGTGTATAACAANAAGAGCTGGACTAACTATGAAGCAAAAAC  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

CATGCTTTTCGCCGAGGCTCCTCTGAACCTTGGTTGCTTGGTTTCACATAGTGTAAACT  
 CATGCTTTTCGCCGAGGCTCCTCTGAACCTTGGTTGCTTGGTTTCACATAGTGTAAACT  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

GTTAATAAGCGAGGTTAATAATAGTTCGGGTGAATAATACTACTGGGACGAACTGATATC  
 GTTAATAAGCGAGGTTAATAATAGTTCGGGTGAATAATACTACTGGGACGAACTGATATC  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

TACCACATAATCTACTGTAAGCTGCCTAATTTTGCTGTAGCTAGATTTCCCGGAAGGGGG  
 TACCACATAATCTACTGTAAGCTGCCTAATTTTGCTGTAGCTAGATTTCCCGGAAGGGGG  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

CTCTTGATGCTGCAGAGGACATAATCAACTGATCAATTTATAGACAGAAAGTTGCATAT  
 CTCTTGATGCTGCAGAGGACATAATCAACTGATCAATTTATAGACAGAAAGTTGCATAT  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

TCTATCATTTGTCCTAGCCCCCTAGCTATATGATCAGCATAAATCAGCAATCCATCATATCT  
 TCTATCATTTGTCCTAGCCCCCTAGCTATATGATCAGCATAAATCAGCAATCCATCATATCT  
 \*\*\*\*\*

P-SETit.Prx3-1:1:3  
 P-SETit.Prx3-1:1:4

FIG. 9e

TC TTC CAGT GTTTTTTTT TGTGGCGAAACAATA TCTTTATTT CAGTTC AATCCT  
TC TTC CAGT GTTTTTTTT TGTGGCGAAACAATA TCTTTATTT CAGTTC AATCCT  
\*\*\*\*\*

P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4

GTTAAGCAGCAC TTGCTGTTTCTGCAGTAACCTGTAAACCGTACTGTACTACTGCTGAC  
GTTAAGCAGCAC TTGCTGTTTCTGCAGTAACCTGTAAACCGTACTGTACTACTGCTGAC  
\*\*\*\*\*

P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4

TTGTCGCTTCTGAGTGGAAAAATGCTCACCCTGATCAGAAAACGATTTCTTCGCCTACAAT  
TTGTCGCTTCTGAGTGGAAAAATGCTCACCCTGATCAGAAAACGATTTCTTCGCCTACAAT  
\*\*\*\*\*

P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4

TATTTGGGACTAGAAGCTCAA TGCTCCATGCAAATTCGGTTAAAACTGAAGCATCAA  
TATTTGGGACTAGAAGCTCAA TGCTCCATGCAAATTCGGTTAAAACTGAAGCATCAA  
\*\*\*\*\*

P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4

CAGTCAAAAATGTCAGCTAAAACTGAGGCATCAACAGTCA TACTTGCAGCCAGATGGAAGA  
CAGTCAAAAATGTCAGCTAAAACTGAGGCATCAACAGTCA TACTTGCAGCCAGATGGAAGA  
\*\*\*\*\*

P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4

ACAACCGTAGCAGTTGGACACAAATGTGGAGTCGACCTAGCTCGCTCGTTTGTCCACCCTC  
ACAACCGTAGCAGTTGGACACAAATGTGGAGTCGACCTAGCTCGCTCGTTTGTCCACCCTC  
\*\*\*\*\*

P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4

TTATTTCTATAAGCATCACCCCTAACAAAATCCCTAACCCACTTCCCTTCGTTACACACATGC  
TTATTTCTATAAGCATCACCCCTAACAAAATCCCTAACCCACTTCCCTTCGTTACACACATGC  
\*\*\*\*\*

P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4

TAGCTGCCTCCCTCCAGCTATAAA TAGCACCCCAAAGCCCATCTCATCTGGCCAT  
TAGCTGCCTCCCTCCAGCTATAAA TAGCACCCCAAAGCCCATCTCATCTGGCCAT  
\*\*\*\*\*

P-SETit.Prx3-1:1:3  
P-SETit.Prx3-1:1:4

FIG. 9f

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

TATCGCGACCGCTAAGAGAAGACATTTAAAATAAGTAGTCGGCATTGTGATAAAAAGAG  
 -----  
 -----  
 -----GTCGGCATTGTGATAAAAAGAG

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

AGCGGATTACTGATGIGCAGGTTCTCGATTGTTGATGAAGTCGACTAGTCGGAGTCGAT  
 -----  
 -----  
 AGCGGATTACTGATGIGCAGGTTCTCGATTGTTGATGAAGTCGACTAGTCGGAGTCGAT

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

TCTGACTAGTTATTGATTGTATGGAACCCCGCCCTCGACTAGTTTTCTAGTCGAGACGAGT  
 -----  
 -----  
 TCTGACTAGTTATTGATTGTATGGAACCCCGCCCTCGACTAGTTTTCTAGTCGAGACGAGT

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

AGTCGAAGTAGACCATCCTCAACTACTTTTTCTAGTTGAGATGAGTAGTCGAAAGTAGTCGT  
 -----  
 -----  
 AGTCGAAGTAGACCATCCTCAACTACTTTTTCTAGTTGAGATGAGTAGTCGAAAGTAGTCGT

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

CGGCGACTTGATTATTTGCCCTCAATCTGTGTGACTTGATCGACGAGCCGTTATGCAGCA  
 -----  
 -----  
 CGGCGACTTGATTATTTGCCCTCAATCTGTGTGACTTGATCGACGAGCCGTTATGCAGCA

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

GCTTGCGGTGGAAACCGACTCAGTCTTCGATTGGAAACACGGAGCCGTCAAATTCATGTC  
 -----  
 -----  
 GCTTGCGGTGGAAACCGACTCAGTCTTCGATTGGAAACACGGAGCCGTCAAATTCATGTC

