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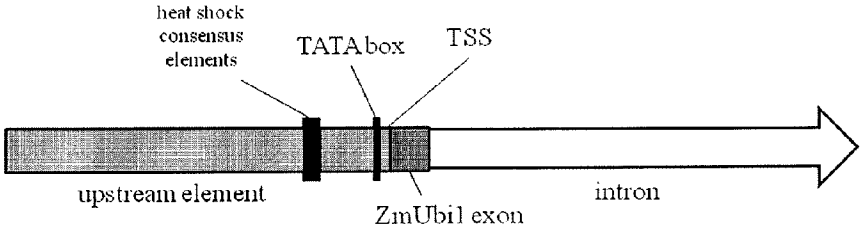
(54) Title: METHOD AND CONSTRUCT FOR SYNTHETIC BIDIRECTIONAL SCBV PLANT PROMOTER



maize Ubi1 promoter

(57) Abrégé/Abstract:
Provided are constructs and methods for expressing multiple genes in plant cells and/or plant tissues. The constructs provided comprise at least one bi directional promoter linked to multiple gene expression cassettes, wherein the bi directional promoter comprises a functional promoter nucleotide sequence from Sugar Cane Bacilliform Virus promoter. In some embodiments, the constructs and methods provided employs a bi directional promoter based on a minimal core promoter element from a Zea mays Ubiquitin 1 gene, or a functional equivalent thereof, and nucleotide sequence elements from a Sugar Cane Bacilliform Virus promoter. In some embodiments, the constructs and methods provided allow expression of genes between three and twenty.

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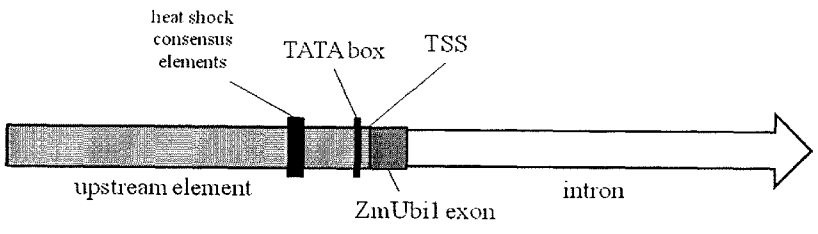
(54) Title: METHOD AND CONSTRUCT FOR SYNTHETIC BIDIRECTIONAL SCBV PLANT PROMOTER



maize Ubi1 promoter

(57) Abstract: Provided are constructs and methods for expressing multiple genes in plant cells and/or plant tissues. The constructs provided comprise at least one bi directional promoter linked to multiple gene expression cassettes, wherein the bi directional promoter comprises a functional promoter nucleotide sequence from Sugar Cane Bacilliform Virus promoter. In some embodiments, the constructs and methods provided employs a bi directional promoter based on a minimal core promoter element from a Zea mays Ubiquitin 1 gene, or a functional equivalent thereof, and nucleotide sequence elements from a Sugar Cane Bacilliform Virus promoter. In some embodiments, the constructs and methods provided allow expression of genes between three and twenty.

METHOD AND CONSTRUCT FOR SYNTHETIC BIDIRECTIONAL SCBV PLANT PROMOTER

PRIORITY CLAIM

This application claims the benefit of the filing date of U.S. Provisional Patent Application Serial No. 61/582,148 filed December 30, 2011. This application also claims benefit of the filing date of U.S. Provisional Patent Application Serial No. 61/641,956 filed May 3, 2012.

TECHNICAL FIELD

This invention is generally related to the field of plant molecular biology, and more specifically the field of stable expression of multiple genes in transgenic plants.

BACKGROUND

Many plant species are capable of being transformed with transgenes from other species to introduce agronomically desirable traits or characteristics, for example, improving nutritional value quality, increasing yield, conferring pest or disease resistance, increasing drought and stress tolerance, improving horticultural qualities (such as pigmentation and growth), imparting herbicide resistance, enabling the production of industrially useful compounds and/or materials from the plant, and/or enabling the production of pharmaceuticals. The introduction of transgenes into plant cells and the subsequent recovery of fertile transgenic plants that contain a stably integrated copy of the transgene can be used to produce transgenic plants that possess the desirable traits.

Control and regulation of gene expression can occur through numerous mechanisms. Transcription initiation of a gene is a predominant controlling mechanism of gene expression. Initiation of transcription is generally controlled by polynucleotide sequences located in the 5'- flanking or upstream region of the transcribed gene. These sequences are collectively referred to as promoters. Promoters generally contain signals for RNA polymerase to begin transcription so that messenger RNA (mRNA) can be produced. Mature mRNA is translated by ribosome, thereby synthesizing proteins. DNA-binding proteins interact specifically with promoter DNA

sequences to promote the formation of a transcriptional complex and initiate the gene expression process. There are a variety of eukaryotic promoters isolated and characterized from plants that are functional for driving the expression of a transgene in plants. Promoters that affect gene expression in response to environmental stimuli, nutrient availability, or adverse conditions including heat shock, anaerobiosis, or the presence of heavy metals have been isolated and characterized. There are also promoters that control gene expression during development or in a tissue, or organ specific fashion. In addition, prokaryotic promoters isolated from bacteria and virus have been isolated and characterized that are functional for driving the expression of a transgene in plants.

A typical eukaryotic promoter consists of a minimal promoter and other *cis*-elements. The minimal promoter is essentially a TATA box region where RNA polymerase II (polII), TATA-binding protein (TBP), and TBP-associated factors (TAFs) may bind to initiate transcription. However in most instances, sequence elements other than the TATA motif are required for accurate transcription. Such sequence elements (*e.g.*, enhancers) have been found to elevate the overall level of expression of the nearby genes, often in a position- and/or orientation-independent manner. Other sequences near the transcription start site (*e.g.*, INR sequences) of some polII genes may provide an alternate binding site for factors that also contribute to transcriptional activation, even alternatively providing the core promoter binding sites for transcription in promoters that lack functional TATA elements. See *e.g.*, Zenzie-Gregory et al. (1992) *J. Biol. Chem.* 267: 2823-30.

Other gene regulatory elements include sequences that interact with specific DNA-binding factors. These sequence motifs are sometimes referred to as *cis*-elements, and are usually position- and orientation-dependent, though they may be found 5′ or 3′ to a gene's coding sequence, or in an intron. Such *cis*-elements, to which tissue-specific or development-specific transcription factors bind, individually or in combination, may determine the spatiotemporal expression pattern of a promoter at the transcriptional level. The arrangement of upstream *cis*-elements, followed by a minimal promoter, typically establishes the polarity of a particular promoter. Promoters in plants that have been cloned and widely used for both basic research and biotechnological application are generally unidirectional, directing only one gene that

has been fused at its 3′ end (*i.e.*, downstream). See, for example, Xie et al. (2001) *Nat. Biotechnol.* 19(7):677-9; U.S. Patent No. 6,388,170.

Many *cis*-elements (or "upstream regulatory sequences") have been identified in plant promoters. These *cis*-elements vary widely in the type of control they exert on operably linked genes. Some elements act to increase the transcription of operably linked genes in response to environmental responses (*e.g.*, temperature, moisture, and wounding). Other *cis*-elements may respond to developmental cues (*e.g.*, germination, seed maturation, and flowering) or to spatial information (*e.g.*, tissue specificity). See, for example, Langridge et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:3219-23. The type of control of specific promoter elements is typically an intrinsic quality of the promoter; *i.e.*, a heterologous gene under the control of such a promoter is likely to be expressed according to the control of the native gene from which the promoter element was isolated. These elements also typically may be exchanged with other elements and maintain their characteristic intrinsic control over gene expression.

It is often necessary to introduce multiple genes into plants for metabolic engineering and trait stacking, which genes are frequently controlled by identical or homologous promoters. However, homology-based gene silencing (HBGS) is likely to arise when multiple introduced transgenes have homologous promoters driving them. *See, e.g.*, Mol et al. (1989) *Plant Mol. Biol.* 13:287-94. HBGS has been reported to occur extensively in transgenic plants. *See, e.g.*, Vaucheret and Fagard (2001) *Trends Genet.* 17:29-35. Several mechanisms have been suggested to explain the phenomena of HBGS, all of which include the feature that sequence homology in the promoter triggers cellular recognition mechanisms that result in silencing of the repeated genes. *See, e.g.*, Matzke and Matzke (1995) *Plant Physiol.* 107:679-85; Meyer and Saedler (1996) *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 47:23-48; Fire (1999) *Trends Genet.* 15:358-63; Hamilton and Baulcombe (1999) *Science* 286:950-2; and Steimer et al. (2000) *Plant Cell* 12:1165-78.

Strategies to avoid HBGS in transgenic plants frequently involve the development of synthetic promoters that are functionally equivalent but have minimal sequence homology. When such synthetic promoters are used for expressing transgenes in crop plants, they may aid in avoiding or reducing HBGS. *See, e.g.*, Mourrain et al. (2007) *Planta* 225(2):365-79; Bhullar et al. (2003) *Plant Physiol.*

132:988-98. Such promoters can be generated by introducing known *cis*-elements in a novel or synthetic stretch of DNA, or alternatively by "domain swapping," wherein domains of one promoter are replaced with functionally equivalent domains from other heterologous promoters.

Thus, there remains a need for constructs and methods for stable expression of multiple transgenes effectively with minimum risk for recombination or loss of transgenes through breeding or multiple generations in transgenic plants.

DISCLOSURE

Described herein are particular synthetic promoters comprising a Ubi1 minimal promoter. In embodiments, a synthetic promoter comprising a Ubi1 minimal promoter further comprises at least one sequence element of a SCBV promoter or functional equivalent thereof. In some examples, such a synthetic promoter (a "synthetic SCBV promoter") can be a promoter that is able to control transcription of an operably linked nucleotide sequence in a plant cell. In other examples, a synthetic SCBV promoter may be a synthetic bidirectional SCBV promoter, for example, a nucleic acid comprising a minimal Ubi1 promoter element nucleotide sequences oriented in the opposite direction with respect to the SCBV promoter elements that is able to control transcription in a plant cell of two operably linked nucleotide sequences that flank the promoter. Additional elements that may be engineered to be included in a synthetic SCBV bidirectional promoter include introns (*e.g.*, an alcohol dehydrogenase (ADH) intron), exons, and/or all or part of an upstream promoter region. In certain examples, a synthetic bidirectional promoter may comprise more than one of any of the foregoing.

A particular embodiment relates to a synthetic polynucleotide comprising (a) a minimal core promoter element from an Ubiquitin-1 gene of *Zea mays* wherein the minimal core promoter element comprises SEQ ID NO: 1 or its complement; (b) a functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter; and (c) an intron from an alcohol dehydrogenase gene; wherein the functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter and the intron from the alcohol dehydrogenase gene comprise SEQ ID NO: 6 or its complement.

Particular embodiments of the invention include cells (*e.g.*, plant cells) comprising a synthetic SCBV promoter or functional equivalent thereof. For example, specific embodiments may include a cell comprising a synthetic bidirectional SCBV promoter or functional equivalent thereof. Plant cells according to particular embodiments may be present in a cell culture, a tissue, a plant part, and/or a whole plant. Thus, a plant (*e.g.*, a monocot or dicot) comprising a cell having a synthetic SCBV promoter or functional equivalent thereof are included in some embodiments.

Other embodiments of the invention include a means for initiating transcription of two operably linked nucleotide sequences of interest. Means for initiating transcription of two operably linked nucleotide sequences of interest include the synthetic bidirectional SCBV promoter of SEQ ID NO: 5.

Also provided are constructs and methods for expressing multiple genes in plant cells and/or plant tissues. The constructs provided comprise at least one bi-directional promoter linked to multiple gene expression cassettes, wherein the bi-directional promoter comprises a functional promoter nucleotide sequence from Sugar Cane Bacilliform Virus (SCBV) promoter. In some embodiments, the constructs and methods provided employs a bi-directional promoter based on a minimal core promoter element from a *Zea mays* Ubiquitin-1 gene, or a functional equivalent thereof, and nucleotide sequence elements from a Sugar Cane Bacilliform Virus promoter. In some embodiments, the constructs and methods provided allow expression of genes between three and twenty.

In one aspect, provided is a synthetic polynucleotide comprising a minimal core promoter element from an Ubiquitin-1 gene of *Zea mays* or *Zea luxurians* and a functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter. In one embodiment, the minimal core promoter element comprises a polynucleotide sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 1 or its complement. In a further or alternative embodiment, the minimal core promoter element comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 16-40. In a further embodiment, the minimal core promoter element comprises SEQ ID NO: 1 or its complement. In a further embodiment, the minimal core promoter element consists essentially of SEQ ID NO: 1 or its complement. In another embodiment, the synthetic polynucleotide provided further comprises an exon from an Ubiquitin-1 gene and an intron from an Ubiquitin-1 gene. In a further embodiment, the exon is from an Ubiquitin-1 gene of *Zea mays* or *Zea luxurians*. In another embodiment, the synthetic polynucleotide provided further comprises an intron from an alcohol dehydrogenase gene. In another embodiment, the synthetic polynucleotide provided further comprises an upstream regulatory sequence from the Sugar Cane Bacilliform Virus promoter. In another embodiment, the functional promoter nucleotide sequence from a Sugar Cane

Bacilliform Virus promoter and the alcohol dehydrogenase gene a polynucleotide sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 6 or its complement. In a further or alternative embodiment, the functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter and the intron from the alcohol dehydrogenase gene comprise SEQ ID NO: 6 or its complement. In a further embodiment, the functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter and the intron from the alcohol dehydrogenase gene consist essentially of SEQ ID NO: 6 or its complement.

In one embodiment, the synthetic polynucleotide provided further comprises at least one element selected from a list comprising an upstream regulatory sequence (URS), an enhancer element, an exon, an intron, a transcription start site, a TATA box, a heat shock consensus element, and a translation START and/or STOP nucleotide sequence. In another embodiment, the synthetic polynucleotide provided further comprises an element selected from the group consisting of an upstream regulatory sequence (URS), an enhancer element, an exon, an intron, a transcription start site, a TATA box, a heat shock consensus element, a translation START and/or STOP nucleotide sequence, and combinations thereof. In another embodiment, the synthetic polynucleotide provided further comprises a nucleotide sequence of interest operably linked to the minimal core promoter element. In another embodiment, the minimal core promoter element from a *Zea mays* Ubiquitin-1 gene and the functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter are in reverse complementary orientation with respect to each other in the polynucleotide.

In another embodiment, the synthetic polynucleotide provided comprises an exon from an Ubiquitin-1 gene, an intron from an Ubiquitin-1 gene, and an intron from an alcohol dehydrogenase gene. In a further or alternative embodiment, the synthetic polynucleotide provided comprises a second coding nucleotide sequence of interest operably linked to the functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter. In a further embodiment, the synthetic polynucleotide provided comprises a polynucleotide sequence that is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 5 or its complement. In a further embodiment, the synthetic polynucleotide provided comprises SEQ ID NO: 5 or its complement. In a further embodiment, the synthetic polynucleotide provided consists

essentially of SEQ ID NO: 5 or its complement. In a further embodiment, the exon or intron is from an Ubiquitin-1 gene of *Zea mays* or *Zea luxurians*.

In a further embodiment, the synthetic polynucleotide provided comprises a first coding nucleotide sequence of interest operably linked to the minimal core promoter element from a *Zea mays* Ubiquitin-1 gene. In another further embodiment, the synthetic polynucleotide provided comprises a second coding nucleotide sequence of interest operably linked to the functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter.

In another aspect, provided is a method for producing a transgenic cell, the method comprising transforming the cell with the polynucleotide provided herein. In one embodiment, the cell is a plant cell. In another aspect, provided is a plant cell comprising the polynucleotide provided herein. In another aspect, provided is a plant comprising the plant cell provided herein.

In another aspect, provided is a method for expressing a nucleotide sequence of interest in a plant cell, the method comprising introducing into the plant cell the nucleotide sequence of interest operably linked to a means for initiating transcription of two operably linked nucleotide sequences of interest. In one embodiment, the method provided comprises introducing into the plant cell a nucleic acid comprising (a) the nucleotide sequence of interest operably linked to the means for initiating transcription of two operably linked nucleotide sequences of interest; and (b) a second nucleotide sequence of interest operably linked to the means for initiating transcription of two operably linked nucleotide sequences of interest.

In one embodiment, the means for initiating transcription of two operably linked nucleotide sequences of interest comprises SEQ ID NO: 5 or its complement. In another embodiment, the means for initiating transcription of two operably linked nucleotide sequences of interest comprises SEQ ID NO: 5. In another embodiment, the means for initiating transcription of two operably linked nucleotide sequences of interest comprises the reverse complement of SEQ ID NO: 5. In another embodiment, the nucleic acid is introduced into the plant cell so as to target to a predetermined site in the DNA of the plant cell the nucleotide sequence of interest operably linked to the means for initiating transcription of two operably linked nucleotide sequences of interest. In a further or alternative embodiment, the nucleotide sequence of interest

operably linked to the means for initiating transcription of two operably linked nucleotide sequences of interest is targeted to the predetermined site utilizing Zinc finger nuclease-mediated recombination.

In some embodiments, the exon is from an Ubiquitin-1 gene of a *Zea spp.* In some embodiments, the intron is from an Ubiquitin-1 gene of a *Zea spp.* In some embodiments, the *Zea spp.* is *Zea mays* or *Zea luxurians*.

In another aspect, provided is a nucleic acid construct for expressing multiple genes in plant cells and/or tissues. The nucleic acid construct comprises (a) a bi-directional promoter, wherein the bi-directional promoter comprises a functional promoter nucleotide sequence from Sugar Cane Bacilliform Virus (SCBV) promoter; and (b) two gene expression cassettes on opposite ends of the bi-directional promoter; wherein at least one of the gene expression cassettes comprises two or more genes linked via a translation switch.

In one embodiment, the bi-directional promoter comprises at least one enhancer. In another embodiment, the bi-directional promoter does not comprise an enhancer. In another embodiment, the nucleic acid construct comprises a binary vector for *Agrobacterium*-mediated transformation. In one embodiment, the bi-directional promoter comprises an element selected from the group consisting of an upstream regulatory sequence (URS), an enhancer element, an exon, an intron, a transcription start site, a TATA box, a heat shock consensus element, and combinations thereof. In another embodiment, the bi-directional promoter comprises a minimal core promoter element from an Ubiquitin-1 gene of *Zea mays* or *Zea luxurians*. In another embodiment, the core promoter element from an Ubiquitin-1 gene and the promoter nucleotide sequence from Sugar Cane Bacilliform Virus (SCBV) promoter are in reverse complimentary orientation with respect to each other. In a further or alternative embodiment, the minimal core promoter element comprises a polynucleotide sequence at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 1 or its complement. In a further or alternative embodiment, the minimal core promoter element comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 16-40. In a further embodiment, the minimal core promoter element comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and

16-35. In a further embodiment, the minimal core promoter element comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 16-30. In a further embodiment, the minimal core promoter element comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 16-25. In a further embodiment, the minimal core promoter element comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 16-20. In a further embodiment, the minimal core promoter element comprises a polynucleotide sequence of SEQ ID NO: 1.

In a further or alternative embodiment, the bi-directional promoter comprises an exon from an Ubiquitin-1 gene and/or an intron from an Ubiquitin gene. In another embodiment, the bi-directional promoter comprises an intron from an alcohol dehydrogenase gene. In one embodiment, the nucleic acid construct is stably transformed into transgenic plants. In one embodiment, the plants are monocotyledons plants. In another embodiment, the plants are dicotyledons plants. In another embodiment, the plants are not monocotyledons plants. In another embodiment, the plants are not dicotyledons plants.

In a further or alternative embodiment, the bi-directional promoter comprises an upstream regulatory sequence from an Ubiquitin gene or the Sugar Cane Bacilliform Virus (SCBV) promoter. In a further embodiment, the bi-directional promoter comprises an upstream regulatory sequence from an Ubiquitin gene. In another embodiment, the bi-directional promoter comprises an upstream regulatory sequence from an Ubiquitin gene or the Sugar Cane Bacilliform Virus (SCBV) promoter.

In a further embodiment, the bi-directional promoter comprises a polynucleotide of at least 75%, 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 5 or its complement. In a further embodiment, the bi-directional promoter comprises a polynucleotide of SEQ ID NO: 5 or its complement. In a further embodiment, the bi-directional promoter comprises a polynucleotide of at least 75%, 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 6 or its complement. In a further embodiment, the bi-directional promoter comprises a polynucleotide of SEQ ID NO: 6 or its complement.

In one embodiment, both the gene expression cassettes comprise two or more genes linked via a translation switch. In a further or alternative embodiment, the translation switch is selected from the group consisting of an internal ribosome entry site (IRES), an alternative splicing site, a ribozyme cleavage site, a polynucleotide sequence coding a 2A peptide, a polynucleotide sequence coding a 2A-like peptide, a polynucleotide sequence coding an intein, a polynucleotide sequence coding a protease cleavage site, and combinations thereof. In a further or alternative embodiment, the translation switch comprises a *cis*-acting hydrolase element (CHYSEL). In a further embodiment, the CHYSEL is a 2A or 2A-like peptide sequence. In another embodiment, a gene upstream of the translational switch does not comprise a translation stop codon. In another embodiment, the nucleic acid construct enables or allows expression of at least four genes. In a further embodiment, all four genes are transgenes. In another embodiment, the nucleic acid construct enables expression of genes between three and twenty. In another embodiment, the nucleic acid construct enables expression of genes between four and eight. In a further or alternative embodiment, the genes are transgenes. In another embodiment, at least one gene expression cassette comprises a polynucleotide sequence encoding a fusion protein. In a further embodiment, the fusion protein comprises three to five genes.

In some embodiments, expression of genes from the bi-directional promoter is at least four-fold higher as compared to a uni-directional promoter. In some embodiments, expression of genes from the bi-directional promoter is from three to ten folds higher as compared to a uni-directional promoter. In some embodiments, expression of genes from the bi-directional promoter is from four to eight folds higher as compared to a uni-directional promoter. In some embodiments, a selection marker gene is placed at far end from the promoter (*i.e.*, at the 3′ end of a gene expression cassette downstream of another gene).

In another aspect, provided is a method for generating a transgenic plant comprising transforming a plant cell with the nucleic acid construct provided herein. In another aspect, provided is a method for generating a transgenic cell comprising transforming the cell with the nucleic acid construct provided herein. In another aspect, provided is a plant cell comprising the nucleic acid construct provided

herein. In a further or alternative embodiment, the nucleic acid construct is stably transformed into the plant cell. In another aspect, provided is a transgenic plant comprising the nucleic acid construct provided herein. In a further or alternative embodiment, the nucleic acid construct is stably transformed into cells of the transgenic plant. In another aspect, provide is a method for expressing multiple genes in plant cells and/or tissues, comprising introducing into the plant cells and/or tissues the nucleic acid construct provided herein. In a further or alternative embodiment, the plant cells and/or tissues are stably transformed with the nucleic acid construct provided herein. In another aspect, provided is a binary vector for *Agrobacterium*-mediated transformation. In one embodiment, the binary vector comprises the nucleic acid construct provided herein. In another embodiment, the binary vector comprises the synthetic polynucleotide provided herein. In another aspect, provided is the use of the bi-directional promoter provided herein for multiple-transgenes expression in plants.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCES

FIG. 1 shows an exemplary (not to scale) maize Ubi1 (ZmUbi1) promoter, which comprises an approximately 900 bp Upstream Element located 5′ of the transcription start site (TSS). The upstream element contains a TATA box (located approximately -30 bp of the TSS), and two overlapping heat shock consensus elements (located approximately -200 bp of the TSS). This promoter also comprises about 1100 bp 3′ of the TSS region. This 3′ region contains an adjacent leader sequence (ZmUbi1 exon), and an intron.

FIG. 2 shows an exemplary embodiment of the synthetic Ubi1 bidirectional promoter provided, which includes a minUbi1P minimal core element cloned upstream of a ZmUbi1 promoter.

FIG. 3 shows an exemplary schematic drawing of *YFP* and *GUS* gene expression cassettes, which are each operably linked to the synthetic Ubi1 bidirectional promoter.

FIG. 4 shows a representative plasmid map of pDAB105801.

FIG. 5 shows a schematic drawing of an exemplary Sugar Cane Bacilliform Virus (SCBV) bidirectional promoter, which includes a Min-Ubi1Pminimal core element cloned upstream of a SCBV promoter.

FIG. 6 shows a representative plasmid map of pDAB105806.

FIG. 7 shows an exemplary schematic drawing of *YFP* and *GUS* gene expression cassettes, which are each operably linked to a synthetic SCBV bidirectional promoter.

FIG. 8 shows exemplary schematic presentations of multi-gene constructs provided herein. Translation switches are shown using a special (vertical dumbbell) symbol.

FIG. 9 shows representative plasmid maps of pDAB108708 and pDAB101556.

FIG. 10A shows SEQ ID NO: 1, which comprises a 215 bp region of a *Zea mays* Ubiquitin 1 minimal core promoter (minUbi1P). FIG. 10B shows SEQ ID NO: 2, which comprises the reverse complement of a polynucleotide comprising a *Z. mays* minUbi1P minimal core promoter (underlined); a *Z. mays* Ubi1 leader (ZmUbi1 exon; bold font); and a *Z. mays* Ubi1 intron (lower case).

FIG. 11 shows SEQ ID NO: 3, which comprises an exemplary synthetic Ubi1 bidirectional promoter, wherein the reverse complement of a first minUbi1P, and a second minUbi1P, are underlined.

FIG. 12 shows SEQ ID NO: 4, which comprises an exemplary nucleic acid comprising *YFP* and *GUS* gene expression cassettes driven by a synthetic Ubi1 bidirectional promoter.

FIG. 13 shows SEQ ID NO: 5, which comprises an exemplary SCBV bidirectional promoter comprising a minUbi1P minimal core promoter, wherein the reverse complement of the minUbi1P is underlined.

FIG. 14 shows SEQ ID NO: 6, which comprises a SCBV promoter containing ADH1 exon 6 (underlined), intron 6 (lower case font), and exon 7 (bold font).

FIG. 15 shows SEQ ID NO: 7, which comprises a nucleic acid comprising *YFP* and *GUS* gene expression cassettes driven by an exemplary SCBV bidirectional promoter.

SEQ ID NO: 8 shows the YFP Forward Primer: 5′-GATGCCTCAG TGGGAAAGG-3′. SEQ ID NO: 9 comprises a YFP Reverse Primer:

5'-CCATAGGTGA GAGTGGTGAC AA-3'. SEQ ID NO: 10 comprises an Invertase Forward Primer: 5'-TGGCGGACGA CGACTTGT-3'. SEQ ID NO: 11 comprises an Invertase Reverse Primer: 5'-AAAGTTTGGA GGCTGCCGT-3'. SEQ ID NO: 12 comprises an Invertase Probe: 5'-CGAGCAGACC GCCGTGTACT TCTACC-3'. SEQ ID NO: 13 comprises an AAD1 Forward Primer: 5'-TGTTCGGTTC CCTCTACCAA-3'. SEQ ID NO: 14 comprises an AAD1 Reverse Primer: 5'-CAACATCCAT CACCTTGACT GA-3'. SEQ ID NO: 15 comprises an AAD1 Probe: 5'-CACAGAACCG TCGCTTCAGC AACA-3' (see also Table 7).

FIG. 16 shows a Western blot analysis for stable YFP expression driven by a bidirectional SCBV Promoter construct (pDAB108708) in maize T_0 plants. Representative plants showed stable YFP expression in leaf driven by the Min-Ubi1P minimal core promoter element. The amount of protein which is produced is indicated as parts per million (ppm).

FIG. 17 shows a Western blot analysis for stable YFP expression from the control construct containing a ZmUbi1 promoter that only drives expression of YFP (pDAB101556); a *GUS* coding sequence is not contained in this construct. The amount of protein which is produced is indicated as parts per million (ppm).

FIG. 18 shows exemplary constructs of four-gene cassette stacks pDAB105849 (AAD1-2A-YFP plus Cry34-2A-Cry35) and pDAB105865 (YFP-2A-AAD1 plus Cry34-2A-Cry35). Shaded arrows indicate direction of transcription from the bi-directional promoter. Ubi1-mimP comprises 200nt sequence upstream of transcriptional start site of maize Ubi1 promoter. SCBV-URS comprises upstream regulatory sequence of SCBV promoter excluding the core promoter (shown as arrow). Ubi1-Int comprises an intron of maize Ubi1 promoter. FIG. 19 shows two additional exemplary constructs of four-gene cassette stacks.

FIG. 20 shows representative maps for plasmids pDAB105818 and pDAB105748.

FIGS. 21A-21E shows additional minimal core promoters (min-Ubi1P or Ubi1-minP) of SEQ ID NOs: 16-40.

FIG. 22 shows representative maps for plasmids pDAB105841 and pDAB105847.

FIG. 23 shows representative maps for plasmids pDAB105840 and pDAB105849.

FIG. 24 shows representative maps for plasmids pDAB101917 and pDAB108719.

FIG. 25 shows representative maps for plasmids pDAB105844 and pDAB105848.

FIG. 26 shows representative maps for plasmids pDAB105865 and pDAB108720.

FIG. 27A-J shows nucleic acid sequence for gene expression cassettes of pDAB108719, where each gene and element is illustrated.

FIG. 28 shows exemplary protein expression data among various constructs tested for Cry34 (FIG. 28A), AAD-1 (FIG. 28B), and Cry35 (FIG. 28C).

FIG. 29 shows two exemplary sequences for yellow fluorescent proteins from Phialidium sp. SL-2003 (PhiYFP, SEQ ID NO: 51; and PhiYFPv3, SEQ ID NO: 52).

FIG. 30 shows exemplary embodiments of the synthetic Ubi1 bidirectional promoter and constructs provided, including pDAB108706 (ZMUbi bidirectional (-200)), pDAB108707 (ZMUbi bidirectional (-90)), pDAB108708 (SCBV bidirectional (-200)), and pDAB108709 (SCBV bidirectional (-90)). pDAB101556 (ZmUbi1-YFP control), pDAB108715 (SCBV without minimal promoter), and pDAB108716 (ZMUbi1 without minimal promoter) serve as control constructs with uni-directional promoters.

FIG. 31A shows exemplary expression results (V6) from the seven constructs shown in FIG. 30 for YFP protein (LCMS) in ng/cm^2. FIG. 31B shows exemplary relative expression results (V6) from the seven constructs shown in FIG. 30 for YFP RNA.

FIG. 32A shows exemplary expression results (V6) from the seven constructs shown in FIG. 30 for GUS protein (LCMS) in ng/cm^2. FIG. 32B shows exemplary relative expression results (V6) from the seven constructs shown in FIG. 30 for GUS RNA.

FIG. 33A shows exemplary expression results (V6) from the seven constructs shown in FIG. 30 for AAD1 protein (LCMS) in ng/cm^2. FIG. 33B shows exemplary

relative expression results (V6) from the seven constructs shown in FIG. 30 for AAD1 RNA.

FIG. 34A shows a statistical analysis of expression results (V6) from the seven constructs shown in FIG. 30 for YFP protein (LCMS) in ng/cm^2. FIG. 34B shows a statistical analysis of relative expression results (V6) from the seven constructs shown in FIG. 30 for YFP RNA. The mean values and statistical results are listed.

FIG. 35A shows a statistical analysis of expression results (V6) from the seven constructs shown in FIG. 30 for GUS protein (LCMS) in ng/cm^2. FIG. 35B shows a statistical analysis of relative expression results (V6) from the seven constructs shown in FIG. 30 for GUS RNA. The mean values and statistical results are listed.

FIG. 36A shows a statistical analysis of expression results (V6) from the seven constructs shown in FIG. 30 for AAD1 protein (LCMS) in ng/cm^2. FIG. 36B shows a statistical analysis of relative expression results (V6) from the seven constructs shown in FIG. 30 for AAD1 RNA. The mean values and statistical results are listed.

FIGS. 37A, 37B, and 37C show exemplary expression results (V10) from the seven constructs shown in FIG. 30 for YFP, AAD1, and GUS protein (LCMS) in ng/cm^2 respectively.

FIGS. 38A, 38B, and 38C show statistical analysis of expression results (V10) from the seven constructs shown in FIG. 30 for YFP, GUS, and AAD1 protein (LCMS) in ng/cm^2 respectively. The mean values and statistical results are listed.

FIGS. 39A, 39B, and 39C show exemplary expression results (R3) from the seven constructs shown in FIG. 30 for YFP, GUS, and AAD1 protein (LCMS) in ng/cm^2, respectively.

FIGS. 40A, 40B, and 40C show statistical analysis of expression results (R3) from the seven constructs shown in FIG. 30 for YFP, GUS, and AAD1 protein (LCMS) in ng/cm^2, respectively. The mean values and statistical results are listed.

FIG. 41 shows additional multi-transgene constructs using Ubi1 promoter, including pDAB108717 and pDAB108718.