FIG. 10a

P-SETit.Rcc3-1:1:1	GACGTAGATTTGCTCTGCTTGGAACCTCCAACCTCAGTGACTTGCTTCTTGTGAGTAGATTG
P-SETit.Rcc3-1:1:10	-----
P-SETit.Rcc3-1:1:11	-----
P-SETit.Rcc3-1:1:16	GACGTAGATTTGCTCTGCTTGGAACCTCCAACCTCAGTGACTTGCTTCTTGTGAGTAGATTG
P-SETit.Rcc3-1:1:1	ATCTTGATGATGAGGTCCCTTCAAGCTCGCCCTGATGATCTCGACGATCACCTCTACCTGAC
P-SETit.Rcc3-1:1:10	-----
P-SETit.Rcc3-1:1:11	-----
P-SETit.Rcc3-1:1:16	ATCTTGATGATGAGGTCCCTTCAAGCTCGCCCTGATGATCTCGACGATCACCTCTACCTGAC
P-SETit.Rcc3-1:1:1	GGCCAACTGTTGGTGTCTAGACCCAGCAACCTACCGAGGGGTACCCGAGGTAGTGT
P-SETit.Rcc3-1:1:10	-----AGACCCAGCAACCTACCGAGGGGTACCCGAGGTAGTGT
P-SETit.Rcc3-1:1:11	-----
P-SETit.Rcc3-1:1:16	GGCCAACTGTTGGTGTCTAGACCCAGCAACCTACCGAGGGGTACCCGAGGTAGTGT
P-SETit.Rcc3-1:1:1	TGTGGTGGGCTCGTGAAGATCAGGAACTTGAAGGTGAACCTCGAACACACGATTTAGAC
P-SETit.Rcc3-1:1:10	TGTGGTGGGCTCGTGAAGATCAGGAACTTGAAGGTGAACCTCGAACACACGATTTAGAC
P-SETit.Rcc3-1:1:11	-----
P-SETit.Rcc3-1:1:16	TGTGGTGGGCTCGTGAAGATCAGGAACTTGAAGGTGAACCTCGAACACACGATTTAGAC
P-SETit.Rcc3-1:1:1	AAGTTCGGGCTGCTTATGCCGCAATAATACCCCATGTCAATGTGTGTTGGATTGTATT
P-SETit.Rcc3-1:1:10	AAGTTCGGGCTGCTTATGCCGCAATAATACCCCATGTCAATGTGTGTTGGATTGTATT
P-SETit.Rcc3-1:1:11	-----
P-SETit.Rcc3-1:1:16	AAGTTCGGGCTGCTTATGCCGCAATAATACCCCATGTCAATGTGTGTTGGATTGTATT
P-SETit.Rcc3-1:1:1	GATTGATCAGATAATTTGGAGGGGCCCTGCTCGCCTTATATGCCCCATTGCCGGAGGCA
P-SETit.Rcc3-1:1:10	GATTGATCAGATAATTTGGAGGGGCCCTGCTCGCCTTATATGCCCCATTGCCGGAGGCA
P-SETit.Rcc3-1:1:11	-----
P-SETit.Rcc3-1:1:16	GATTGATCAGATAATTTGGAGGGGCCCTGCTCGCCTTATATGCCCCATTGCCGGAGGCA

**FIG. 10b**

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

GGGTACAGGTCGGTTGTTGTACAAGAGTACTAGTCGGTTTTGACCAGCGAGTCCCTACTC  
 GGGCTACAGGTCGGTTGTTGTACAAGAGTACTAGTCGGTTTTGACCAGCGAGTCCCTACTC  
 -----  
 GGGCTACAGGTCGGTTGTTGTACAAGAGTACTAGTCGGTTTTGACCAGCGAGTCCCTACTC

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

TAAITGCTACAAGTAGTTCCCTAATCCTTGACTAGTCCTTGTCCGCCACGTAGACCACCGA  
 TAAITGCTACAAGTAGTTCCCTAATCCTTGACTAGTCCTTGTCCGCCACGTAGACCACCGA  
 -----  
 TAAITGCTACAAGTAGTTCCCTAATCCTTGACTAGTCCTTGTCCGCCACGTAGACCACCGA

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

CGTCTTGCACCCTAGTCTCTGTGTTTGATACATCTTGGTGACAGTCCGGATATTGTAGGAC  
 CGTCTTGCACCCTAGTCTCTGTGTTTGATACATCTTGGTGACAGTCCGGATATTGTAGGAC  
 -----  
 CGTCTTGCACCCTAGTCTCTGTGTTTGATACATCTTGGTGACAGTCCGGATATTGTAGGAC

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

TATCCAAGCTTCCCAGTAGGCCCATAGATGTATGGCCGACAACCTGGATAATGTAACTCTG  
 TATCCAAGCTTCCCAGTAGGCCCATAGATGTATGGCCGACAACCTGGATAATGTAACTCTG  
 -----  
 TATCCAAGCTTCCCAGTAGGCCCATAGATGTATGGCCGACAACCTGGATAATGTAACTCTG

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

GGTCAGTACTATCCTTATCTATA TAGACACAAAACAACGTACTATAGCAGAAGTTTAAGCT  
 GGTCAGTACTATCCTTATCTATA TAGACACAAAACAACGTACTATAGCAGAAGTTTAAGCT  
 -----  
 GGTCAGTACTATCCTTATCTATA TAGACACAAAACAACGTACTATAGCAGAAGTTTAAGCT

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

CGTAACCCACCAATATTTGGTGGCATA TAGACCACCGTATTGCTGATATAGTGCCTCGTAACCC  
 CGTAACCCACCAATATTTGGTGGCATA TAGACCACCGTATTGCTGATATAGTGCCTCGTAACCC  
 -----  
 CGTAACCCACCAATATTTGGTGGCATA TAGACCACCGTATTGCTGATATAGTGCCTCGTAACCC

FIG. 10c

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

ACCAATATTCGTGGCATAAGAGATCTCTTAGGCAATAAAATTAGCAGTACGAAACAATCTA  
 ACCAATATTCGTGGCATAAGAGATCTCTTAGGCAATAAAATTAGCAGTACGAAACAATCTA  
 -----  
 ACCAATATTCGTGGCATAAGAGATCTCTTAGGCAATAAAATTAGCAGTACGAAACAATCTA

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

TGTCCACGTTGTCTAATAACAATGTTCTAAACCTTACAGCCTACTGGACAGTTCTCTAGC  
 TGTCCACGTTGTCTAATAACAATGTTCTAAACCTTACAGCCTACTGGACAGTTCTCTAGC  
 -----GTGTTGCTAATAACAATGTTCTAAACCTTACAGCCTACTGGACAGTTCTCTAGC  
 TGTCCACGTTGTCTAATAACAATGTTCTAAACCTTACAGCCTACTGGACAGTTCTCTAGC  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

CATGATACATGTGCATGTCCGAAACAAATATTTATGGGTACCCGAAAGGTTAAATTTTGT  
 CATGATACATGTGCATGTCCGAAACAAATATTTATGGGTACCCGAAAGGTTAAATTTTGT  
 CATGATACATGTGCATGTCCGAAACAAATATTTATGGGTACCCGAAAGGTTAAATTTTGT  
 CATGATACATGTGCATGTCCGAAACAAATATTTATGGGTACCCGAAAGGTTAAATTTTGT  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