FIG. 42A shows exemplary relative expression results (V6) of Cry34 RNA from six constructs pDAB105748 (ZMUbi1-YFP), pDAB105818 (ZMUbi1-Cry34/ZMUbi1 -Cry35/ZMUbi1-AAD1), pDAB108717 (YFP/AAD-1-ZMUbi1 bidirectional-Cry34-Cry35), pDAB108718 (AAD1/YFP-ZMUbi1 bidirectinal-Cry34-Cry35), pDAB108719 (YFP/AAD1-SCBV bidirectional-Cry34-Cry35), and pDAB108720 (AAD1/YFP – SCBV bidirectional-Cry34-Cry35). FIG. 42B shows exemplary relative expression results (V6) of Cry34 protein (LCMS) from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

FIG. 43A shows exemplary relative expression results (V6) of AAD1 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. FIG. 43B shows exemplary relative expression results (V6) of AAD1 protein (LCMS) from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

FIG. 44A shows exemplary relative expression results (V6) of YFP RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. FIG. 44B shows exemplary relative expression results (V6) of YFP protein (LCMS) from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

FIG. 45A shows exemplary relative expression results (V6) of Cry35 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. FIG. 45B shows exemplary relative expression results (V6) of Cry35 protein (ELISA) from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

FIG. 46 shows exemplary relative expression results (V6) of PAT RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

FIG. 47A shows a statistical analysis of expression results (V6) of Cry34 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. FIG. 47B shows a statistical analysis of expression results (V6) of Cry34 protein from the same six constructs pDAB105748,

pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIG. 48A shows a statistical analysis of expression results (V6) of AAD1 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. FIG. 48B shows a statistical analysis of expression results (V6) of AAD1 protein from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIG. 49A shows a statistical analysis of expression results (V6) of YFP RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. FIG. 49B shows a statistical analysis of expression results (V6) of YFP protein from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIG. 50A shows a statistical analysis of expression results (V6) of Cry35 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. FIG. 50B shows a statistical analysis of expression results (V6) of Cry35 protein from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIG. 51 shows a statistical analysis of expression results (V6) of PAT RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIGS. 52A, 52B, 52C, and 52D show exemplary protein expression results (V10) of YFP, AAD1, Cry34, and Cry35 respectively from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

FIGS. 53A, 53B, 53C, and 53D show statistical analysis of protein expression results (V10) of YFP, AAD1, Cry34, and Cry35 respectively from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIGS. 54A, 54B, 54C, and 54D show exemplary protein expression results (R3) of YFP, AAD1, Cry34, and Cry35 respectively from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

FIGS. 55A, 55B, 55C, and 55D show statistical analysis of protein expression results (R3) of YFP, AAD1, Cry34, and Cry35 respectively from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIG. 56 shows exemplary results of Western blot for protein expression of Cry34, Cry35, and AAD1 from pDAB108718, pDAB108717, pDAB108719, and pDAB108720.

MODE(S) FOR CARRYING OUT THE INVENTION

Development of transgenic products is becoming increasingly complex, which requires pyramiding multiple transgenes into a single locus. Traditionally each transgene usually requires a unique promoter for expression, so multiple promoters are required to express different transgenes within one gene stack. In addition to increasing the size of the gene stack, this frequently leads to repeated use of the same promoter to obtain similar levels of expression patterns of different transgenes controlling the same trait. Multi-gene constructs driven by the same promoter are known to cause gene silencing, thus making transgenic products less efficacious in the field. Excess of transcription factor (TF)-binding sites due to promoter repetition can cause depletion of endogenous TFs leading to transcriptional inactivation. The silencing of transgenes will likely undesirably affect the performance of a transgenic plant produced to express the transgenes. Repetitive sequences within a transgene may lead to gene intra-locus homologous recombination resulting in polynucleotide rearrangements.

Provided are methods and constructs combining the bidirectional promoter system with bicistronic organization of genes on either one or both ends of the promoter, for example with the use of a 2A sequence from Thosea asigna virus. The 2A protein, which is only 16–20 amino acids long, cleaves the polyprotein at its own carboxyl-terminus. This "self-cleavage" or "ribosome skip" property of the 2A or

2A-like peptide can be used to process artificial polyproteins produced in transgenic plants. In one embodiment, Cry34 and Cry35 genes are fused in one gene expression cassette, while YFP (or PhiYFP) and AAD1 genes are fused into another gene expression cassette (with a single open reading frame (ORF) with a copy of the 2A protein gene placed between the two genes in each combination). For example, each of these gene expression cassettes (or gene pairs) can be placed on the either end of the bidirectional promoter to drive 4 transgenes using a single promoter. Thus, the constructs and methods provided herein are useful to avoid repeated use of the same promoter and significantly reduce the size of commercial constructs. In addition, driving four or more genes with one promoter also provides ability to co-express genes controlling a single trait.

Plant promoters used for basic research or biotechnological application are generally unidirectional, directing only one gene that has been fused at its 3′ end (downstream). It is often necessary to introduce multiple genes into plants for metabolic engineering and trait stacking and therefore, multiple promoters are typically required in future transgenic crops to drive the expression of multiple genes. It is desirable to design strategies that can save the number of promoters deployed and allow simultaneous co-regulated expression for gene pyramiding. In some embodiment, the bi-directional promoters provided can drive transcription of multiple transcription units, including RNAi, artificial miRNA, or haipin-loop RNA sequences.

Embodiments herein utilize a process wherein a unidirectional promoter from a maize ubiquitin-1 gene (*e.g.*, ZmUbi1) and a SCBV promoter to design a synthetic bidirectional promoter, such that one promoter can direct the expression of two genes, one on each end of the promoter. Synthetic bidirectional promoters may allow those in the art to stack transgenes in plant cells and plants while lessening the repeated use of the same promoter and reducing the size of transgenic constructs. Furthermore, regulating the expression of two genes with a single synthetic bidirectional promoter may also provide the ability to co-express the two genes under the same conditions, such as may be useful, for example, when the two genes each contribute to a single trait in the host. The use of bidirectional function of promoters in plants has been reported in some cases, including the CaMV 35 promoters (Barfield and Pua (1991) *Plant Cell Rep.* 10(6-7):308-14; Xie et al. (2001)), and the mannopine synthase promoter (mas)

promoters (Velten et al. (1984) *EMBO J.* 3(12):2723-30; Langridge et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:3219-23).

Transcription initiation and modulation of gene expression in plant genes is directed by a variety of DNA sequence elements that are collectively arranged within the promoter. Eukaryotic promoters consist of minimal core promoter element (minP), and further upstream regulatory sequences (URSs). The core promoter element is a minimal stretch of contiguous DNA sequence that is sufficient to direct accurate initiation of transcription. Core promoters in plants also comprise canonical regions associated with the initiation of transcription, such as CAAT and TATA boxes. The TATA box element is usually located approximately 20 to 35 nucleotides upstream of the initiation site of transcription.

The activation of the minP is dependent upon the URS, to which various proteins bind and subsequently interact with the transcription initiation complex. URSs comprise of DNA sequences, which determine the spatiotemporal expression pattern of a promoter comprising the URS. The polarity of a promoter is often determined by the orientation of the minP, while the URS is bipolar (*i.e.*, it functions independent of its orientation). For example, the CaMV 35S synthetic unidirectional polar promoter may be converted to a bidirectional promoter by fusing a minP at the 5′ end of the promoter in the opposite orientation. See, for example, Xie et al. (2001) *Nat. Biotechnol.* 19(7):677-9.

In specific examples of some embodiments, a minimal core promoter element (minUbi1P) of a modified maize Ubi1 promoter (ZmUbi1) originally derived from the *Z. mays* inbred line, B73, is used to engineer a synthetic bidirectional SCBV promoter that may function in plants to provide expression control characteristics that are unique with respect to previously available bidirectional promoters. Embodiments include a synthetic bidirectional SCBV promoter that further includes nucleotide sequence derived from a native SCBV promoter. Particular embodiments may further include a synthetic bidirectional SCBV promoter comprising an intron (*e.g.*, an ADH intron) in close proximity to SCBV and minUbi1P sequence elements in the synthetic bidirectional SCBV promoter.

The ZmUbi1 promoter originally derived from B73 comprises sequences located in the maize genome within about 899 bases 5′ of the transcription start site,

and further within about 1093 bases 3′ of the transcription start site. Christensen et al. (1992) *Plant Mol. Biol.* 18(4):675-89 (describing a B73 ZmUbi1 gene). A modified ZmUbi1 promoter derived from B73 that is used in some examples is an approximately 2 kb promoter that contains a TATA box; two overlapping heat shock consensus elements; an 82 or 83 nucleotide (depending on the reference strand) leader sequence immediately adjacent to the transcription start site, which is referred to herein as ZmUbi1 exon; and a 1015-1016 nucleotide intron (see FIG. 1 for example). Other maize ubiquitin promoter variants derived from *Zea* species and *Zea mays* genotypes may exhibit high sequence conservation around the minP element consisting of the TATA element and the upstream heat shock consensus elements. Thus, embodiments of the invention are exemplified by the use of this short (~200 nt) highly conserved region (*e.g.*, SEQ ID NO: 1) of a ZmUbi1 promoter as a minimal core promoter element for constructing synthetic bidirectional plant promoters.

Certain abbreviations disclosed are listed in Table 1.

Table 1. Abbreviations used in the disclosure

Phrase	Abbreviation
bicinchoninic acid	BCA
cauliflower mosaic virus	CaMV
chloroplast transit peptide	CTP
homology-based gene silencing	HBGS
ZmUbi1 minimal core promoter	minUbi1P
oligo ligation amplification	OLA
phosphate buffered saline	PBS
phosphate buffered saline with 0.05% Tween 20	PBST
polymerase chain reaction	PCR
rolling circle amplification	RCA
reverse transcriptase PCR	RT-PCR
single nucleotide primer extension	SNuPE
upstream regulatory sequence	URS
Zea mays Ubiquitin-1 gene	ZmUbi1

As used herein, the articles, "a," "an," and "the" include plural references unless the context clearly and unambiguously dictates otherwise.

As used herein, the phrase "backcrossing" refers to a process in which a breeder crosses hybrid progeny back to one of the parents, for example, a first generation hybrid $_{F1}$ with one of the parental genotypes of the F1 hybrid.

As used herein, the phrase "intron" refers to any nucleic acid sequence comprised in a gene (or expressed nucleotide sequence of interest) that is transcribed but not translated. Introns include untranslated nucleic acid sequence within an expressed sequence of DNA, as well as the corresponding sequence in RNA molecules transcribed therefrom.

As used herein, the phrase "isolated" refers to biological component (including a nucleic acid or protein) has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs (*i.e.*, other chromosomal and extra-chromosomal DNA and RNA, and proteins), while effecting a chemical or functional change in the component (*e.g.*, a nucleic acid may be isolated from a chromosome by breaking chemical bonds connecting the nucleic acid to the remaining DNA in the chromosome). Nucleic acid molecules and proteins that have been "isolated" include nucleic acid molecules and proteins purified by standard purification methods. The phrase "isolated" also embraces nucleic acids and proteins prepared by recombinant expression in a host cell, as well as chemically synthesized nucleic acid molecules, proteins, and peptides.

As used herein, the phrase "gene expression" refers to a process by which the coded information of a nucleic acid transcriptional unit (including, *e.g.*, genomic DNA) is converted into an operational, non-operational, or structural part of a cell, often including the synthesis of a protein. Gene expression can be influenced by external signals; for example, exposure of a cell, tissue, or organism to an agent that increases or decreases gene expression. Expression of a gene can also be regulated anywhere in the pathway from DNA to RNA to protein. Regulation of gene expression occurs, for example, through controls acting on transcription, translation, RNA transport and processing, degradation of intermediary molecules such as mRNA, or through activation, inactivation, compartmentalization, or degradation of specific protein molecules after they have been made, or by combinations thereof. Gene expression can be measured at the RNA level or the protein level by any method known in the art,

including, without limitation, Northern blot, RT-PCR, Western blot, or *in vitro*, *in situ*, or *in vivo* protein activity assay(s).

As used herein, the phrase "homology-based gene silencing" (HBGS) refers to a generic term that includes both transcriptional gene silencing and posttranscriptional gene silencing. Silencing of a target locus by an unlinked silencing locus can result from transcription inhibition (transcriptional gene silencing; TGS) or mRNA degradation (post-transcriptional gene silencing; PTGS), owing to the production of double-stranded RNA (dsRNA) corresponding to promoter or transcribed sequences, respectively. The involvement of distinct cellular components in each process suggests that dsRNA-induced TGS and PTGS likely result from the diversification of an ancient common mechanism. However, a strict comparison of TGS and PTGS has been difficult to achieve because it generally relies on the analysis of distinct silencing loci. A single transgene locus can be described to trigger both TGS and PTGS, owing to the production of dsRNA corresponding to promoter and transcribed sequences of different target genes. See, for example, Mourrain et al. (2007) *Planta* 225:365-79. It is likely that siRNAs are the actual molecules that trigger TGS and PTGS on homologous sequences: the siRNAs would in this model trigger silencing and methylation of homologous sequences in *cis* and in *trans* through the spreading of methylation of transgene sequences into the endogenous promoter.

As used herein, the phrase "nucleic acid molecule" (or "nucleic acid" or "polynucleotide") refers to a polymeric form of nucleotides, which may include both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide may refer to a ribonucleotide, deoxyribonucleotide, or a modified form of either type of nucleotide. A "nucleic acid molecule" as used herein is synonymous with "nucleic acid" and "polynucleotide." A nucleic acid molecule is usually at least 10 bases in length, unless otherwise specified. The term may refer to a molecule of RNA or DNA of indeterminate length. The term includes single- and double-stranded forms of DNA. A nucleic acid molecule may include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages.

Nucleic acid molecules may be modified chemically or biochemically, or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by

those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications (*e.g.*, uncharged linkages: for example, methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.; charged linkages: for example, phosphorothioates, phosphorodithioates, etc.; pendent moieties: for example, peptides; intercalators: for example, acridine, psoralen, etc.; chelators; alkylators; and modified linkages: for example, alpha anomeric nucleic acids, etc.). The term "nucleic acid molecule" also includes any topological conformation, including single-stranded, double-stranded, partially duplexed, triplexed, hairpinned, circular, and padlocked conformations.

Transcription proceeds in a 5′ to 3′ manner along a DNA strand. This means that RNA is made by the sequential addition of ribonucleotide-5′-triphosphates to the 3′ terminus of the growing chain (with a requisite elimination of the pyrophosphate). In either a linear or circular nucleic acid molecule, discrete elements (*e.g.*, particular nucleotide sequences) may be referred to as being "upstream" relative to a further element if they are bonded or would be bonded to the same nucleic acid in the 5′ direction from that element. Similarly, discrete elements may be "downstream" relative to a further element if they are or would be bonded to the same nucleic acid in the 3′ direction from that element.

As used herein, the phrase "base position," refers to the location of a given base or nucleotide residue within a designated nucleic acid. The designated nucleic acid may be defined by alignment (see below) with a reference nucleic acid.

As used herein, the phrase "hybridization" refers to a process where oligonucleotides and their analogs hybridize by hydrogen bonding, which includes Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary bases. Generally, nucleic acid molecules consist of nitrogenous bases that are either pyrimidines (cytosine (C), uracil (U), and thymine (T)) or purines (adenine (A) and guanine (G)). These nitrogenous bases form hydrogen bonds between a pyrimidine and a purine, and the bonding of the pyrimidine to the purine is referred to as "base pairing." More specifically, A will hydrogen bond to T or U, and G will bond to C. "Complementary" refers to the base pairing that occurs between two

distinct nucleic acid sequences or two distinct regions of the same nucleic acid sequence.

As used herein, the phrases "specifically hybridizable" and "specifically complementary" refers to a sufficient degree of complementarity such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. The oligonucleotide need not be 100% complementary to its target sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA, and there is sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions where specific binding is desired, for example under physiological conditions in the case of *in vivo* assays or systems. Such binding is referred to as specific hybridization.

Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the chosen hybridization method and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (especially the Na+ and/or Mg2+ concentration) of the hybridization buffer will contribute to the stringency of hybridization, though wash times also influence stringency. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are discussed in Sambrook et al. (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, chs. 9 and 11.

As used herein, the phrase "stringent conditions" encompass conditions under which hybridization will only occur if there is less than 50% mismatch between the hybridization molecule and the DNA target. "Stringent conditions" include further particular levels of stringency. Thus, as used herein, "moderate stringency" conditions are those under which molecules with more than 50% sequence mismatch will not hybridize; conditions of "high stringency" are those under which sequences with more than 20% mismatch will not hybridize; and conditions of "very high stringency" are those under which sequences with more than 10% mismatch will not hybridize.

In particular embodiments, stringent conditions can include hybridization at 65°C, followed by washes at 65°C with 0.1x SSC/0.1% SDS for 40 minutes.

The following are representative, non-limiting hybridization conditions:

Very High Stringency: Hybridization in 5x SSC buffer at 65°C for 16 hours; wash twice in 2x SSC buffer at room temperature for 15 minutes each; and wash twice in 0.5x SSC buffer at 65°C for 20 minutes each.

High Stringency: Hybridization in 5x-6x SSC buffer at 65-70°C for 16-20 hours; wash twice in 2x SSC buffer at room temperature for 5-20 minutes each; and wash twice in 1x SSC buffer at 55-70°C for 30 minutes each.