AGTATTTATGAGGGGAGGGGGCGTTGACGAAAAAAATAACTTAGCTAAGCGTAATTGG  
 AGTATTTATGAGGGGAGGGGGCGTTGACGAAAAAAATAACTTAGCTAAGCGTAATTGG  
 AGTATTTATGAGGGGAGGGGGCGTTGACGAAAAAAATAACTTAGCTAAGCGTAATTGG  
 AGTATTTATGAGGGGAGGGGGCGTTGACGAAAAAAATAACTTAGCTAAGCGTAATTGG  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

CTAAAAACATACAATGTTGTTCCAGCATCAAGCCTACGTGATCATTTACAAAAACCAAC  
 CTAAAAACATACAATGTTGTTCCAGCATCAAGCCTACGTGATCATTTACAAAAACCAAC  
 CTAAAAACATACAATGTTGTTCCAGCATCAAGCCTACGTGATCATTTACAAAAACCAAC  
 CTAAAAACATACAATGTTGTTCCAGCATCAAGCCTACGTGATCATTTACAAAAACCAAC  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

TCAAAAGATAGGTGCATGTTCCTTTTAGTGCAAAACTTAAGGACACCTACCTTGCAAAA  
 TCAAAAGATAGGTGCATGTTCCTTTTAGTGCAAAACTTAAGGACACCTACCTTGCAAAA  
 TCAAAAGATAGGTGCATGTTCCTTTTAGTGCAAAACTTAAGGACACCTACCTTGCAAAA  
 TCAAAAGATAGGTGCATGTTCCTTTTAGTGCAAAACTTAAGGACACCTACCTTGCAAAA  
 \*\*\*\*\*

FIG. 10d



CTTAGCTTTGTTACCCAGAAATGAACCCGCTAAGCTCGAGGAGCTCTGAACTTACATGACCA  
 CTTAGCTTTGTTACCCAGAAATGAACCCGCTAAGCTCGAGGAGCTCTGAACTTACATGACCA  
 CTTAGCTTTGTTACCCAGAAATGAACCCGCTAAGCTCGAGGAGCTCTGAACTTACATGACCA  
 CTTAGCTTTGTTACCCAGAAATGAACCCGCTAAGCTCGAGGAGCTCTGAACTTACATGACCA  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

AAATATAATAACACAAAAGTCATGCATGATTTTCTTTAATAAGTATCGAGCAATAATGGTT  
 AAATATAATAACACAAAAGTCATGCATGATTTTCTTTAATAAGTATCGAGCAATAATGGTT  
 AAATATAATAACACAAAAGTCATGCATGATTTTCTTTAATAAGTATCGAGCAATAATGGTT  
 AAATATAATAACACAAAAGTCATGCATGATTTTCTTTAATAAGTATCGAGCAATAATGGTT  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

CGGGTGTCTTTCGTCTCATAACCTCTATTGTCTCCTCCGTGATCAACAAGGGTGGATCCGGGT  
 CGGGTGTCTTTCGTCTCATAACCTCTATTGTCTCCTCCGTGATCAACAAGGGTGGATCCGGGT  
 CGGGTGTCTTTCGTCTCATAACCTCTATTGTCTCCTCCGTGATCAACAAGGGTGGATCCGGGT  
 CGGGTGTCTTTCGTCTCATAACCTCTATTGTCTCCTCCGTGATCAACAAGGGTGGATCCGGGT  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

GGTGCAAGGGGGCTCAAGCCCCCTACCTCTCCCAAGGAGAAAGAGGAGAAAGAAAGT  
 GGTGCAAGGGGGCTCAAGCCCCCTACCTCTCCCAAGGAGAAAGAGGAGAAAGAAAGT  
 GGTGCAAGGGGGCTCAAGCCCCCTACCTCTCCCAAGGAGAAAGAGGAGAAAGAAAGT  
 GGTGCAAGGGGGCTCAAGCCCCCTACCTCTCCCAAGGAGAAAGAGGAGAAAGAAAGT  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

GAAGGAAGAAGAAACCCCTATAATTAATGCTACCTCCGCCACTGCTGATCAACACAAC  
 GAAGGAAGAAGAAACCCCTATAATTAATGCTACCTCCGCCACTGCTGATCAACACAAC  
 GAAGGAAGAAGAAACCCCTATAATTAATGCTACCTCCGCCACTGCTGATCAACACAAC  
 GAAGGAAGAAGAAACCCCTATAATTAATGCTACCTCCGCCACTGCTGATCAACACAAC  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

ATTCCTAAAACCATTTCCCTTGGCATTGGCGCATGTTACAAGGTACAAAAGAGCCAGCCCCA  
 ATTCCTAAAACCATTTCCCTTGGCATTGGCGCATGTTACAAGGTACAAAAGAGCCAGCCCCA  
 ATTCCTAAAACCATTTCCCTTGGCATTGGCGCATGTTACAAGGTACAAAAGAGCCAGCCCCA  
 ATTCCTAAAACCATTTCCCTTGGCATTGGCGCATGTTACAAGGTACAAAAGAGCCAGCCCCA  
 \*\*\*\*\*

P-SETit.Rcc3-1:1:1  
 P-SETit.Rcc3-1:1:10  
 P-SETit.Rcc3-1:1:11  
 P-SETit.Rcc3-1:1:16

FIG. 10e

TATGCCAAGTTACTAAACTAAACTATGATCCACCATGGAGCGAGAACAACGTC AACAGG  
TATGCCAAGTTACTAAACTAAACTATGATCCACCATGGAGCGAGAACAACGTC AACAGG  
TATGCCAAGTTACTAAACTAAACTATGATCCACCATGGAGCGAGAACAACGTC AACAGG  
TATGCCAAGTTACTAAACTAAACTATGATCCACCATGGAGCGAGAACAACGTC AACAGG  
\*\*\*\*\*

P-SETit.Rcc3-1:1:1  
P-SETit.Rcc3-1:1:10  
P-SETit.Rcc3-1:1:11  
P-SETit.Rcc3-1:1:16

CATCAACCAATGCAGCAATCTTGATCGCTAGTACTGTCCGGCATTATCTGAAACAAAAT  
CATCAACCAATGCAGCAATCTTGATCGCTAGTACTGTCCGGCATTATCTGAAACAAAAT  
CATCAACCAATGCAGCAATCTTGATCGCTAGTACTGTCCGGCATTATCTGAAACAAAAT  
CATCAACCAATGCAGCAATCTTGATCGCTAGTACTGTCCGGCATTATCTGAAACAAAAT  
\*\*\*\*\*

P-SETit.Rcc3-1:1:1  
P-SETit.Rcc3-1:1:10  
P-SETit.Rcc3-1:1:11  
P-SETit.Rcc3-1:1:16