Moderate Stringency: Hybridization in 6x SSC buffer at room temperature to 55°C for 16-20 hours; wash at least twice in 2x-3x SSC buffer at room temperature to 55°C for 20-30 minutes each.

In particular embodiments, specifically hybridizable nucleic acid molecules can remain bound under very high stringency hybridization conditions. In these and further embodiments, specifically hybridizable nucleic acid molecules can remain bound under high stringency hybridization conditions. In these and further embodiments, specifically hybridizable nucleic acid molecules can remain bound under moderate stringency hybridization conditions.

As used herein, the phrase "oligonucleotide" refers to a short nucleic acid polymer. Oligonucleotides may be formed by cleavage of longer nucleic acid segments, or by polymerizing individual nucleotide precursors. Automated synthesizers allow the synthesis of oligonucleotides up to several hundred base pairs in length. Because oligonucleotides may bind to a complementary nucleotide sequence, they may be used as probes for detecting DNA or RNA. Oligonucleotides composed of DNA (oligodeoxyribonucleotides) may be used in PCR, a technique for the amplification of small DNA sequences. In PCR, the oligonucleotide is typically referred to as a "primer," which allows a DNA polymerase to extend the oligonucleotide and replicate the complementary strand.

As used herein, the phrase "sequence identity" or "identity," refers to a context where two nucleic acid or polypeptide sequences, may refer to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window.

As used herein, the phrase "percentage of sequence identity" refers to the value determined by comparing two optimally aligned sequences (*e.g.*, nucleic acid

sequences, and amino acid sequences) over a comparison window, wherein the portion of the sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleotide or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window, and multiplying the result by 100 to yield the percentage of sequence identity.

Methods for aligning sequences for comparison are well-known in the art. Various programs and alignment algorithms are described in, for example: Smith and Waterman (1981) *Adv. Appl. Math.* 2:482; Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443; Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85:2444; Higgins and Sharp (1988) *Gene* 73:237-44; Higgins and Sharp (1989) *CABIOS* 5:151-3; Corpet et al. (1988) *Nucleic Acids Res.* 16:10881-90; Huang et al. (1992) *Comp. Appl. Biosci.* 8:155-65; Pearson et al. (1994) *Methods Mol. Biol.* 24:307-31; Tatiana et al. (1999) *FEMS Microbiol. Lett.* 174:247-50. A detailed consideration of sequence alignment methods and homology calculations can be found in, *e.g.*, Altschul et al. (1990) *J. Mol. Biol.* 215:403-10.

The National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST™; Altschul et al. (1990)) is available from several sources, including the National Center for Biotechnology Information (Bethesda, MD), and on the internet, for use in connection with several sequence analysis programs. A description of how to determine sequence identity using this program is available on the internet under the "help" section for BLAST™. For comparisons of nucleic acid sequences, the "Blast 2 sequences" function of the BLAST™ (Blastn) program may be employed using the default parameters. Nucleic acid sequences with even greater similarity to the reference sequences will show increasing percentage identity when assessed by this method.

As used herein, the phrase "operably linked" refers to a context where the first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked with a coding sequence when

the promoter affects the transcription or expression of the coding sequence. When recombinantly produced, operably linked nucleic acid sequences are generally contiguous and, where necessary to join two protein-coding regions, in the same reading frame. However, elements need not be contiguous to be operably linked.

As used herein, the phrase "promoter" refers to a region of DNA that generally is located upstream (towards the 5′ region of a gene) that is needed for transcription. Promoters may permit the proper activation or repression of the gene which they control. A promoter may contain specific sequences that are recognized by transcription factors. These factors may bind to the promoter DNA sequences and result in the recruitment of RNA polymerase, an enzyme that synthesizes RNA from the coding region of the gene.

As used herein, the phrase "transforms" or "transduces" refers to a process where a virus or vector transfers nucleic acid molecules into a cell. A cell is "transformed" by a nucleic acid molecule "transduced" into the cell when the nucleic acid molecule becomes stably replicated by the cell, either by incorporation of the nucleic acid molecule into the cellular genome or by episomal replication. As used herein, the term "transformation" encompasses all techniques by which a nucleic acid molecule can be introduced into such a cell. Examples include, but are not limited to: transfection with viral vectors; transformation with plasmid vectors; electroporation (Fromm et al. (1986) *Nature* 319:791-3); lipofection (Felgner et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7); microinjection (Mueller et al. (1978) *Cell* 15:579-85); *Agrobacterium*-mediated transfer (Fraley et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:4803-7); direct DNA uptake; whiskers-mediated transformation; and microprojectile bombardment (Klein et al. (1987) *Nature* 327:70).

As used herein, the phrase "transgene" refers to an exogenous nucleic acid sequence. In one example, a transgene is a gene sequence (*e.g.*, a herbicide-resistant gene), a gene encoding an industrially or pharmaceutically useful compound, or a gene encoding a desirable agricultural trait. In yet another example, the transgene is an antisense nucleic acid sequence, wherein expression of the antisense nucleic acid sequence inhibits expression of a target nucleic acid sequence. A transgene may contain regulatory sequences operably linked to the transgene (*e.g.*, a promoter). In some embodiments, a nucleic acid sequence of interest is a transgene. However, in

other embodiments, a nucleic acid sequence of interest is an endogenous nucleic acid sequence, wherein additional genomic copies of the endogenous nucleic acid sequence are desired, or a nucleic acid sequence that is in the antisense orientation with respect to the sequence of a target nucleic acid molecule in the host organism.

As used herein, the phrase "vector" refers to a nucleic acid molecule as introduced into a cell, thereby producing a transformed cell. A vector may include nucleic acid sequences that permit it to replicate in the host cell, such as an origin of replication. Examples include, but are not limited to, a plasmid, cosmid, bacteriophage, or virus that carries exogenous DNA into a cell. A vector can also include one or more genes, antisense molecules, and/or selectable marker genes and other genetic elements known in the art. A vector may transduce, transform, or infect a cell, thereby causing the cell to express the nucleic acid molecules and/or proteins encoded by the vector. A vector may optionally include materials to aid in achieving entry of the nucleic acid molecule into the cell (*e.g.*, a liposome).

As used herein, the phrase "plant" includes plants and plant parts including but not limited to plant cells and plant tissues such as leaves, stems, roots, flowers, pollen, and seeds. The class of plants that can be used in the present invention is generally as broad as the class of higher and lower plants amenable to mutagenesis including angiosperms (monocotyledonous and dicotyledonous plants), gymnosperms, ferns and multicellular algae. Thus, "plant" includes dicotyledons plants and monocotyledons plants. Examples of dicotyledons plants include tobacco, Arabidopsis, soybean, tomato, papaya, canola, sunflower, cotton, alfalfa, potato, grapevine, pigeon pea, pea, Brassica, chickpea, sugar beet, rapeseed, watermelon, melon, pepper, peanut, pumpkin, radish, spinach, squash, broccoli, cabbage, carrot, cauliflower, celery, Chinese cabbage, cucumber, eggplant, and lettuce. Examples of monocotyledons plants include corn, rice, wheat, sugarcane, barley, rye, sorghum, orchids, bamboo, banana, cattails, lilies, oat, onion, millet, and triticale.

As used herein, the phrase "plant material" refers to leaves, stems, roots, flowers or flower parts, fruits, pollen, egg cells, zygotes, seeds, cuttings, cell or tissue cultures, or any other part or product of a plant. In some embodiment, plant material includes cotyledon and leaf.

As used herein, the phrase "translation switch" refers to a mechanism at end of a gene allowing translation of an immediate downstream gene. The mechanism of translation switch can function at nucleic acid level (for example, viral or eukaryotic internal ribosome entry site (IRES), an alternative splicing site, or a ribozyme cleavage site) or at peptide/protein level (for example, a 2A peptide, a 2A-like peptide, an intein peptide, or a protease cleavage site).

These mechanisms of translation switch at nucleic acid level or at peptide/protein level are well known in the art. *See, e.g.*, Z. Li, H.M. Schumacher, et al. (2010) *J. Biotechnol.* 145(1): 9-16; Y. Chen, K. Perumal, et al. (2000) *Gene Expr.* 9(3):133-143; T.D. Dinkova, H. Zepeda, et al. (2005) *Plant J.* 41(5): 722-731; Y.L. Dorokhov, M.V. Skulachev, et al. (2002) *Proc. Natl. Acad. Sci. U. S. A.* 99(8): 5301-5306; O. Fernandez-Miragall and C. Hernandez (2011) *PLoS One* 6(7): e22617; E. Groppelli, G.J. Belsham, et al. (2007) *J. Gen. Virol.* 88(Pt 5): 1583-1588; S.H. Ha, Y.S. Liang, et al. (2010) *Plant Biotechnol J.* 8(8): 928-938; A. Karetnikov and K. Lehto (2007) *J. Gen. Virol.* 88(Pt 1): 286-297; A. Karetnikov and K. Lehto (2008) *Virology* 371(2): 292-308; M.A. Khan, H. Yumak, et al. (2009) *J. Biol. Chem.* 284(51): 35461-35470; and D.C. Koh, S.M. Wong, et al. (2003) *J. Biol. Chem.* 278(23): 20565-20573. Multi-gene expression constructs containing modified inteins have been disclosed in U.S. Patent Nos. 7,026,526 and 7,741,530, as well as U.S. Patent application 2008/0115243.

As used herein, the phrase "selectable marker" or "selectable marker gene" refers to a gene that is optionally used in plant transformation to, for example, protect the plant cells from a selective agent or provide resistance/tolerance to a selective agent. Only those cells or plants that receive a functional selectable marker are capable of dividing or growing under conditions having a selective agent. Examples of selective agents can include, for example, antibiotics, including spectinomycin, neomycin, kanamycin, paromomycin, gentamicin, and hygromycin. These selectable markers include gene for neomycin phosphotransferase (npt II), which expresses an enzyme conferring resistance to the antibiotic kanamycin, and genes for the related antibiotics neomycin, paromomycin, gentamicin, and G418, or the gene for hygromycin phosphotransferase (hpt), which expresses an enzyme

conferring resistance to hygromycin. Other selectable marker genes can include genes encoding herbicide resistance including Bar (resistance against BASTA® (glufosinate ammonium), or phosphinothricin (PPT)), acetolactate synthase (ALS, resistance against inhibitors such as sulfonylureas (SUs), imidazolinones (IMIs), triazolopyrimidines (TPs), pyrimidinyl oxybenzoates (POBs), and sulfonylamino carbonyl triazolinones that prevent the first step in the synthesis of the branched-chain amino acids), glyphosate, 2,4-D, and metal resistance or sensitivity. The phrase "marker-positive" refers to plants that have been transformed to include the selectable marker gene.

Various selectable or detectable markers can be incorporated into the chosen expression vector to allow identification and selection of transformed plants, or transformants. Many methods are available to confirm the expression of selection markers in transformed plants, including for example DNA sequencing and PCR (polymerase chain reaction), Southern blotting, RNA blotting, immunological methods for detection of a protein expressed from the vector, *e g.*, precipitated protein that mediates phosphinothricin resistance, or other proteins such as reporter genes β-glucuronidase (GUS), luciferase, green fluorescent protein (GFP), DsRed, β-galactosidase, chloramphenicol acetyltransferase (CAT), alkaline phosphatase, and the like (see Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor Press, N.Y., 2001).

Selectable marker genes are utilized for the selection of transformed cells or tissues. Selectable marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT) as well as genes conferring resistance to herbicidal compounds. Herbicide resistance genes generally code for a modified target protein insensitive to the herbicide or for an enzyme that degrades or detoxifies the herbicide in the plant before it can act. For example, resistance to glyphosate or has been obtained by using genes coding for the mutant target enzymes, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Genes and mutants for EPSPS have been disclosed in U.S. Patent Nos. 4,940,835, 5,188,642, 5,310,667, 5,633,435, 5,633,448, and 6,566,587. Resistance to glufosinate ammonium, bromoxynil, and 2,4-dichlorophenoxyacetate (2,4-D) have been obtained by using

bacterial genes encoding phosphinothricin acetyltransferase, a nitrilase, or a 2,4-dichlorophenoxyacetate monooxygenase, which detoxify the respective herbicides. Enzymes/genes for glufosinate resistance/tolerance have been disclosed in U.S. Patent Nos. 5,273,894, 5,276,268, 5,550,318, and 5,561,236. Enzymes/genes for 2,4-D resistance have been previously disclosed in U.S. Patent Nos. 6,100,446 and 6,153,401, as well as patent applications US 2009/0093366 and WO 2007/053482. Enzymes/genes for nitrilase has been previously disclosed in U.S. Patent Nos. 4,810,648.

Other herbicides can inhibit the growing point or meristem, including imidazolinone or sulfonylurea, and genes for resistance/tolerance of acetohydroxyacid synthase (AHAS) and acetolactate synthase (ALS) for these herbicides have been described. Genes and mutants for AHAS and mutants have been disclosed in U.S. Patent Nos. 4,761,373, 5,304,732, 5,331,107, 5,853,973, and 5,928,937. Genes and mutants for ALS have been disclosed in U.S. Patent Nos. 5,013,659 and 5,141,870.

Glyphosate resistance genes include mutant 5-enolpyruvylshikimate-3 -phosphate synthase (EPSPs) genes (via the introduction of recombinant nucleic acids and/or various forms of *in vivo* mutagenesis of native EPSPs genes), aroA genes and glyphosate acetyl transferase (GAT) genes, respectively). Resistance genes for other phosphono compounds include glufosinate (phosphinothricin acetyl transferase (PAT) genes from Streptomyces species, including Streptomyces hygroscopicus and Streptomyces viridichromogenes), and pyridinoxy or phenoxy proprionic acids and cyclohexones (ACCase inhibitor-encoding genes). Herbicide resistance/tolerance genes of acetyl coemzyme A carboxylase (ACCase) have been described in U.S. Patents 5,162,602 and 5,498,544.

A DNA molecule encoding a mutant aroA gene can be obtained under ATCC accession number 39256, and the nucleotide sequence of the mutant gene is disclosed in U.S. Pat. No. 4,769,061 to Comai, European patent application No. 0 333 033 to Kumada et al., and U.S. Pat. No. 4,975,374 to Goodman et al., disclosing nucleotide sequences of glutamine synthetase genes which confer resistance to herbicides such as L-phosphinothricin. The nucleotide sequence of a PAT gene is provided in European application No. 0 242 246 to Leemans et al. Also DeGreef et

al., *Bio/Technology* 7:61 (1989), describes the production of transgenic plants that express chimeric bar genes coding for PAT activity. Exemplary of genes conferring resistance to phenoxy proprionic acids and cyclohexones, including sethoxydim and haloxyfop, are the Acc1-S1, Acc1-S2 and Acc1-S3 genes described by Marshall et al., *Theon. Appl. Genet.* 83:435 (1992). GAT genes capable of conferring glyphosate resistance are described in WO 2005012515 to Castle et al. Genes conferring resistance to 2,4-D, fop and pyridyloxy auxin herbicides are described in WO 2005107437 and U.S. patent application Ser. No. 11/587,893.

Other herbicides can inhibit photosynthesis, including triazine (psbA and ls+ genes) or benzonitrile (nitrilase gene). Przibila et al., *Plant Cell* 3:169 (1991), describes the transformation of Chlamydomonas with plasmids encoding mutant psbA genes. Nucleotide sequences for nitrilase genes are disclosed in U.S. Pat. No. 4,810,648 to Stalker, and DNA molecules containing these genes are available under ATCC Accession Nos. 53435, 67441, and 67442. Cloning and expression of DNA coding for a glutathione S-transferase is described by Hayes et al., *Biochem. J.* 285:173 (1992).

For purposes of the present invention, selectable marker genes include, but are not limited to genes encoding: neomycin phosphotransferase II (Fraley et al. (1986) *CRC Critical Reviews in Plant Science* 4:1-25); cyanamide hydratase (Maier-Greiner et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:4250-4264); aspartate kinase; dihydrodipicolinate synthase (Perl et al. (1993) *Bio/Technology* 11:715-718); tryptophan decarboxylase (Goddijn et al. (1993) *Plant Mol. Bio.* 22:907-912); dihydrodipicolinate synthase and desensitized aspartade kinase (Perl et al. (1993) *Bio/Technology* 11:715-718); bar gene (Toki et al. (1992) *Plant Physiol.* 100:1503-1507; and Meagher et al. (1996), *Crop Sci.* 36:1367); tryptophan decarboxylase (Goddijn et al. (1993) *Plant Mol. Biol.* 22:907-912); neomycin phosphotransferase (NEO) (Southern et al. (1982) *J. Mol. Appl. Gen.* 1:327; hygromycin phosphotransferase (HPT or HYG) (Shimizu et al. (1986) *Mol. Cell Biol.* 6:1074); dihydrofolate reductase (DHFR) (Kwok et al. (1986) *PNAS USA* 4552); phosphinothricin acetyltransferase (DeBlock et al. (1987) *EMBO J.* 6:2513); 2,2-dichloropropionic acid dehalogenase (Buchanan-Wollatron et al. (1989) *J. Cell. Biochem.* 13D:330); acetohydroxyacid synthase (Anderson et al., U.S. Pat. No.