CCAGATCACCCATCTCATCACAGTCACATGCATTCATGGTACGGGAACCGTTAGCAAAC  
CCAGATCACCCATCTCATCACAGTCACATGCATTCATGGTACGGGAACCGTTAGCAAAC  
CCAGATCACCCATCTCATCACAGTCACATGCATTCATGGTACGGGAACCGTTAGCAAAC  
CCAGATCACCCATCTCATCACAGTCACATGCATTCATGGTACGGGAACCGTTAGCAAAC  
\*\*\*\*\*

P-SETit.Rcc3-1:1:1  
P-SETit.Rcc3-1:1:10  
P-SETit.Rcc3-1:1:11  
P-SETit.Rcc3-1:1:16

CACCAACTAATCAGCATTTGCAACACTCTTCTCCTATAAAATGCAGCGAGCGGGGACACC  
CACCAACTAATCAGCATTTGCAACACTCTTCTCCTATAAAATGCAGCGAGCGGGGACACC  
CACCAACTAATCAGCATTTGCAACACTCTTCTCCTATAAAATGCAGCGAGCGGGGACACC  
CACCAACTAATCAGCATTTGCAACACTCTTCTCCTATAAAATGCAGCGAGCGGGGACACC  
\*\*\*\*\*

P-SETit.Rcc3-1:1:1  
P-SETit.Rcc3-1:1:10  
P-SETit.Rcc3-1:1:11  
P-SETit.Rcc3-1:1:16

ATAAACCATCACAGGCACTTAG  
ATAAACCATCACAGGCACTTAG  
ATAAACCATCACAGGCACTTAG  
ATAAACCATCACAGGCACTTAG  
\*\*\*\*\*

P-SETit.Rcc3-1:1:1  
P-SETit.Rcc3-1:1:10  
P-SETit.Rcc3-1:1:11  
P-SETit.Rcc3-1:1:16

FIG. 10f



P-SETit. Ssp1-1:1:1  
GTCATAGCGGTATTTTTCACCTCAACAAGTGTCTCCTTATCCCTTTCATCCATGAGTAATG  
P-SETit. Ssp1-1:1:2  
GTCATAGCGGTATTTTTCACCTCAACAAGTGTCTCCTTATCCCTTTCATCCATGAGTAATG  
\*\*\*\*\*  
P-SETit. Ssp1-1:1:1  
GCTCTTGGAAACATAATCCTACTTTAGATAGGTGACAAAATTCAGCTTAAAAAGATTTAGATC  
P-SETit. Ssp1-1:1:2  
GCTCTTGGAAACATAATCCTACTTTAGATAGGTGACAAAATTCAGCTTAAAAAGATTTAGATC  
\*\*\*\*\*  
P-SETit. Ssp1-1:1:1  
CACATCACCATATCACAACTTTACAGACTCTCTTTAAATAAAGTGGAAATGCCGTATGATGTG  
P-SETit. Ssp1-1:1:2  
CACATCACCATATCACAACTTTACAGACTCTCTTTAAATAAAGTGGAAATGCCGTATGATGTG  
\*\*\*\*\*  
P-SETit. Ssp1-1:1:1  
ACCGATGCCCTTCACATGTCCTTATGGTGAACTTGTGATGATATTTGTCACCCACGGTATCAA  
P-SETit. Ssp1-1:1:2  
ACCGATGCCCTTCACATGTCCTTATGGTGAACTTGTGATGATATTTGTCACCCACGGTATCAA  
\*\*\*\*\*  
P-SETit. Ssp1-1:1:1  
GAAAAACCAACAAGAAAGTCACCTTTTTCCTTTTAAATAAAGTGCATATTTTGTGGCCAA  
P-SETit. Ssp1-1:1:2  
GAAAAACCAACAAGAAAGTCACCTTTTTCCTTTTAAATAAAGTGCATATTTTGTGGCCAA  
\*\*\*\*\*  
P-SETit. Ssp1-1:1:1  
CACATACCGGTACAACCTAAGAAAATTAATCACTAAATGTTGAAAATTGACTAGATACCATG  
P-SETit. Ssp1-1:1:2  
CACATACCGGTACAACCTAAGAAAATTAATCACTAAATGTTGAAAATTGACTAGATACCATG  
\*\*\*\*\*  
P-SETit. Ssp1-1:1:1  
TCATGGCTTGGTTATCTAGATAAATAATTGCTTAGGAAAAGCAAAAATCATGCTAGAGCT  
P-SETit. Ssp1-1:1:2  
TCATGGCTTGGTTATCTAGATAAATAATTGCTTAGGAAAAGCAAAAATCATGCTAGAGCT  
\*\*\*\*\*  
P-SETit. Ssp1-1:1:1  
ATATGATCTCTCAAGAGTATAAATAGGTCCCGGATATGTAGCCAAATCT  
P-SETit. Ssp1-1:1:2  
ATATGATCTCTCAAGAGTATAAATAGGTCCCGGATATGTAGCCAAATCT  
\*\*\*\*\*

FIG. 11b

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4

-----  
GACTTTTCACGCTGAGAAACTGTCAGGAAGCTCAAACAACACAGCCTATACAGTGATTC  
-----  
TGTACCAATCAAACCACACTCACAACCTCACCAATGTACCTGTTTATTTTCCAAAATCAACCAT  
-----  
TGCTGTGGGGCTTGTGGGTTATGACTTATGAGTATGAGCAGTATTTTCCCCCTGGAAATG  
-----  
CAAGTGGCAGATAGCTTCTGAGATCTACCATGCACCCTGGTAAAGCTCCTACCATTTTAT  
-----  
TTGCAATAGTAGATGCATATAAATTGATGGACACAGAGTTCTGAACCGTGAGATTTTCTTT  
-----  
AACTTCAGAGTTCTGTCACTATGTTAACGTGGATCTGGAGTTTTCAGGAACCTGTGAAGA  
-----  
AGGAACCGTACCTAAAAGTCTCACGAAGACCAAATGGGCAAGCCAGACAGTCAG  
-----  
AGAGACAGGGCTTCTTCGGCCAACTGGACAAGGATGTGCCATCATGTGCTGAGAGATTA  
-----  
CCAAATCCGCAATTGCGAGACGTGCCCTTCTCAATCAGTTTGCACAAGCGATAAGGTAAA  
-----  
CAACCATTGGAGCATTTGTTAACAGCAGCCTAGCATGGCAGGATGCGTCACTGAATAATG  
-----  
CGGAGGGCAGGACAGTAGGACAGGACACCTAATCCCTGCAAAATCTAGTGGCTCTGGGAC