4,761,373; Haughn et al. (1988) *Mol. Gen. Genet.* 221:266); 5-enolpyruvyl-shikimate-phosphate synthase (aroA) (Comai et al. (1985) *Nature* 317:741); haloarylnitrilase (Stalker et al., published PCT application WO87/04181); acetyl-coenzyme A carboxylase (Parker et al. (1990) *Plant Physiol.* 92:1220); dihydropteroate synthase (sul I) (Guerineau et al. (1990) *Plant Mol. Biol.* 15:127); and 32 kD photosystem II polypeptide (psbA) (Hirschberg et al. (1983) *Science* 222:1346).

Also included are genes encoding resistance to: chloramphenicol (Herrera-Estrella et al. (1983) *EMBO J.* 2:987-992); methotrexate (Herrera-Estrella et al. (1983) *Nature* 303:209-213; Meijer et al. (1991) *Plant Mol Bio.* 16:807-820 (1991); hygromycin (Waldron et al. (1985) *Plant Mol. Biol.* 5:103-108; Zhijian et al. (1995) *Plant Science* 108:219-227; and Meijer et al. (1991) *Plant Mol. Bio.* 16:807-820); streptomycin (Jones et al. (1987) *Mol. Gen. Genet.* 210:86-91); spectinomycin (Bretagne-Sagnard et al. (1996) *Transgenic Res.* 5:131-137); bleomycin (Hille et al. (1986) *Plant Mol. Biol.* 7:171-176); sulfonamide (Guerineau et al. (1990) *Plant Mol. Bio.* 15:127-136); bromoxynil (Stalker et al. (1988) *Science* 242:419-423); 2,4-D (Streber et al. (1989) *Bio/Technology* 7:811-816); glyphosate (Shaw et al. (1986) *Science* 233:478-481); and phosphinothricin (DeBlock et al. (1987) *EMBO J.* 6:2513-2518).

The above list of selectable marker and reporter genes are not meant to be limiting. Any reporter or selectable marker gene are encompassed by the present invention. If necessary, such genes can be sequenced by methods known in the art.

The reporter and selectable marker genes are synthesized for optimal expression in the plant. That is, the coding sequence of the gene has been modified to enhance expression in plants. The synthetic marker gene is designed to be expressed in plants at a higher level resulting in higher transformation efficiency. Methods for synthetic optimization of genes are available in the art. In fact, several genes have been optimized to increase expression of the gene product in plants.

The marker gene sequence can be optimized for expression in a particular plant species or alternatively can be modified for optimal expression in plant families. The plant preferred codons may be determined from the codons of highest frequency in the proteins expressed in the largest amount in the particular plant

species of interest. See, for example, EPA 0359472; EPA 0385962; WO 91/16432; Perlak et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:3324-3328; and Murray et al. (1989) *Nucleic Acids Research* 17: 477-498; U.S. Pat. No. 5,380,831; and U.S. Pat. No. 5,436,391. In this manner, the nucleotide sequences can be optimized for expression in any plant. It is recognized that all or any part of the gene sequence may be optimized or synthetic. That is, fully optimized or partially optimized sequences may also be used.

Genes that Confer Resistance to an Herbicide:

A. Resistance/tolerance of acetohydroxyacid synthase (AHAS) and acetolactate synthase (ALS) against herbicides imidazolinone or sulfonylurea. Genes and mutants for AHAS and mutants have been disclosed in U.S. Patent Nos. 4,761,373, 5,304,732, 5,331,107, 5,853,973, and 5,928,937. Genes and mutants for ALS have been disclosed in U.S. Patent Nos. 5,013,659 and 5,141, 870.

B. Resistance/tolerance genes of acetyl coemzyme A carboxylase (ACCase) against herbicides cyclohexanediones and/or aryloxyphenoxypropanoic acid (including Haloxyfop, Diclofop, Fenoxyprop, Fluazifop, Quizalofop) have been described in U.S. Patents 5,162,602 and 5,498,544.

C. Genes for glyphosate resistance/tolerance. Gene of 5-enolpyruvyl -3-phosphoshikimate synthase (ES3P synthase) has been described in U.S. Patent No. 4,769,601. Genes of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and mutants have been described in U.S. Patent Nos. 4,940,835, 5,188,642, 5,310,667, 5,633,435, 5,633,448, and 6,566,587.

D. Genes for glufosinate (bialaphos, phosphinothricin (PPT)) resistance/tolerance. Gene for phosphinothricin acetyltransferase (Pat) has been described in U.S. Patent Nos. 5,273,894, 5,276,268, and 5,550,318; and gene for bialaphos resistance gene (Bar) has been described in U.S. Patent Nos. 5,561,236 and 5,646,024, 5,648,477, and 7,112,665. Gene for glutamine synthetase (GS) has been described in U.S. Patent No. 4,975,372 and European patent application EP 0333033 A1.

E. Resistance/tolerance genes of hydroxy phenyl pyruvate dioxygcnase (HPPD) against herbicides isoxazole, diketonitriles, and/or triketones including

sulcotrione and mesotrione have been described in U.S. Patent Nos. 6,268,549 and 6,069,115.

F. Genes for 2,4-D resistance/tolerance. Gene of 2,4-D-monooxygenase has been described in U.S. Patent No. 6,100,446 and 6,153,401. Additional genes for 2,4-D resistance/tolerance are disclosed in US 2009/0093366 and WO 2007/053482.

G. Gene of imidazoleglycerol phosphate dehydratase (IGPD) against herbicides imidazole and/or triazole has been described in U.S. Patent No. 5,541,310. Genes of Dicamba degrading enzymes (oxygenase, ferredoxin, and reductase) against herbicide Dicamba have been disclosed in U.S. Patent Nos. 7,022,896 and 7,105,724.

H. Genes for herbicides that inhibit photosynthesis, including triazine (psbA and ls+ genes) or a benzonitrile (nitrilase gene). *See, e.g.*, Przibila et al., *Plant Cell* 3:169 (1991) disclosing transformation of *Chlamydomonas* with plasmids encoding mutant psbA genes. Nucleotide sequences for nitrilase genes are disclosed in U.S. Patent No. 4,810,648 and DNA molecules containing these genes are available under ATCC Accession Nos. 53435, 67441, and 67442. Cloning and expression of DNA coding for a glutathione S-transferase is described by Hayes et al., *Biochem. J.* 285:173 (1992).

Unless otherwise specifically explained, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this disclosure belongs. Definitions of common terms in molecular biology can be found in, for example: Lewin, *Genes V*, Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Meyers (ed.), *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

This disclosure provides nucleic acid molecules comprising a synthetic nucleotide sequence that may function as a bidirectional promoter. In some embodiments, a synthetic bidirectional promoter may be operably linked to one or two nucleotide sequence(s) of interest. For example, a synthetic bidirectional promoter may be operably linked to one or two nucleotide sequence(s) of interest (*e.g.*, two

genes, one on each end of the promoter), so as to regulate transcription of at least one (*e.g.*, one or both) of the nucleotide sequence(s) of interest. By incorporating a URS from a SCBV promoter in the synthetic bidirectional promoter, particular expression and regulatory patterns (*e.g.*, such as are exhibited by genes under the control of the SCBV promoter) may be achieved with regard to a nucleotide sequence of interest that is operably linked to the synthetic bidirectional promoter.

Some embodiments of the invention are exemplified herein by incorporating a minimal core promoter element from a unidirectional maize ubiquitin-1 gene (ZmUbi1) promoter into a molecular context different from that of the native promoter to engineer a synthetic bidirectional promoter. This minimal core promoter element is referred to herein as "minUbi1P," and is approximately 200 nt in length. Sequencing and analysis of minUbi1P elements from multiple *Zea* species and *Z. mays* genotypes has revealed that functional minUbi1P elements are highly conserved, such that a minUbi1P element may element may preserve its function as an initiator of transcription if it shares, for example, at least about 75%; at least about 80%; at least about 85%; at least about 90%; at least about 91%; at least about 92%; at least about 93%; at least about 94%; at least about 95%; at least about 96%; at least about 97%; at least about 98%; at least about 99%; and/or at least about 100% sequence identity to the minUbi1P element of SEQ ID NO:1. Characteristics of minUbi1P elements that may be useful in some embodiments of the invention may include, for example and without limitation, the aforementioned high conservation of nucleotide sequence; the presence of at least one TATA box; and/or the presence of at least one (*e.g.*, two) heat shock consensus element(s). In particular minUbi1P elements, more than one heat shock consensus elements may be overlapping within the minUbi1P sequence.

In some embodiments, the process of incorporating a minUbi1P element into a molecular context different from that of a native promoter to engineer a synthetic bidirectional promoter may comprise incorporating the minUbi1P element into a SCBV promoter nucleic acid, while reversing the orientation of the minUbi1P element with respect to the remaining sequence of the SCBV promoter. Thus, a synthetic SCBV bidirectional promoter may comprise a minUbi1P minimal core promoter element located 3′ of, and in reverse orientation with respect to, a SCBV promoter nucleotide sequence, such that it may be operably linked to a nucleotide sequence of

interest located 3′ of the SCBV promoter nucleotide sequence. For example, the minUbi1P element may be incorporated at the 3′ end of a SCBV promoter in reverse orientation.

A synthetic bidirectional SCBV promoter may also comprise one or more additional sequence elements in addition to a minUbi1P element and elements of a native SCBV promoter. In some embodiments, a synthetic bidirectional SCBV promoter may comprise a promoter URS; an exon (*e.g.*, a leader or signal peptide); an intron; a spacer sequence; and or combinations of one or more of any of the foregoing. For example and without limitation, a synthetic bidirectional SCBV promoter may comprise a URS sequence from a SCBV promoter; an intron from a ADH gene; an exon encoding a leader peptide from a Ubi1 gene; an intron from a Ubi1 gene; and combinations of these.

In some of those examples comprising a synthetic bidirectional SCBV promoter comprising a promoter URS, the URS may be selected to confer particular regulatory properties on the synthetic promoter. Known promoters vary widely in the type of control they exert on operably linked genes (*e.g.*, environmental responses, developmental cues, and spatial information), and a URS incorporated into a heterologous promoter typically maintains the type of control the URS exhibits with regard to its native promoter and operably linked gene(s). Langridge et al. (1989), *supra*. Examples of eukaryotic promoters that have been characterized and may contain a URS comprised within a synthetic bidirectional Ubi1 promoter according to some embodiments include, for example and without limitation: those promoters described in U.S. Patent Nos. 6,437,217 (maize RS81 promoter); 5,641,876 (rice actin promoter); 6,426,446 (maize RS324 promoter); 6,429,362 (maize PR-1 promoter); 6,232,526 (maize A3 promoter); 6,177,611 (constitutive maize promoters); 6,433,252 (maize L3 oleosin promoter); 6,429,357 (rice actin 2 promoter, and rice actin 2 intron); 5,837,848 (root-specific promoter); 6,294,714 (light-inducible promoters); 6,140,078 (salt-inducible promoters); 6,252,138 (pathogen-inducible promoters); 6,175,060 (phosphorous deficiency-inducible promoters); 6,388,170 (bidirectional promoters); 6,635,806 (gamma-coixin promoter); and U.S. Patent Application Serial No. 09/757,089 (maize chloroplast aldolase promoter).

Additional exemplary prokaryotic promoters include the nopaline synthase (NOS) promoter (Ebert et al. (1987) *Proc. Natl. Acad. Sci. USA* 84(16):5745-9); the octopine synthase (OCS) promoter (which is carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*); the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter (Lawton et al. (1987) *Plant Mol. Biol.* 9:315-24); the CaMV 35S promoter (Odell et al. (1985) *Nature* 313:810-2; the figwort mosaic virus 35S-promoter (Walker et al. (1987) *Proc. Natl. Acad. Sci. USA* 84(19):6624-8); the sucrose synthase promoter (Yang and Russell (1990) *Proc. Natl. Acad. Sci. USA* 87:4144-8); the R gene complex promoter (Chandler et al. (1989) *Plant Cell* 1:1175-83); CaMV35S (U.S. Patent Nos. 5,322,938, 5,352,605, 5,359,142, and 5,530,196); FMV35S (U.S. Patent Nos. 6,051,753, and 5,378,619); a PC1SV promoter (U.S. Patent No. 5,850,019); the SCP1 promoter (U.S. Patent No. 6,677,503); and AGRtu.nos promoters (GenBank Accession No. V00087; Depicker et al. (1982) *J. Mol. Appl. Genet.* 1:561-73; Bevan et al. (1983) *Nature* 304:184-7), and the like.

In some embodiments, a synthetic bidirectional SCBV promoter may further comprise an exon. For example, in examples it may be desirable to target or traffic a polypeptide encoded by a nucleotide sequence of interest operably linked to the promoter to a particular subcellular location and/or compartment. In these and other embodiments, a coding sequence (exon) may be incorporated into a nucleic acid molecule between the remaining synthetic bidirectional SCBV promoter sequence and a nucleotide sequence encoding a polypeptide. These elements may be arranged according to the discretion of the skilled practitioner such that the synthetic bidirectional SCBV promoter promotes the expression of a polypeptide (or one or both of two polypeptide-encoding sequences that are operably linked to the promoter) comprising the peptide encoded by the incorporated coding sequence in a functional relationship with the remainder of the polypeptide. In particular examples, an exon encoding a leader, transit, or signal peptide (*e.g.*, a Ubi1 leader peptide) may be incorporated.

Peptides that may be encoded by an exon incorporated into a synthetic bidirectional Ubi1 promoter include, for example and without limitation: a Ubiquitin (*e.g.*, Ubi1) leader peptide; a chloroplast transit peptide (CTP) (*e.g.*, the *A. thaliana* EPSPS CTP (Klee et al. (1987) *Mol. Gen. Genet.* 210:437-42), and the *Petunia hybrida*

EPSPS CTP (della-Cioppa et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:6873-7)), as exemplified for the chloroplast targeting of dicamba monooxygenase (DMO) in International PCT Publication No. WO 2008/105890.

Introns may also be incorporated in a synthetic bidirectional SCBV promoter in some embodiments of the invention, for example, between the remaining synthetic bidirectional SCBV promoter sequence and a nucleotide sequence of interest that is operably linked to the promoter. In some examples, an intron incorporated into a synthetic bidirectional SCBV promoter may be, without limitation, a 5′ UTR that functions as a translation leader sequence that is present in a fully processed mRNA upstream of the translation start sequence (such a translation leader sequence may affect processing of a primary transcript to mRNA, mRNA stability, and/or translation efficiency). Examples of translation leader sequences include maize and petunia heat shock protein leaders (U.S. Patent No. 5,362,865), plant virus coat protein leaders, plant rubisco leaders, and others. *See, e.g.*, Turner and Foster (1995) *Molecular Biotech.* 3(3):225-36. Non-limiting examples of 5′ UTRs include GmHsp (U.S. Patent No. 5,659,122); PhDnaK (U.S. Patent No. 5,362,865); AtAnt1; TEV (Carrington and Freed (1990) *J. Virol.* 64:1590-7); and AGRtunos (GenBank Accession No. V00087; and Bevan et al. (1983) *Nature* 304:184-7). In particular examples, a Ubi1 and/or ADH intron(s) may be incorporated in a synthetic bidirectional SCBV promoter.

Additional sequences that may optionally be incorporated into a synthetic bidirectional SCBV promoter include, for example and without limitation: 3′ non-translated sequences; 3′ transcription termination regions; and polyadenylation regions. These are genetic elements located downstream of a nucleotide sequence of interest (*e.g.*, a sequence of interest that is operably linked to a synthetic bidirectional SCBV promoter), and include polynucleotides that provide polyadenylation signal, and/or other regulatory signals capable of affecting transcription, mRNA processing, or gene expression. A polyadenylation signal may function in plants to cause the addition of polyadenylate nucleotides to the 3′ end of a mRNA precursor. The polyadenylation sequence may be derived from the natural gene, from a variety of plant genes, or from T-DNA genes. A non-limiting example of a 3′ transcription termination region is the nopaline synthase 3′ region (nos 3′; Fraley et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:4803-7). An example of the use of different 3′ nontranslated regions is provided in

Ingelbrecht et al. (1989), *Plant Cell* 1:671-80. Non-limiting examples of polyadenylation signals include one from a *Pisum sativum* RbcS2 gene (Ps.RbcS2-E9; Coruzzi et al. (1984) *EMBO J.* 3:1671-9) and AGRtu.nos (GenBank Accession No. E01312).

In some embodiments, a synthetic bidirectional SCBV promoter comprises one or more nucleotide sequence(s) that facilitate targeting of a nucleic acid comprising the promoter to a particular locus in the genome of a target organism. For example, one or more sequences may be included that are homologous to segments of genomic DNA sequence in the host (*e.g.*, rare or unique genomic DNA sequences). In some examples, these homologous sequences may guide recombination and integration of a nucleic acid comprising a synthetic bidirectional SCBV promoter at the site of the homologous DNA in the host genome. In particular examples, a synthetic bidirectional SCBV promoter comprises one or more nucleotide sequences that facilitate targeting of a nucleic acid comprising the promoter to a rare or unique location in a host genome utilizing engineered nuclease enzymes that recognize sequence at the rare or unique location and facilitate integration at that rare or unique location. Such a targeted integration system employing zinc-finger endonucleases as the nuclease enzyme is described in U.S. Patent Application No. 13/011,735.

Nucleic acids comprising a synthetic bidirectional SCBV promoter may be produced using any technique known in the art, including for example and without limitation: RCA; PCR amplification; RT-PCR amplification; OLA; and SNuPE. These and other equivalent techniques are well known to those of skill in the art, and are further described in detail in, for example and without limitation: Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory, 2001; and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, 1998.

Delivery and/or transformation: The present disclosure also provides methods for transforming a cell with a nucleic acid molecule comprising a synthetic bidirectional SCBV promoter. Any of the large number of techniques known in the art for introduction of nucleic acid molecules into plants may be used to transform a plant with a nucleic acid molecule comprising a synthetic bidirectional SCBV promoter according to some embodiments, for example, to introduce one or more synthetic

bidirectional SCBV promoters into the host plant genome, and/or to further introduce one or more nucleic acid molecule(s) of interest operably linked to the promoter.

Suitable methods for transformation of plants include any method by which DNA can be introduced into a cell, for example and without limitation: electroporation (*see, e.g.*, U.S. Patent 5,384,253); microprojectile bombardment (*see, e.g.*, U.S. Patents 5,015,580, 5,550,318, 5,538,880, 6,160,208, 6,399,861, and 6,403,865); *Agrobacterium*-mediated transformation (*see, e.g.*, U.S. Patents 5,635,055, 5,824,877, 5,591,616; 5,981,840, and 6,384,301); and protoplast transformation (*see, e.g.*, U.S. Patent 5,508,184). Through the application of techniques such as the foregoing, the cells of virtually any plant species may be stably transformed, and these cells may be developed into transgenic plants by techniques known to those of skill in the art. For example, techniques that may be particularly useful in the context of cotton transformation are described in U.S. Patents 5,846,797, 5,159,135, 5,004,863, and 6,624,344; techniques for transforming *Brassica* plants in particular are described, for example, in U.S. Patent 5,750,871; techniques for transforming soya are described, for example, in U.S. Patent 6,384,301; and techniques for transforming maize are described, for example, in U.S. Patents 7,060,876 and 5,591,616, and International PCT Publication WO 95/06722.

After effecting delivery of an exogenous nucleic acid to a recipient cell, the transformed cell is generally identified for further culturing and plant regeneration. In order to improve the ability to identify transformants, one may desire to employ a selectable or screenable marker gene with the transformation vector used to generate the transformant. In this case, the potentially transformed cell population can be assayed by exposing the cells to a selective agent or agents, or the cells can be screened for the desired marker gene trait.

Cells that survive the exposure to the selective agent, or cells that have been scored positive in a screening assay, may be cultured in media that supports regeneration of plants. In some embodiments, any suitable plant tissue culture media (*e.g.*, MS and N6 media) may be modified by including further substances, such as growth regulators. Tissue may be maintained on a basic media with growth regulators until sufficient tissue is available to begin plant regeneration efforts, or following repeated rounds of manual selection, until the morphology of the tissue is suitable for

regeneration (*e.g.*, at least 2 weeks), then transferred to media conducive to shoot formation. Cultures are transferred periodically until sufficient shoot formation has occurred. Once shoots are formed, they are transferred to media conducive to root formation. Once sufficient roots are formed, plants can be transferred to soil for further growth and maturity.

To confirm the presence of the desired nucleic acid molecule comprising a synthetic bidirectional SCBV promoter in the regenerating plants, a variety of assays may be performed. Such assays include, for example: molecular biological assays, such as Southern and Northern blotting and PCR; biochemical assays, such as detecting the presence of a protein product, *e.g.*, by immunological means (ELISA and/or Western blots) or by enzymatic function; plant part assays, such as leaf or root assays; and analysis of the phenotype of the whole regenerated plant.

Targeted integration events may be screened, for example, by PCR amplification using, *e.g.*, oligonucleotide primers specific for nucleic acid molecules of interest. PCR genotyping is understood to include, but not be limited to, polymerase-chain reaction (PCR) amplification of genomic DNA derived from isolated host plant callus tissue predicted to contain a nucleic acid molecule of interest integrated into the genome, followed by standard cloning and sequence analysis of PCR amplification products. Methods of PCR genotyping have been well described (*see, e.g.*, Rios et al. (2002), *Plant J.* 32:243-53), and may be applied to genomic DNA derived from any plant species or tissue type, including cell cultures. Combinations of oligonucleotide primers that bind to both target sequence and introduced sequence may be used sequentially or multiplexed in PCR amplification reactions. Oligonucleotide primers designed to anneal to the target site, introduced nucleic acid sequences, and/or combinations of the two may be produced. Thus, PCR genotyping strategies may include, for example and without limitation: amplification of specific sequences in the plant genome; amplification of multiple specific sequences in the plant genome; amplification of non-specific sequences in the plant genome; and combinations of any of the foregoing. One skilled in the art may devise additional combinations of primers and amplification reactions to interrogate the genome. For example, a set of forward and reverse oligonucleotide primers may be designed to anneal to nucleic acid

sequence(s) specific for the target outside the boundaries of the introduced nucleic acid sequence.

Forward and reverse oligonucleotide primers may be designed to anneal specifically to an introduced nucleic acid molecule, for example, at a sequence corresponding to a coding region within a nucleotide sequence of interest comprised therein, or other parts of the nucleic acid molecule. These primers may be used in conjunction with the primers described above. Oligonucleotide primers may be synthesized according to a desired sequence, and are commercially available (*e.g.*, from Integrated DNA Technologies, Inc., Coralville, IA). Amplification may be followed by cloning and sequencing, or by direct sequence analysis of amplification products. One skilled in the art might envision alternative methods for analysis of amplification products generated during PCR genotyping. In one embodiment, oligonucleotide primers specific for the gene target are employed in PCR amplifications.

Some embodiments of the present invention also provide cells comprising a synthetic bidirectional SCBV promoter, for example, as may be present in a nucleic acid construct. In particular examples, a synthetic bidirectional SCBV promoter according to some embodiments may be utilized as a regulatory sequence to regulate the expression of transgenes in plant cells and plants. In some such examples, the use of a synthetic bidirectional SCBV promoter operably linked to a nucleotide sequence of interest (*e.g.*, a transgene) may reduce the number of homologous promoters needed to regulate expression of a given number of nucleotide sequences of interest, and/or reduce the size of the nucleic acid construct(s) required to introduce a given number of nucleotide sequences of interest. Furthermore, use of a synthetic bidirectional SCBV promoter may allow co-expression of two operably linked nucleotide sequence of interest under the same conditions (*i.e.*, the conditions under which the SCBV promoter is active). Such examples may be particularly useful, *e.g.*, when the two operably linked nucleotide sequences of interest each contribute to a single trait in a transgenic host comprising the nucleotide sequences of interest, and co-expression of the nucleotide sequences of interest advantageously impacts expression of the trait in the transgenic host.

In some embodiments, a transgenic plant comprising one or more synthetic bidirectional SCBV promoter(s) and/or nucleotide sequence(s) of interest may have one or more desirable traits conferred (*e.g.*, introduced, enhanced, or contributed to) by expression of the nucleotide sequence(s) of interest in the plant. Such traits may include, for example and without limitation: resistance to insects, other pests, and disease-causing agents; tolerances to herbicides; enhanced stability, yield, or shelf-life; environmental tolerances; pharmaceutical production; industrial product production; and nutritional enhancements. In some examples, a desirable trait may be conferred by transformation of a plant with a nucleic acid molecule comprising a synthetic bidirectional SCBV promoter operably linked to a nucleotide sequence of interest. In some examples, a desirable trait may be conferred to a plant produced as a progeny plant *via* breeding, which trait may be conferred by one or more nucleotide sequences of interest operably linked to a synthetic bidirectional SCBV promoter that is/are passed to the plant from a parent plant comprising a nucleotide sequence of interest operably linked to a synthetic bidirectional SCBV promoter.

A transgenic plant according to some embodiments may be any plant capable of being transformed with a nucleic acid molecule of the invention, or of being bred with a plant transformed with a nucleic acid molecule of the invention. Accordingly, the plant may be a dicot or monocot. Non-limiting examples of dicotyledonous plants for use in some examples include: alfalfa; beans; broccoli; cabbage; canola, carrot; cauliflower; celery; Chinese cabbage; cotton; cucumber; eggplant; lettuce; melon; pea; pepper; peanut; potato; pumpkin; radish; rapeseed; spinach; soybean; squash; sugarbeet; sunflower; tobacco; tomato; and watermelon. Non-limiting examples of monocotyledonous plants for use in some examples include: corn; onion; rice; sorghum; wheat; rye; millet; sugarcane; oat; triticale; switchgrass; and turfgrass.

In some embodiments, a transgenic plant may be used or cultivated in any manner, wherein presence a synthetic bidirectional SCBV promoter and/or operably linked nucleotide sequence of interest is desirable. Accordingly, such transgenic plants may be engineered to, *inter alia*, have one or more desired traits, by being transformed with nucleic acid molecules according to the invention, and may be cropped and/or cultivated by any method known to those of skill in the art.

While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications and changes may be made without departing from the invention.

The following examples are provided to illustrate certain particular features and/or embodiments. The examples should not be construed to limit the disclosure to the particular features or embodiments exemplified.

EXAMPLES

EXAMPLE 1: Transformation and Expression

Transformation of *Agrobacterium tumefaciens*: The pDAB108706 binary vector is transformed into *Agrobacterium tumefaciens* strain DAt13192 ternary (U.S. Prov. Pat. No. 61/368965). Bacterial colonies are isolated and binary plasmid DNA is isolated and confirmed *via* restriction enzyme digestion.

Corn Transformation: Ear Sterilization and Embryo Isolation. To obtain maize immature embryos, plants of *Zea mays* (c.v. B104) are grown in the greenhouse and self or sib-pollinated to produce ears. The ears are harvested approximately 9-12 days post-pollination. On the day of the experiment, ears are surface-sterilized by immersion in a 20% solution of household bleach, which contains 5% sodium hypochlorite, and shaken for 20-30 minutes, followed by three rinses in sterile water. After sterilization, immature zygotic embryos (1.5–2.2 mm) are aseptically dissected from each ear and randomly distributed into micro-centrifuge tubes containing liquid infection media (LS Basal Medium, 4.43 gm/L; N6 Vitamin Solution [1000X], 1.00 mL/L; L-proline, 700.0 mg/L; sucrose, 68.5 gm/L; glucose, 36.0 gm/L; 2,4-D, 1.50 mg/L. For a given set of experiments, pooled embryos from 2-3 ears are used for each treatment.

Agrobacterium Culture Initiation: Glycerol stocks of *Agrobacterium* containing the binary vectors described above are streaked on AB minimal medium plates containing appropriate antibiotics and are grown at 20°C for 3-4 days. A single colony is picked and streaked onto YEP plates containing the same antibiotics and was incubated at 28°C for 1-2 days.

Agrobacterium Culture and Co-cultivation: On the day of the experiment, *Agrobacterium* colonies are taken from the YEP plate, suspended in 10 mL of infection

medium in a 50 mL disposable tube, and the cell density is adjusted to OD600 = 0.2-0.4 nm using a spectrophotometer. The *Agrobacterium* cultures are placed on a rotary shaker at 100 rpm, room temperature, while embryo dissection is performed. Immature zygotic embryos between 1.5-2.2 mm in size are isolated from the sterilized maize kernels and placed in 1 mL of the infection medium and washed once in the same medium. The *Agrobacterium* suspension (2 mL) is added to each tube and the tubes are inverted for about 20 times then shaken for 10-15 minutes. The embryos are transferred onto co-cultivation media (MS Salts, 4.33 gm/L; L-proline, 700.0 mg/L; myo-inositol, 100.0 mg/L; casein enzymatic hydrolysate 100.0 mg/L; Dicamba- 3.30 mg/L; sucrose, 30.0 gm/L; Gelzan™, 3.00 gm/L; modified MS-Vitamin [1000X], 1.00 ml/L, $AgNo_3$, 15.0 mg/L; Acetosyringone, 100 μM), oriented with the scutellum facing up, and incubated for 3-4 days in the light at 25°C.

GUS and YFP/PhiYFP Transient expression: Transient YFP/PhiYFP and GUS expression can be observed in transformed embryos and after 3 days of co-cultivation with *Agrobacterium*. The embryos are observed under a stereomicroscope (Leica Microsystems, Buffalo Grove, IL) using YFP filter and 500 nm light source. Embryos showing YFP/PhiYFP expression are selected for GUS histochemical assay. GUS staining solution is prepared as described in Maniatis et al. (1989) and embryos are incubated in 1 mL solution for 24 hours at 37°C. The embryos are observed for GUS transient expression under the microscope.

Callus Selection and Regeneration of Putative Events: Following the co-cultivation period, embryos are transferred to resting media (MS salts, 4.33 gm/L; L-proline, 700.0 mg/L; myo-inositol, 100.0 mg/L; MES [(2-(n-morpholino)-ethanesulfonic acid), free acid] 500.0 mg/L; casein enzymatic hydrolysate 100.0 mg/L; Dicamba, 3.30 mg/L; sucrose, 30.0 gm/L; Gelzan 2.30 gm/L; modified MS-Vitamin [1000X], 1.00 ml/L; $AgNo_3$, 15.0 mg/L; Carbenicillin, 250.0 mg/L) without selective agent and incubated in the light for 7 days at 28°C. Embryos are transferred onto Selection 1 media (MS salts, 4.33 gm/L; L-proline, 700.0 mg/L; myo-inositol , 100.0 mg/L; MES [(2-(n-morpholino)-ethanesulfonic acid), free acid] 500.0 mg/L; casein enzymatic hydrolysate 100.0 mg/L; Dicamba, 3.30 mg/L; sucrose, 30.0 gm/L; Gelzan™ 2.30 gm/L; modified MS-Vitamin [1000X], 1.00 ml/L; $AgNo_3$, 15.0 mg/L; Carbenicillin, 250.0 mg/L) containing 100

nM haloxyfop and incubated in 24 hours light with light intensity of 50 μmol $m^{-2}s^{-1}$ for 7 days at 28°C.

Embryos with proliferating embryogenic calli are transferred onto Selection 2 media (MS salts, 4.33 gm/L; myo-inositol, 100.0 mg/L; L-proline, 700.0 mg/L; MES [(2-(n-morpholino)-ethanesulfonic acid), free acid] 500.0 mg/L; casein enzymatic hydrolysate 100.0 mg/L; Dicamba, 3.30 mg/L; sucrose, 30.0 gm/L; Gelzan™ 2.30 gm/L; modified MS-Vitamin [1000X], 1.00 ml/L; $AgNo_3$, 15.0 mg/L; Carbenicillin, 250.0 mg/L) containing 500 nM haloxyfop and are incubated in 24 hours light with light intensity of 50 μmol $m^{-2}s^{-1}$ for another 14 days at 28°C. This selection step allows transgenic callus to further proliferate and differentiate. The callus selection period lasts for three weeks. Proliferating, embryogenic calli are transferred onto Regeneration 1 media (MS salts, 4.33 gm/L; myo-inositol, 100.0 mg/L; L-proline, 350.0 mg/L; MES [(2-(n-morpholino)-ethanesulfonic acid), free acid] 250.0 mg/L; casein enzymatic hydrolysate 50.0 mg/L; NAA 0.500 mg/L; ABA 2.50 mg/L; BA 1.00 mg/L; sucrose, 45.0 gm/L; Gelzan™ 2.50 gm/L; modified MS-Vitamin [1000X], 1.00 ml/L; $AgNo_3$, 1.00 mg/L; Carbenicillin, 250.0 mg/L) containing 500 nM haloxyfop and cultured in 24 hours light with light intensity of 50 μmol $m^{-2}s^{-1}$ for 7 days at 28°C. Embryogenic calli with shoot-like buds are transferred onto Regeneration 2 media (MS salts, 4.33 gm/L; modified MS-Vitamin [1000X], 1.00 ml/L; myo-inositol, 100.0 mg/L; sucrose, 60.0 gm/L; Gellan Gum G434™ 3.00 gm/L; Carbenicillin, 250.0 mg/L) containing 500 nM haloxyfop. The cultures are incubated under 24 hours light with light intensity of 50 μmol $m^{-2}s^{-1}$ for 7-10 days at 28°C. Small shoots with primary roots are transferred to shoot elongation and rooting media (MS salts, 4.33 gm/L; modified MS-Vitamin [1000X], 1.00 ml/L; myo-inositol, 100.0 mg/L; sucrose, 60.0 gm/L; Gellan Gum G434™ 3.00 gm/L; Carbenicillin, 250.0 mg/L) in MAGENTA™ boxes (Sigma-Aldrich, St. Louis, MO), and are incubated under 16/8 hours light/dark for 7 days at 28°C. Putative transgenic plantlets are analyzed for transgene copy number and transferred to the greenhouse.