FIG. 12a

```

P-SETit.Tip-1:1:1
P-SETit.Tip-1:1:4
-----
ACTGATCATGGCCCCAAAAGTGCTAACCCACTGCATTACTAAAAACGTGTGGGTGTGGGAGT
-----
P-SETit.Tip-1:1:1
P-SETit.Tip-1:1:4
-----
TCCAAGTTGGCTGTTGTTCATGGAAATGGTATGAAAAATGCAAAAAGATTCTCGTTGTTTTGTT
-----
P-SETit.Tip-1:1:1
P-SETit.Tip-1:1:4
-----
GAGAGTTAGTAAATTGGGATGACTTCGGCATGACAACAGCACAAAACCTTACATAATTTGGT
-----
P-SETit.Tip-1:1:1
P-SETit.Tip-1:1:4
-----
AAGACTTGCAACAATAAATCAAAAAGATGCTAGTAGATACCAACAAAAAAGAAAAACAGAG
-----
P-SETit.Tip-1:1:1
P-SETit.Tip-1:1:4
-----
CTAGCATGAACACTTCTTTGATCACAGTTGATCAAAAGGTGATGGCCCTCAGACAAAAGCT
-----
P-SETit.Tip-1:1:1
P-SETit.Tip-1:1:4
-----
GTTGATGATGGTCAAACACTTTTGCTACCGAATATCGCAGTTGTCCTTTGGTACATTCAC
-----
P-SETit.Tip-1:1:1
P-SETit.Tip-1:1:4
-----
AAGTTTGATCTTATCATCACCATCAGAAAGTTCAGAAAGTCTCGATGAAAAACAATAAGAAA
-----
P-SETit.Tip-1:1:1
P-SETit.Tip-1:1:4
-----
TGAATACTGCTTACTTAGCTCAAATTCATAATCCGTTGTACAGGATACTTAAAAAAGGT
-----
P-SETit.Tip-1:1:1
P-SETit.Tip-1:1:4
-----
ACCAAAGGCTGTTCCCTAATCATACGCTGAAGTCGTTGCCACCAATGGCAGCTGTACTGTC
-----
TGTACTGTC
*****
ATATTGTCGTGGTTTTTCAATTGCTGTACCTGATGCAAAACGTAATGGGTTTACTAATCTT
P-SETit.Tip-1:1:1
ATAATGTCGTGGTTTTTCAATTGCTGTACCTGATGCAAAACGTAATGGGTTTACTAATCTT
P-SETit.Tip-1:1:4
*****

```

FIG. 12b

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
GCACCCGCGGCTTCAAAATGAAGAGTGTAAATTTGGTCCACGTCACCATCACCGGTTCC  
GCACCCGCGGACTTCAAAATGAAGAGTGTAAATTTGGTCCACGTCACCATCACCGGTTCC  
\*\*\*\*\*

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
AATGTCTAGAAATGGCAGGCAAAGATGATTGGACAGGCATGCAGGGGAAAAAGACCCGT  
AATGTCTAGAAATGGCAGGCAAAGATGATTGGACAGGCATGCAGGGGAAAAAGACCCGA  
\*\*\*\*\*

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
TGACGATGTAIGCGAGTTCACCACCATTCGGAGCAATGATTATCAGCCACACGACTTACTC  
TGACGATGATGCGAGTTCACCACCATTCGGAGCAATGATTATCAGCCACACGACTTACTC  
\*\*\*\*\*

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
TTCAGAGCTAACCCACTGCCATGCAGAGAAAAAGTGAATCATAATTGTCATGATCTACAACG  
TTCAGAGCTAACCCACTGCCATGCAGAGAAAAAGTGAATCATAATTGTCATGATCTACAACG  
\*\*\*\*\*

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
AAGTGAACAACATCAGGCATGCTAAAAGTGTGAAACTTTACTGATCTCTCATGTTGGACAA  
AAGTGAACAACATCAGGCATGCTAAAAGTGTGAAACTTTACTGATCTCTCATGTTGGACAA  
\*\*\*\*\*

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
CAAAGAATACGGGAATACATCAGCAACGCAACTCTTGAGCTTTGCTTGCCGAATGACCAG  
CAAAGAATACGGGAATACATCAGCAACGCAACTCTTGAGCTTTGCTTGCCGAATGACCAG  
\*\*\*\*\*

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
CTAGAAATTTCCAAGCATTTACAGAAACAATGACTTTAAGTTTCAGAAAAACAATAACAAGG  
CTAGAAATTTCCAAGCATTTACAGAAACAATGACTTTAAGTTTCAGAAAAACAATAACAAGG  
\*\*\*\*\*

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
CCACTAAAATAAGCGTGGGATAACAATATCCTCCAGATGACAGGCAATCTGCAACTTGCAG  
CCACTAAAATAAGCGTGGGATAACAATATCCTCCAGATGACAGGCAATCTGCAACTTGCAG  
\*\*\*\*\*

P-SETit.Tip-1:1:1  
P-SETit.Tip-1:1:4  
CCATTCAAATGTACGATTAACAAAAATTTAAGCGCCACATGAGATAATATATCCTCCAA  
CCATTCAAATGTACGATTAACAAAAATTTAAGCGCTACATGAGATAATATATCCTCCAA  
\*\*\*\*\*

FIG. 12c

P-SETit.Tip-1:1:1  
 P-SETit.Tip-1:1:4  
 TTAGGGCCTTTAGTATTGTCAATTAGTTCATAAACCAATGGTGCAATCCCTCACATGGACGCTGC  
 TTAGGGCCTTTAGTATTGTCAATTAGTTCATAAACCAATGGTGCAATCCCTCACATGGACGCTGC  
 \*\*\*\*\*

P-SETit.Tip-1:1:1  
 P-SETit.Tip-1:1:4  
 ATAAGAAGTTCAATAATAGCAACAGACATAATGAACAAAGCAATGGTGCCCTGCCCGGCCGG  
 ATAAGAAGTTCAATAATAGCAACAGACATAATGAACAAAGCAATGGTGCCCTGCCCGGCCGG  
 \*\*\*\*\*

P-SETit.Tip-1:1:1  
 P-SETit.Tip-1:1:4  
 ACTAGCTAGTACTACCAATCATGGAATAAGCTAGTACCCCTAAATGAAATTAATAATGGTTT  
 ACTAGCTAGTACTACCAATCATGGAATAAGCTAGTACCCCTAAATGAAATTAATAATGGTTT  
 \*\*\*\*\*

P-SETit.Tip-1:1:1  
 P-SETit.Tip-1:1:4  
 TTAGCGATTATCCACGCCGTCAGAACTACTCTAATCCACAAGTTGAGGCCGCCCATGAAG  
 TTAGCGATTATCCACGCCGTCAGAACTACTCTAATCCACAAGTTGAGGCCGCCCATGAAG  
 \*\*\*\*\*

P-SETit.Tip-1:1:1  
 P-SETit.Tip-1:1:4  
 CCGCGAGAGGGCGACGCCATGTGTATAAAAGGGCCCTAAGCTGAGTGGACTTGTGCATC  
 CCGCGAGAGGGCGACGCCATGTGTATAAAAGGGCCCTAAGCTGAGTGGACTTGTGCATC  
 \*\*\*\*\*