EXAMPLE 2: Construction of a Synthetic Bidirectional SCBV Promoter and pDAB108708 Vector

An exemplary schematic drawing of the maize Ubiquitin-1 promoter (Ubi1) is shown in FIG. 1. An Ubi1 promoter is cloned from maize. A plasmid which contained the promoter is PCR amplified using a high-fidelity PCR amplification system. An approximately 200 nt region of the maize Ubi1 promoter is identified as a *Zea mays* Ubi1 minimal core promoter (minUbi1P) (SEQ ID NO: 1). The minUbi1P of SEQ ID NO: 1 is then added to a polynucleotide comprising a *Zea mays* Ubiquitin-1 exon (ZmUbi1 exon) and a *Zea mays* Ubiquitin-1 intron (ZmUbi1 intron) using cloning methods commonly known in the art to produce the polynucleotide of SEQ ID NO: 2. The resulting polynucleotide was then cloned upstream in reverse orientation of a nucleic acid comprising the maize Ubi1 promoter (including the Ubi1 URS) to produce the synthetic bidirectional Ubi1 promoter of SEQ ID NO: 3.

Reporter gene coding sequences are cloned downstream of each end of the synthetic bidirectional Ubi1 promoter. A yellow fluorescence protein (YFP) coding sequence is inserted downstream of the polynucleotide fragment which contained the minUbi1P, ZmUbi1 exon, and ZmUbi1 intron promoter elements. In addition, a downstream leader sequence containing a 3-frame stop polynucleotide sequence and the maize consensus polynucleotide sequence is added to the minUbi1P, ZmUbi1, exon and ZmUbi1 intron promoter elements fragment. A *uidA* (*GUS*) coding sequence was also inserted downstream of the synthetic bidirectional Ubi1 promoter in reverse orientation with respect to the *YFP* sequence to produce the nucleic acid of SEQ ID NO: 4. The resulting polynucleotide comprising the synthetic bidirectional Ubi1 promoter operably linked to the *YFP* and *GUS* genes was cloned into plasmid pDAB105801. FIG. 4 shows the orientation of the YFP and GUS expression cassette in relation to the synthetic bidirectional Ubi1 promoter in plasmid pDAB105801.

The native Ubi1 promoter sequence is removed from the bidirectional Ubi1 promoter of plasmid pDAB105801 and replaced with a PCR amplified fragment containing the SCBV promoter and ADH intron (SEQ ID NO: 6). The resulting exemplary synthetic bidirectional SCBV promoter is set forth as SEQ ID NO: 5 (also see FIG. 5). The addition of this SCBV promoter resulted in the completion of vector pDAB105806 (FIG. 6). This vector contained the *YFP* and *GUS* gene expression

cassettes which were driven by the SCBV bi-directional promoter (SEQ ID NO: 7; also see FIG. 7).

A binary vector which contained the *GUS* and *YFP* gene expression cassettes from plasmid pDAB105806 is completed *via* a GATEWAY L-R CLONASE reaction (Invitrogen, Carlsbad, CA). The resulting vector, pDAB108708, contained the *GUS*, *YFP*, and *AAD-1* gene expression cassettes within the T-strand region (see FIG. 9).

EXAMPLE 3: Expression of Genes Operably linked to a Synthetic Bidirectional SCBV Promoter

Representative examples of YFP and GUS transient expression in *Zea mays* embryos transformed with pDAB108708 can be imaged. Both sides of the bidirectional SCBV promoter can drive robust expression of the operably linked *YFP* and *GUS* coding sequences. The YFP expression levels are comparable to the GUS expression levels. These observations confirm that both sides of the bidirectional SCBV promoter are biologically functional. Moreover, the minUbi1P element of the synthetic bidirectional SCBV promoter can express YFP at similar expression levels as compared to *Zea mays* callus transformed with a binary plasmid (pDAB101556) that contained only a unidirectional ZmUbi1 promoter driving the *YFP* coding sequence. Expression of YFP or GUS is not detected in negative control immature embryos which are not transformed with a binary construct, and did not contain the *YFP* or *GUS* coding sequences.

EXAMPLE 4: Stable Expression of Genes Operably linked to a Synthetic Bidirectional SCBV Promoter

Images of *Zea mays* callus cells that are stably transformed with the pDAB108708 binary vector, which contains a *YFP* coding sequence, can be observed. These cells are obtained from *Z. mays* embryos that have been propagating on Selection 2 medium. The microscopy conditions and protocol that are used to generate the images of the embryos. The bidirectional SCBV promoter can drive robust expression of the *YFP* coding sequences. These results confirm that the Min-Ubi1P minimal promoter element of the bidirectional SCBV promoter is capable of expressing a reporter gene in stably transformed *Z. mays* callus cells. The

levels of expression of the YFP protein are similar as compared to YFP expression in *Z. mays* callus transformed with a control binary vector that contained the unidirectional ZmUbi1 promoter driving the *YFP* coding sequence (pDAB101556). Expression of YFP is not detected in the negative control callus that was not transformed with a binary construct and did not contain a *YFP* or *GUS* coding sequence.

EXAMPLE 5: Transgene Copy Number Estimation Using Real Time TaqMan™ PCR

Zea mays embryos are transformed with a binary vector containing a bidirectional SCBV promoter, pDAB108708, and other plants are transformed with a control binary vector, pDAB101556. The presence of *YFP* transgenes within the genome of both set of *Z. mays* plants is confirmed *via* a hydrolysis probe assay. Stably transformed transgenic *Z. mays* plantlets that developed from the callus are obtained and analyzed to identify events that contain a low copy number (1-2 copies) of full-length T-strand inserts from the pDAB108708 binary vector and pDAB101556 control binary vector. Identified plantlets are advanced to the green house and grown.

The Roche Light Cycler480™ system is used to determine the transgene copy number for events that are transformed with the pDAB108708 binary vector. The method utilizes a biplex TAQMAN® reaction that employs oligonucleotides specific to the *YFP* gene and to the endogenous *Z. mays* reference gene, invertase (Genbank Accession No: U16123.1), in a single assay. Copy number and zygosity are determined by measuring the intensity of *YFP*-specific fluorescence, relative to the *invertase*-specific fluorescence, as compared to known copy number standards.

In *Z. mays* transformed with the pDAB108708 binary vector, a *YFP* gene-specific DNA fragment is amplified with one TAQMAN® primer/probe set containing a probe labeled with FAM fluorescent dye, and invertase is amplified with a second TAQMAN® primer/probe set containing a probe labeled with HEX fluorescence (Table 2). The PCR reaction mixture is prepared as set forth in Table 3, and the gene-specific DNA fragments are amplified according to the conditions set forth in Table 4. Copy number and zygosity of the samples are determined by

measuring the relative intensity of fluorescence specific for the reporter gene, *YFP*, to fluorescence specific for the reference gene, invertase, as compared to known copy number standards.

Table 2. Forward and reverse nucleotide primer and fluorescent probes (synthesized by Integrated DNA Technologies, Coralville, IA)

Primer Name	**SEQ ID NO:**	**Primer Sequence**
YFP Forward Primer	SEQ ID NO: 8	GATGCCTCAGTGGGAAAGG
YFP Reverse Primer	SEQ ID NO: 9	CCATAGGTGAGAGTGGTGACAA
YFP Probe	SEQ ID NO: 41	ROCHE UPL Probe #125 CTTGGAGC Cat # 04693604001 (Roche, Indianapolis, IN)
Invertase Forward Primer	SEQ ID NO: 10	TGGCGGACGACGACTTGT
Invertase Reverse Primer	SEQ ID NO: 11	AAAGTTTGGAGGCTGCCGT
Invertase Probe	SEQ ID NO: 12	5'HEX/CGAGCAGACCGCCGTGTACTT CTACC /3BHQ_1/3'
AAD1 Forward Primer	SEQ ID NO: 13	TGTTCGGTTCCCTCTACCAA
AAD1 Reverse Primer	SEQ ID NO: 14	CAACATCCATCACCTTGACTGA
AAD1 Probe	SEQ ID NO: 15	CACAGAACCGTCGCTTCAGCAACA

Standards are created by diluting the vector, pDAB108708, into *Z. mays* B104 genomic DNA (gDNA) to obtain standards with a known relationship of pDAB108706:gDNA. For example, samples having one; two; and four cop(ies) of vector DNA per one copy of the *Z. mays* B104 gDNA are prepared. One and two copy dilutions of the pDAB108706 mixed with the *Z. mays* B104 gDNA standard are validated against a control *Z. mays* event that is known to be hemizygous, and a control *Z. mays* event that is known to be homozygous (*Z. mays* event 278; *see* PCT International Patent Publication No. WO 2011/022469 A2). A TAQMAN® biplex assay which utilizes oligonucleotides specific to the *AAD1* gene and oligonucleotides specific to the endogenous *Z. mays* reference gene, invertase, is performed by amplifying and detecting a gene-specific DNA fragment for *AAD1* with one TAQMAN® primer/probe set containing a probe labeled with FAM fluorescent dye, and by amplifying and detecting a gene-specific DNA fragment for invertase with a second TAQMAN® primer/probe set containing a probe labeled with HEX fluorescence (Table 2). The *AAD1* TAQMAN® reaction mixture is

prepared as set forth in Table 3 and the specific fragments are amplified according to the conditions set forth in Table 4.

Table 3. TAQMAN® PCR reaction mixture.

Number of Reactions	µl each	Final Concentration
H_2O	0.5 µL	-
PVP (10%)	0.1 µL	0.1%
ROCHE 2X Master Mix	5 µL	1X
YFP Forward Primer (10 µM)	0.4 µL	0.4 µM
YFP Reverse Primer (10 µM)	0.4 µL	0.4 µM
YFP Probe UPL#125 (5 µM)	0.4 µL	0.2 µM
Invertase Forward Primer (10 µM)	0.4 µL	0.4 µM
Invertase Reverse Primer (10 µM)	0.4 µL	0.4 µM
Invertase Probe (5µM)	0.4 µL	0.2 µM
DNA Template	2.0 µL	-
Total reaction volume	10 µL	-

The level of fluorescence that was generated for each reaction was analyzed using the Roche LightCycler 480™ Thermocycler according to the manufacturer's directions. The FAM fluorescent moiety was excited at an optical density of 465/510 nm, and the HEX fluorescent moiety was excited at an optical density of 533/580 nm. The copy number was determined by comparison of Target/Reference values for unknown samples (output by the LightCycler 480™) to Target/Reference values of four known copy number standards (Null, 1-Copy (hemi), 2-Copy (homo) and 4-Copy).

Table 4. Thermocycler conditions for PCR amplification.

PCR Steps	Temp (°C)	Time	No. of cycles
Step-1	95	10 minutes	1
Step-2	95	10 seconds	40
	59	35 seconds	
	72	1 second	
Step-3	40	10 seconds	1

Results from the transgene copy number analysis of transgenic plants obtained *via* transformation with a bidirectional ZmUbi1 promoter construct (pDAB108706), and of transgenic plants obtained *via* transformation with a control unidirectional ZmUbi1 promoter YFP construct (pDAB101556) is shown in Table 5. Only plants with 1-2 copies of the *yfp* transgene are transferred to the greenhouse for further expression analyses.

Table 5. Transgene copy number estimation of the transgenic plants obtained from bidirectional promoter and control constructs.

Construct	Number of Embryos Transformed	Number of Positive Events	1-2 Copies of *YFP*
pDAB101566	100	31	13
pDAB108708	113	26	16

EXAMPLE 6: Whole Plant Stable Expression of Genes Operably linked to a Synthetic Bidirectional SCBV Promoter.

Whole plants that contain a low copy number of the binary plasmid pDAB108708, and plants that contain a low copy number of the control binary plasmid pDAB101556, are grown in a greenhouse. These plants are analyzed using microscopy, where images can be observed showing *YFP* expression in T_0 *Z. mays* plants that are stably transformed with an exemplary nucleic acid construct comprising a *YFP* expression cassette operably linked to a synthetic SCBV bidirectional promoter (pDAB108708). Representative examples of stable expression of YFP in leaf and root tissue of transgenic T_0 maize plants obtained from *Z. mays* embryos transformed with pDAB108708 show good *YFP* expression. The bidirectional SCBV promoter can drive robust expression of the *YFP* coding sequences both in leaf tissues and root tissues. The microscopy analysis also confirms that the Min-UbiP1 minimal promoter element in the bidirectional SCBV promoter can drive *YFP* expression at similar expression levels as compared to *Z. mays* plants transformed with a control binary plasmid (pDAB101556) that contains a unidirectional ZmUbi1 promoter driving expression of the *YFP* coding sequence. The control plants show stable YFP expression in leaf tissues and root tissues.

EXAMPLE 7: Western Blot Analysis of Genes Operably linked to a Synthetic Bidirectional SCBV Promoter

Total Soluble Protein: Transformed T_0 maize plants are sampled at the V6 developmental stage. A total of four leaf punches from the youngest unfolded leaf are sampled into a matrix tube and placed into a matrix box. As a negative control, four leaf punches of two untransformed B104 maize plants at the V6 developmental stage are sampled into a matrix tube. A steel bead is placed into the matrix tubes with the samples, and then 400 µL PBST is added to each tube. The tubes are capped, and protein is extracted *via* bead beating at 1500 rpm for 5 minutes in a Kleco™ tissue grinder. Debris is pelleted *via* centrifugation.

A 5 µL sample from each tube was diluted to 25 µL with PBST in a 96-well microtiter plate. These samples were analyzed for total soluble protein using a BCA protein assay kit (Thermo Scientific Pierce, Rockford, IL) according to the manufacturer's directions. Bovine serum albumin (BSA) standards provided in the kit were analyzed in duplicate, and the average of the values was used to generate a standard curve that was subsequently used to calculate total soluble protein for each sample. The total soluble protein for each sample was then normalized to mg/µL.

Table 6. Western blot protocol.

Step	Condition	Time
First Wash	PBST	5 min.
Primary Hybridization	2 µg/mL rabbit anti-PhiYFP (Axxora, San Diego, CA) in StartingBlock™ T20 (Thermo Fisher Scientific Inc., Waltham, MA)	60 min.
Rinse	PBST	3 x 5 min.
Secondary Hybridization	horse radish peroxidase (HRP)-conjugated goat anti-rabbit IgG	30 min.
Second Wash	PBST	3 x 5 min.
Rinse	PBS	3 x 2 min

YFP/PhiYFP Western Blot Analysis: In the 96-well microtiter plate, each 5 µL sample of extracted protein is diluted with 5 µL 2x Laemmli Buffer + 2-β-mercaptoethanol. Control samples of purified YFP/PhiYFP in HEPES buffer

(50 mM HEPES, 200 mM KCl, 10% glycerol) is purchased from Axxora (San Diego, CA). The samples are prepared in the same plate by diluting 1:1 with Laemmli buffer to produce a standard curve of the following concentrations: 0.5 ng/μL, 0.25 ng/μL, and 0.125 ng/μL. Samples are heated in a Thermocycler at 95°C for 30 minutes, and then cooled to 4°C. A Bio-Rad Criterion gel™ is then assembled using MES/SDS buffer. The samples are allowed to warm to room temperature, and 10 μL of sample are loaded into each well of two gels. In addition, samples of purified YFP/PhiYFP used for a standard curve, and protein ladder marker, are loaded into wells of the gel. The gels are electrophoretically run at 150 V and 150 mA for 90 min. After the run, the gel casings are opened and the proteins are transferred to a nitrocellulose membrane using the iBlot System™ (Invitrogen). Protein is transferred from the gel to the membrane by running a current of 20 V for 10 minutes. The nitrocellulose membrane is removed and placed in StartingBlock T20™ blocking buffer overnight at 4°C. The blocking buffer is then discarded, and the membrane is processed using the protocol set forth in Table 6.