P-SETit.Tip-1:1:1  
 P-SETit.Tip-1:1:4  
 AGATTAGT  
 AGATTAGT  
 \*\*\*\*\*

FIG. 12d



P-SETit. TubA2-1-1:1:2	TAGCGTAGCAGGCTAGCAGCCTGAACCTCTCGGGTGAATTCGTGGCGAGTTAGCTTGTCTCG
P-SETit. TubA2-1-1:1:3	-----
P-SETit. TubA2-1-1:1:2	CTGCCGTCAATGGAGTGAATAITGGCAGTACGAAAGTCACACGGCAGGCAGAGATTGGGA
P-SETit. TubA2-1-1:1:3	-----
P-SETit. TubA2-1-1:1:2	ACATGGTGGAAACAAGAACCATCGGCATCGTCTTCTGATCAATATAGTGATTAAGTACTGGAA
P-SETit. TubA2-1-1:1:3	-----
P-SETit. TubA2-1-1:1:2	GAATCAAGAACAAGATAAAACCAAGGCATCCTGGATCTACCATGTCAATCTTTGCTTTCA
P-SETit. TubA2-1-1:1:3	-----
P-SETit. TubA2-1-1:1:2	TCCTCGTCGTTTGGAGCGAGCCTGTCTTGTITGAGGGCGGCTCGACCCTGCCACGGAAGT
P-SETit. TubA2-1-1:1:3	-----
P-SETit. TubA2-1-1:1:2	AACAGGTGCATGAGGTCTGCAGTCTGCAGTACTAGAACTTCCCAGCTCCCAAATTC AACGAAA
P-SETit. TubA2-1-1:1:3	-----
P-SETit. TubA2-1-1:1:2	CTGAAAAAAGTCATTGGTAACATCAAAGTCTTGCTTTAGGATAGCTGCTGTTCTTGACGC
P-SETit. TubA2-1-1:1:3	-----
P-SETit. TubA2-1-1:1:2	TGGTTAGATTTGTCCAATAAAATTACATGACCAGCGCACATGTACTTGGTATTCGACTTGA
P-SETit. TubA2-1-1:1:3	-----
P-SETit. TubA2-1-1:1:2	TGATTTGTATGTACTGACGGACTTGAATAAAGTAGGGGAAATTAGGACTCAGAAGTCTAC
P-SETit. TubA2-1-1:1:3	-----
P-SETit. TubA2-1-1:1:2	GATTTTGGTCCAATAATTGATTTGTGATGTTCAAAGTCGACGCTCTTTTCGAGACGAAAA
P-SETit. TubA2-1-1:1:3	-----

FIG. 13a

P-SETit. TubA2-1-1:1:2 TGTGATCGGTAGAACAAAAACGACGCAATTTCTTTTCAAAAAGAAAAAGATTAGGGGCAGA  
P-SETit. TubA2-1-1:1:3 -----

P-SETit. TubA2-1-1:1:2 ACAATTCAGGATCTGTTGGCTGGACTTATAAGCCGGCTGAAAAGCTGAAAACGGTTGATT  
P-SETit. TubA2-1-1:1:3 -----

P-SETit. TubA2-1-1:1:2 TATTGTGAGAGAAAAACAACACTGTTTGGTGGCTGATAAAGCTGAAAACGAAACAGGCTGTCAGTC  
P-SETit. TubA2-1-1:1:3 -----ACTGTTTGGTGGCTGATAAAGCTGAAAACGAAACAGGCTGTCAGTC  
\*\*\*\*\*

P-SETit. TubA2-1-1:1:2 ATTAGGCTGCCGGCTCTGGAAACAGTCCGCCATCAAGAGATCACCCGAACCATGTTGAGCT  
P-SETit. TubA2-1-1:1:3 ATTAGGCTGCCGGCTCTGGAAACAGTCCGCCATCAAGAGATCACCCGAACCATGTTGAGCT  
\*\*\*\*\*

P-SETit. TubA2-1-1:1:2 CATCAACGTGCCCGCTGTGTGCTCATTTGGTGTCCGGTCTGGTGGCGGCATCTTT  
P-SETit. TubA2-1-1:1:3 CATCAACGTGCCCGCTGTGTGCTCATTTGGTGTCCGGTCTGGTGGCGGCATCTTT  
\*\*\*\*\*

P-SETit. TubA2-1-1:1:2 CGTTGCGTCTTGTCAATGGATTATCCACGACGGCAGTGTCTGTTTATGATGACGGCA  
P-SETit. TubA2-1-1:1:3 CGTTGCGTCTTGTCAATGGATTATCCACGACGGCAGTGTCTGTTTATGATGACGGCA  
\*\*\*\*\*

P-SETit. TubA2-1-1:1:2 CTTGATTCGCCATGGATGGTGA AAAACGAAATCGAAGTGGGTCATTTTTATTATTACCAG  
P-SETit. TubA2-1-1:1:3 CTTGATTCGCCATGGATGGTGA AAAACGAAATCGAAGTGGGTCATTTTTATTATTACCAG  
\*\*\*\*\*

P-SETit. TubA2-1-1:1:2 ATGACGTGGTTGGTGACCTGAGAGGAACAGACATGAGAAGATGAGATCAGCAGCTCAATT  
P-SETit. TubA2-1-1:1:3 ATGACGTGGTTGGTGACCTGAGAGGAACAGACATGAGAAGATGAGATCAGCAGCTCAATT  
\*\*\*\*\*

FIG. 13b

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3  
 CCCACATTGACAGACGACCTTTGGAGCAGTACAAGAGACCCAAAATGAAGAATAGTGCAAAA  
 CCCACATTGACAGACGACCTTTGGAGCAGTACAAGAGACCCAAAATGAAGAATAGTGCAAAA  
 \*\*\*\*\*

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3  
 TTACAGAGTATATTAGAACACGAGAAATTAAGTTTACTCGAGGTCAATGAAAGAATT  
 TTACAGAGTATATTAGAACACGAGAAATTAAGTTTACTCGAGGTCAATGAAAGAATT  
 \*\*\*\*\*

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3  
 TAGGACTATTGAGAGCACAGGCTGTGTTCAAGTCCGTCATGAACCAGAGAAAAGTTCCCAC  
 TAGGACTATTGAGAGCACAGGCTGTGTTCAAGTCCGTCATGAACCAGAGAAAAGTTCCCAC  
 \*\*\*\*\*

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3  
 CAAGGCCCTCCATCAGAGGAACGAAATTTGCCCTGGAACCTCCATCCTCACCAGTCACCCTC  
 CAAGGCCCTCCATCAGAGGAACGAAATTTGCCCTGGAACCTCCATCCTCACCAGTCACCCTC  
 \*\*\*\*\*

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3  
 ACCACTTACCAGCCTAATAAAGCGTAGCAGACAATTTGGAGTAGACGATCATCATGTGCA  
 ACCACTTACCAGCCTAATAAAGCGTAGCAGACAATTTGGAGTAGACGATCATCATGTGCA  
 \*\*\*\*\*

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3  
 GTCTCATAGGCCCTCTTCTAGGAATAACCGTATTTGATAGCCAATCCACCTATGTGGTGA  
 GTCTCATAGGCCCTCTTCTAGGAATAACCGTATTTGATAGCCAATCCACCTATGTGGTGA  
 \*\*\*\*\*

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3

FIG. 13c

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3  
 TTTTTTTTCCCTTGTCCATCGGGGTCGGCTTTCCATCCGACGGTGTCTGCCAGTGTAGCGCA  
 TTTTTTTTCCCTTGTCCATCGGGGTCGGCTTTCCATCCGACGGTGTCTGCCAGTGTAGCGCA  
 \*\*\*\*\*

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3  
 ATCCATCTGTCGGCTTTCIGCTTTCIGATGAACGTTCCGGTTGACGCCGCTAAGCTGCCTT<sup>55/59</sup>  
 ATCCATCTGTCGGCTTTCIGCTTTCIGATGAACGTTCCGGTTGACGCCGCTAAGCTGCCTT<sup>59</sup>  
 \*\*\*\*\*

P-SETit. TubA2-1-1:1:2  
 P-SETit. TubA2-1-1:1:3  
 GTCCTCCTTTTCTCCCGTCTGCACAGGGAGGGG  
 GTCCTCCTTTTCTCCCGTCTGCACAGGGAGGGG  
 \*\*\*\*\*

FIG. 13d

P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
ACTGCCGACACGCCCTCACTGGCGGGAGGGCTCCGAGCGCTCTCTCCCGGGCGCGGGC  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
GGAGCAGCGATCTGGATTGGAGAGAAATAGAGGAAAAGAGAGGAAAAGGAGAGAGATAGCG  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
CAAAGAGCTGAAAAGATAAGGTTGTGGGGCTGTGGTGAATTAGAGACCCTAATCCCTC  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
CATCTCCTAATGACGCGGTGCCCAAGACCAGTGCCGCGGCACACCAGCGTCTAAGTGAAC  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
TTCCGCTAACCTTCCGGTCATTGCCCTGAAAAGATGTCAATGTGGGAGGCCCCCTCTCA  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
GTAGATTGCCAACTGCCCTACCGTGCCACTCTTCCATGCATGATTGCTCCCCTATCCCG  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
TTTTCTCACAACAGATAGACAACAGTAAGCATCACTAAAAGCAAGCATGTGTAGAACCCTTAA  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
AAAAAGGCTTATACTACCAGTATACTATCAACCAGCATGCCGTTTTTGAAGTATCCAGGA  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
TTAGAAGCTTCTACTGGCCTTTTATATATATAGCTGTGGACCCTGTGGTAAACCTTTCTCTTT  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
TGGCGCTTGCTTAATCTCGGCCGTGCTGGTCCATGCTTAGGCCTAGGCCAGAGATAGAGC  
-----  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
CGGGGTGAATGGGGCTAAAGCTCAGCTGCTCGAGGGCCGTGGGCTGGTTCCACTAGC  
-----

FIG. 14a



P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3  
P-SETit.Ubq1-1:1:1  
P-SETit.Ubq1-1:1:3

GTGGAGCCGGCAGTATGCGCCCCAGCACGGCCGAGGTGGTGGTGGCCCGTGGCCCTGCTG  
GTGGAGCCGGCAGTATGCGCCCCAGCACGGCCGAGGTGGTGGTGGCCCGTGGCCCTGCTG  
\*\*\*\*\*  
TCTGCGCGGCTCGGGACAAC TTGAAACTGGGCCACCGCCTCGTCGAACTCGCAACCCCGT  
TCTGCGCGGCTCGGGACAAC TTGAAACTGGGCCACCGCCTCGTCGAACTCGCAACCCCGT  
\*\*\*\*\*  
TGGCGGAAGAAAGGAATGGCTCGTAGGGCCCGGTAGAAATCGAAGAAATGTTGCGCTGGG  
TGGCGGAAGAAAGGAATGGCTCGTAGGGCCCGGTAGAAATCGAAGAAATGTTGCGCTGGG  
\*\*\*\*\*  
CTTCGATTACATAAACA TGGCCCTGAAGCTCTAAAACGACGGCCCGGTGCGCCGCGGATG  
CTTCGATTACATAAACA TGGCCCTGAAGCTCTAAAACGACGGCCCGGTGCGCCGCGGATG  
\*\*\*\*\*  
GAAAGAGACCGGATCCTCCTCGTGAAT TCTGGAAGGCCACACGAGAGCGGACCCACCCCGG  
GAAAGAGACCGGATCCTCCTCGTGAAT TCTGGAAGGCCACACGAGAGCGGACCCACCCCGG  
\*\*\*\*\*  
ACGCGGAGGAGTCGTGCGTGTCC AACACGGCCGGCGGGCTGGGCTGCGACCTTAACCAG  
ACGCGGAGGAGTCGTGCGTGTCC AACACGGCCGGCGGGCTGGGCTGCGACCTTAACCAG  
\*\*\*\*\*  
CAAGGCACGCCACGACCCCGCCCTCGAGGCATAAATACCC TCCCATCC  
CAAGGCACGCCACGACCCCGCCCTCGAGGCATAAATACCC TCCCATCC  
\*\*\*\*\*

FIG. 14c

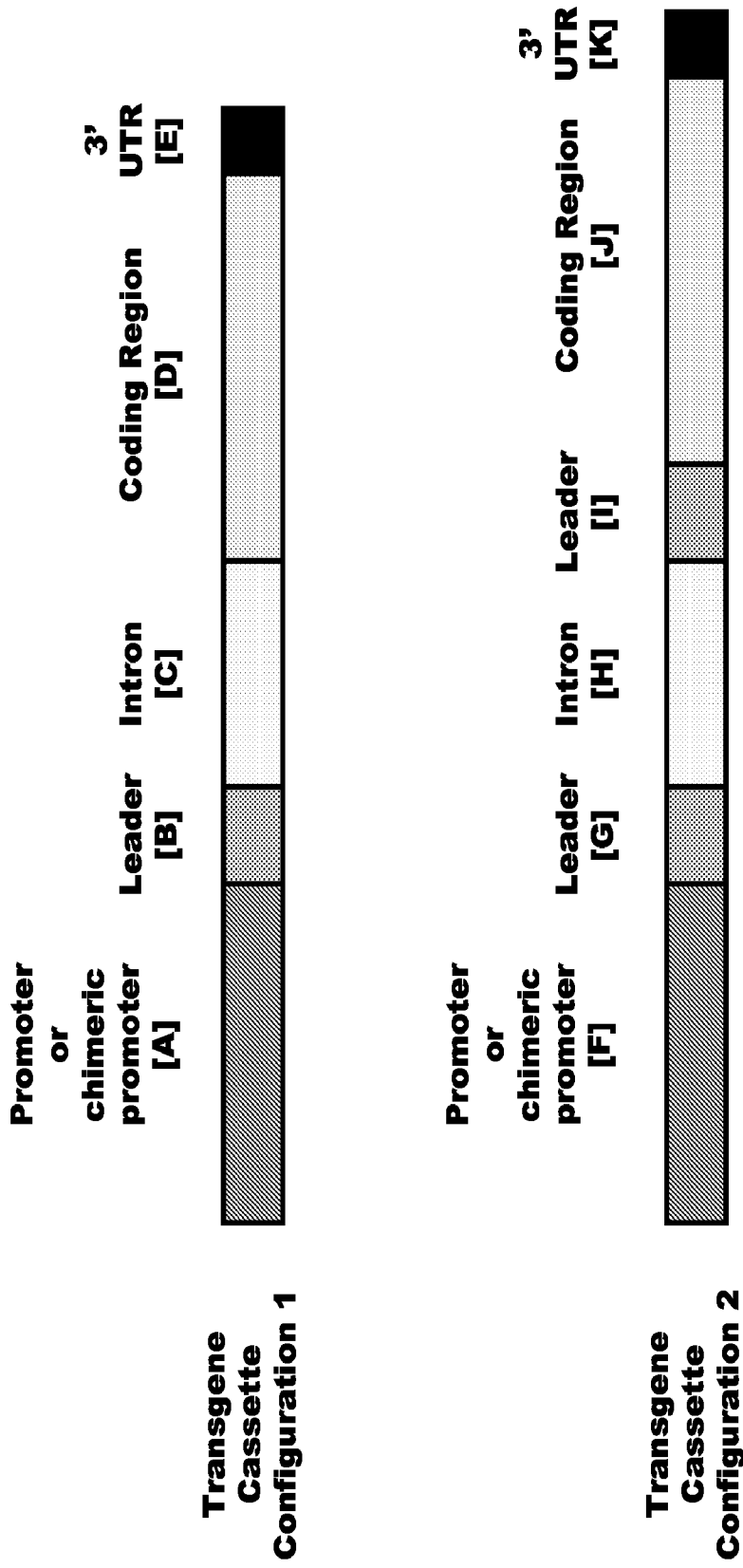


FIG. 15



INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2011/021269

A. CLASSIFICATION OF SUBJECT MATTER  
INV. C12N15/82 C12N9/10  
ADD.  
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED  
Minimum documentation searched (classification system followed by classification symbols)  
C12N  
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)  
EPO-Internal, BIOSIS, MEDLINE, WPI Data, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/073844 A1 (MONSANTO TECHNOLOGY LLC [US]; FLASINSKI STANISLAW [US]; GUO SHIRLEY XI) 11 June 2009 (2009-06-11) the whole document	1-13
X	WO 2006/084868 A2 (BASF PLANT SCIENCE GMBH [DE]; SONG HEE-SOOK [US]; MORRA MARC [US]; DAM) 17 August 2006 (2006-08-17) examples 1-7	1-13
X	WO 99/09188 A2 (VLAAMS INTERUNIV INST BIOTECH [BE]; CHEN CUIYING [BE]; MEYERMANS HUGO) 25 February 1999 (1999-02-25) the whole document	1-13
	----- -/--	

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p>
--	--

Date of the actual completion of the international search  21 March 2011	Date of mailing of the international search report  21/06/2011
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Bilang, Jürg

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2011/021269

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see additional sheet

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2011/021269

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/06752 A1 (VLAAMS INTERUNIV INST BIOTECH [BE]; BOERJAN WOUT [BE]; CHEN CUIYING [B] 10 February 2000 (2000-02-10) page 5, paragraph 5 - page 9, paragraph 1 -----	1-13
Y	LI LAIGENG ET AL: "Secondary xylem-specific expression of caffeoyl-coenzyme A 3-O-methyltransferase plays an important role in the methylation pathway associated with lignin biosynthesis in loblolly pine", PLANT MOLECULAR BIOLOGY, vol. 40, no. 4, July 1999 (1999-07), pages 555-565, XP009146160, ISSN: 0167-4412 the whole document -----	1-13
Y	VEERANAGAMALLAIAH G ET AL: "Proteomic analysis of salt stress responses in foxtail millet ( <i>Setaria italica</i> L. cv. Prasad) seedlings", PLANT SCIENCE, ELSEVIER IRELAND LTD, IE, vol. 175, no. 5, 1 November 2008 (2008-11-01), pages 631-641, XP025428401, ISSN: 0168-9452, DOI: DOI:10.1016/J.PLANTSCI.2008.06.017 [retrieved on 2008-07-10] p. 634, pennultimate line; Table 2 (p. 635), 11th protein -----	1-13

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/US2011/021269
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009073844	A1	11-06-2009	AR 069573 A1 03-02-2010
			CA 2707586 A1 11-06-2009
			US 2009151020 A1 11-06-2009
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WO 2006084868	A2	17-08-2006	AU 2006212223 A1 17-08-2006
			CA 2596600 A1 17-08-2006
			CN 101155922 A 02-04-2008
			EP 1851318 A2 07-11-2007
			EP 2147977 A1 27-01-2010
			EP 2184361 A2 12-05-2010
			EP 2186903 A2 19-05-2010
			EP 2163633 A1 17-03-2010
			US 2008163395 A1 03-07-2008
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WO 9909188	A2	25-02-1999	AU 9072298 A 08-03-1999
-----			
WO 0006752	A1	10-02-2000	AU 5508599 A 21-02-2000
			CA 2336706 A1 10-02-2000
-----			

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13(partially)

Gene regulatory elements derived from *S. italica* Cafeoyl CoA methyltransferase (SEQ ID NOs 1, 35, 116, 373-376, 544-546)

---

2-125. claims: 1-13(partially)

As invention 1, but elements derived from one of glutathione-S-transferase (invention 2), isoflavone reductase (invention 3), peroxidase (invention 4) .... (according to table 1)

---

126. claims: 14-21(partially)

Chloroplast transit peptide derived from *S. italica* casein lytic proteinase B3 heat shock protein-like protein (SEQ ID NO: 324)

---

127-148. claims: 14-21(partially)

As invention 126, but transit peptide derived from one of ascorbate peroxidase (invention 127), anthranilate synthase alpha 2 subunit (invention 128) .... (according to table 1)

---