Antibody binding was detected using the Amersham ECL™ plus chemiluminescent detection system following the manufacturer's directions. Film was exposed at 10 minutes and 30 minutes. The 10 minute exposed film was used to quantify protein, and the 30 minute overexposure film was used to confirm the absence of protein in B104 and other control samples. The membrane was taped to the back of the exposed film, and protein was quantified *via* pixel density analysis. The pixel density of the purified protein standards was first used to generate a standard curve that was used to quantify protein in the samples. Though membrane showed bands for a PhiYFP monomer and dimer even in the purified standard, only the PhiYFP monomer was used to quantify protein expression. Values for the protein were then normalized to ng/μL. The ratio of normalized total soluble protein (TSP) to PhiYFP was calculated to the units of ng YFP/mg TSP, or alternatively, parts per million (ppm).

GUS Western Blot Analysis: Expression of GUS protein is quantified in a similar manner to PhiYFP, with the following exception: a 10 μL sample of extract is diluted 1:1 with 2x Laemmli + 2-β-mercaptoethanol, denatured at 95°C for 30

minutes, and then 15 µL is loaded into the gel. Processed membranes with film (1 minute exposure) are overlayed with the membrane for pixel density analysis.

Results of a Western blot analysis of 12 transgenic T_0 maize plants obtained from *Z. mays* embryos transformed with the binary vector, pDAB108708, are shown in FIG. 16. The bidirectional SCBV promoter shows robust expression of the *YFP* and *GUS* coding sequences from leaf tissue. These observations confirm that the Min-UbiP1 minimal promoter element isolated from a *Zea mays* Ubiquitin1 Promoter and fused to the SCBV promoter can express YFP at similar expression levels as compared to *Z. mays* callus transformed with a binary plasmid containing a unidirectional ZmUbi1 promoter driving the *YFP* coding sequence (pDAB101556; see FIG. 17).

EXAMPLE 8: Construct of a Four-gene Cassette Stack

A plasmid pDAB105806 construct is used as the starting plasmid to generate a four-gene cassette stack (AAD1-2A-PhiYFP and Cry34(8V6)-2A-Cry35) driven by a single SCBV bi-directional promoter. A representative map of plasmid pDAB105806 is shown in FIG. 6, which contains a SCBV bi-directional Promoter.

The AAD1-2A-PhiYFP fragment derived from plasmid pDAB105841 (FIG. 22) is cloned into the PstI and SacI cut vector backbone of the plasmid pDAB105806 using cloning methods commonly known in the art. This resulted in the intermediate plasmid pDAB105847 (FIG. 22). A NotI/XbaI digested Cry34(8V6)-2A-Cry35 fragment obtained from the plasmid pDAB105840 is cloned between NotI/SpeI sites of plasmid pDAB105847 to construct plasmid pDAB105849 (FIG. 23). The plasmid pDAB105849 contains Cry34(8V6)-2A-Cry35 and AAD1-2A-PhiYFP gene cassettes on each side of the SCBV bidirectional promoter.

A binary vector containing the SCBV bidirectional promoter, and gene expression cassettes Cry34(8V6)-2A-Cry35 and AAD1-2A-PhiYFP from plasmid pDAB105849 is generated via a GATEWAY L-R CLONASE reaction (Invitrogen, Carlsbad, CA) into a destination plasmid pDAB101917 (FIG. 24). The resulting vector, pDAB108719, contains the Cry34(8V6)-2A-Cry35, AAD1-2A-PhiYFP, and PAT gene expression cassettes within the T-DNA borders (FIG.24).

EXAMPLE 9: Construct of a Second Four-gene Cassette Stack

A PhiYFP-2A-AAD1 fragment derived from plasmid pDAB105844 (FIG. 25) is cloned into the PstI and SacI cut vector backbone of the plasmid pDAB105806 using cloning methods commonly known in the art. This resulted in the intermediate plasmid pDAB105848 (FIG. 25). A NotI/XbaI digested Cry34(8V6)-2A-Cry35 fragment obtained from the plasmid pDAB105840 is cloned between NotI/SpeI sites of plasmid pDAB105848 to construct plasmid pDAB105865 (FIG. 26). The plasmid pDAB105865 contains Cry34(8V6)-2A-Cry35 and PhiYFP-2A-AAD1 gene cassettes on each side of the SCBV bidirectional promoter.

A binary vector containing the SCBV bidirectional promoter, and gene cassettes Cry34(8V6)-2A-Cry35 and PhiYFP-2A-AAD1 from plasmid pDAB105865 is generated via a GATEWAY L-R CLONASE reaction (Invitrogen, Carlsbad, CA) into a destination plasmid pDAB101917 (FIG. 24). The resulting vector, pDAB108720, contains the Cry34(8V6)-2A-Cry35, PhiYFP-2A-AAD1, and PAT gene expression cassettes within the T-DNA borders (FIG. 26).

EXAMPLE 10: Transformation of *Agrobacterium tumefaciens* Strain DAt13192

The pDAB108719 and pDAB108720 binary vectors are transformed into *Agrobacterium tumefaciens* ternary strain DAt13192 (see U.S. Prov. Pat. App. No. 61/368965). Bacterial colonies are isolated and binary plasmid DNA is extracted and verified via restriction enzyme digestions.

EXAMPLE 11: Transformation into Maize

Ear Sterilization and Embryo Isolation: To obtain maize immature embryos, plants of *Zea mays* (c.v. B104) are grown in the greenhouse and self or sib-pollinated to produce ears. The ears are harvested approximately 9-12 days post-pollination. On the day of the experiment, ears are surface-sterilized by immersion in a 20% solution of household bleach, which contains 5% sodium hypochlorite, and shaken for 20-30 minutes, followed by three rinses in sterile water. After sterilization, immature zygotic

embryos (1.5–2.2 mm) are aseptically dissected from each ear and randomly distributed into micro-centrifuge tubes containing liquid infection media (LS Basal Medium, 4.43 g/L; N6 Vitamin Solution [1000X], 1.00 mL/L; L-proline, 700.0 mg/L; sucrose, 68.5 g/L; glucose, 36.0 g/L; 2,4-D, 1.50 mg/L. For a given set of experiments, pooled embryos from 2-3 ears are used for each treatment.

Agrobacterium Culture Initiation: Glycerol stocks of *Agrobacterium* strains containing the binary vectors described above are streaked on AB minimal medium plates containing appropriate antibiotics and are grown at 20°C for 3-4 days. A single colony is picked and streaked onto YEP plates containing the same antibiotics and is incubated at 28°C for 1-2 days.

Agrobacterium Culture and Co-cultivation: On the day of the experiment, *Agrobacterium* colonies are picked from the YEP plate, suspended in 10 mL of infection medium in a 50 mL disposable tube, and the cell density is adjusted to OD_{600} = 0.2-0.4 nm using a spectrophotometer. The *Agrobacterium* cultures are placed on a rotary shaker at 115 rpm, room temperature, while embryo dissection is performed. Immature zygotic embryos between 1.5-2.2 mm in size are isolated from the sterilized maize kernels and placed in 1 mL of the infection medium and washed once in the same medium. The *Agrobacterium* suspension (2 mL) is added to each tube and the tubes were inverted for about 20 times then shaken for 10-15 minutes. The embryos are transferred onto co-cultivation media (MS Salts, 4.33 g/L; L-proline, 700.0 mg/L; myo-inositol, 100.0 mg/L; casein enzymatic hydrolysate 100.0 mg/L; Dicamba 3.30 mg/L; sucrose, 30.0 g/L; Gelzan™, 3.00 g/L; modified MS-Vitamin [1000X], 1.00 ml/L; $AgNo_3$, 15.0 mg/L; Acetosyringone, 100.0 µM), oriented with the scutellum facing up, and incubated for 3-4 days in the light at 25°C.

YFP/PhiYFP Transient expression: Transient YFP/PhiYFP expression can be observed in transformed embryos after 3 days of co-cultivation with *Agrobacterium*. The embryos are observed under a stereomicroscope (Leica Microsystems, Buffalo Grove, IL) using YFP filter and 500 nm light source.

Callus Selection and Regeneration of Putative Events: Following the co-cultivation period, embryos are transferred to resting media (MS salts, 4.33 g/L; L-proline, 700.0 mg/L; myo-inositol, 100.0 mg/L; MES [(2-(n-morpholino)-ethanesulfonic acid), free acid], 500.0 mg/L; casein enzymatic

hydrolysate, 100.0 mg/L; Dicamba, 3.30 mg/L; sucrose, 30.0 g/L; Gelzan™, 2.30 g/L; modified MS-Vitamin [1000X], 1.00 ml/L; $AgNO_3$, 15.0 mg/L; Carbenicillin, 250.0 mg/L) without selective agent and incubated in 24 hours light with light intensity of 50 μmol $m^{-2}s^{-1}$ for 7 days at 28°C. Embryos are transferred onto selection 1 media (MS salts, 4.33 g/L; L-proline, 700.0 mg/L; myo-inositol , 100.0 mg/L; MES [(2-(n-morpholino)-ethanesulfonic acid), free acid], 500.0 mg/L; casein enzymatic hydrolysate, 100.0 mg/L; Dicamba, 3.30 mg/L; sucrose, 30.0 g/L; Gelzan™, 2.30 g/L; modified MS-Vitamin [1000X], 1.00 ml/L; $AgNO_3$, 15.0 mg/L; Carbenicillin, 250.0 mg/L), containing 3 mg/L Bialaphos and incubated in 24 hours light with light intensity of 50 μmol $m^{-2}s^{-1}$ for 7 days at 28°C.

Embryos with proliferating embryogenic calli are transferred onto selection 2 media (MS salts, 4.33 g/L; myo-inositol, 100.0 mg/L; L-proline, 700.0 mg/L; MES [(2-(n-morpholino)-ethanesulfonic acid), free acid], 500.0 mg/L; casein enzymatic hydrolysate, 100.0 mg/L; Dicamba, 3.30 mg/L; sucrose, 30.0 g/L; Gelzan™ 2.30 g/L; modified MS-Vitamin [1000X], 1.00 ml/L; $AgNo_3$, 15.0 mg/L; Carbenicillin, 250.0 mg/L), containing 5 mg/L Bialaphos and are incubated in 24 hours light with light intensity of 50 μmol $m^{-2}s^{-1}$ for another 14 days at 28°C. This selection step allows transgenic callus to further proliferate and differentiate. The callus selection period may last for three weeks. Proliferating, embryogenic calli are transferred onto regeneration 1 media (MS salts, 4.33 g/L; myo-inositol, 100.0 mg/L; L-proline, 350.0 mg/L; MES [(2-(n-morpholino)-ethanesulfonic acid), free acid], 250.0 mg/L; casein enzymatic hydrolysate, 50.0 mg/L; NAA, 0.500 mg/L; ABA, 2.50 mg/L; BA, 1.00 mg/L; sucrose, 45.0 g/L; Gelzan™ 2.50 g/L; modified MS-Vitamin [1000X], 1.00 ml/L; $AgNO_3$, 1.00 mg/L; Carbenicillin, 250.0 mg/L), containing 3 mg/L Bialaphos and cultured in 24 hours light with light intensity of 50 μmol $m^{-2}s^{-1}$ for 7 days at 28°C.

Embryogenic calli with shoot/buds are transferred onto regeneration 2 media (MS salts, 4.33 g/L; modified MS-Vitamin [1000X], 1.00 ml/L; myo-inositol, 100.0 mg/L; sucrose, 60.0 g/L; Gellan Gum G434™, 3.00 g/L; Carbenicillin, 250.0 mg/L), containing 3 mg/L Bialaphos. The cultures are incubated under 24 hours light with light intensity of 50 μmol $m^{-2}s^{-1}$ for 7-10 days at 28°C. Small shoots with primary roots are transferred to shoot elongation and rooting media (MS salts, 4.33 g/L; N6 Vitamin Solution [1000X], 1.00 mL/L; myo-inositol, 100.0 mg/L; sucrose, 30.0 g/L;

agar 5.50 g/L; in phytatray and are incubated under 16/8 hours light/dark at 90 µmol $m^{-2}s^{-1}$ for 7 days at 28°C. Healthy putative transgenic plantlets are selected then incubated in 16/8 hours light/dark at 200 µmol $m^{-2}s^{-1}$ for another 2-5 days at 25°C and are analyzed for transgene copy number and transferred to the greenhouse.

EXAMPLE 12: Transient PhiYFP Expression

Transient expression of PhiYFP from *Zea mays* embryos transformed with pDAB108719 is performed. The bi-directional SCBV promoter can express PhiYFP from the AAD1-2A-PhiYFP gene expression cassette, where non-transformed embryo does not show any PhiYFP fluorescence. Similar level of PhiYFP expression can be observed from *Zea mays* embryos transformed with a binary plasmid pDAB105748 (FIG. 20) containing a uni-directional *Zea mays* (Zm) Ubi1 promoter driving single *PhiYFP* coding sequence displayed expected level of YFP/PhiYFP expression. Transient expression of PhiYFP can be observed from *Zea mays* embryos transformed with pDAB108720, where bi-directional Zm Ubi1 promoter can express PhiYFP from the PhiYFP-2A-AAD1 gene expression cassette.

EXAMPLE 13: PhiYFP Expression in Stably Transformed Maize

PhiYFP Expression in Stably Transformed *Zea mays* Callus Driven by a Bi-Directional Zm Ubi1 Promoter: *Zea mays* embryos transformed with the pDAB108719 binary vector containing the AAD1-2A-PhiYFP gene expression cassette show good PhiYFP expression. The bi-directional SCBV promoter can drive robust expression of PhiYFP. These results confirm that the Min-UbiP1 minimal promoter element of the bi-directional SCBV promoter is capable of expressing a reporter gene, for example PhiYFP or YFP. The levels of expression of the PhiYFP protein are similar as compared to *Zea mays* callus transformed with a control binary vector which contained the uni-directional Zm Ubi1 promoter driving the *PhiYFP* coding sequence (pDAB105748). Expression of PhiYFP is not detected in the negative control callus which is not transformed with a binary construct and did not contain the *PhiYFP* coding sequences.

Zea mays embryos transformed with the pDAB108720 binary vector which contains the PhiYFP-2A-AAD1 gene expression cassette show good PhiYFP

expression. The bi-directional SCBV promoter can drive robust expression of PhiYFP. These results confirm that the Min-UbiP1 minimal promoter element of the bi-directional SCBV promoter is capable of expressing a reporter gene, for example PhiYFP or YFP.

EXAMPLE 14: Estimation of Transgene Copy Number

Transgene Copy Number Estimation Using Real Time TaqMan™ PCR: *Zea mays* plants were transformed with binary vectors containing a bidirectional SCBV promoter, pDAB108719 and pDAB108720, and other plants are transformed with a control binary vector, pDAB105748. The presence of coding sequence (*PhiYFP*, *AAD1*, *Cry34*, *Cry35*, *Pat*) within the genome of *Z. mays* plants transgenic to pDAB108719 and pDAB108720 is confirmed *via* a TaqMan hydrolysis probe assay. The plants transgenic to control vector pDAB105748 are analyzed for the presence of PhiYFP sequence. Stably transformed transgenic *Z. mays* plantlets that developed from the callus are obtained and analyzed to identify events that contain a low copy number (1-2 copies) of full-length T-strand inserts from the pDAB108719 and pDAB108720 binary vectors, and pDAB105748 control binary vector. Confirmed plantlets are advanced to the green house and grown.

The Roche Light Cycler480™ system is used to determine the transgene copy number for events that are transformed with the pDAB108719 and pDAB108720 binary vector. The method utilized a biplex TAQMAN® reaction that employs oligonucleotides specific to the coding sequence and to the endogenous *Z. mays* reference gene, *invertase* (Genbank Accession No: U16123.1), in a single assay. Copy number and zygosity are determined by measuring the intensity of coding sequence-specific fluorescence, relative to the *invertase*-specific fluorescence, as compared to known copy number standards.

Table 7. Forward and reverse nucleotide primer and fluorescent probes (synthesized by Integrated DNA Technologies, Coralville, IA).

Primer Name	Primer Sequence
YFP Forward Primer	GATGCCTCAGTGGGAAAGG (SEQ ID NO: 8)
YFP Reverse Primer	CCATAGGTGAGAGTGGTGACAA (SEQ ID NO: 9)
YFP Probe	ROCHE UPL Probe #125 CTTGGAGC (SEQ ID NO: 41) Cat # 04693604001 (Roche, Indianapolis, IN)
Invertase Forward Primer	TGGCGGACGACGACTTGT (SEQ ID NO: 10)
Invertase Reverse Primer	AAAGTTTGGAGGCTGCCGT (SEQ ID NO: 11)
Invertase Probe	5'HEX/CGAGCAGACCGCCGTGTACTTCTACC/3BHQ_1/3' (SEQ ID NO: 12)
AAD1 Forward Primer	TGTTCGGTTCCCTCTACCAA (SEQ ID NO: 13)
AAD1 Reverse Primer	CAACATCCATCACCTTGACTGA (SEQ ID NO: 14)
AAD1 Probe	CACAGAACCGTCGCTTCAGCAACA (SEQ ID NO: 15)
Cry34 Forward Primer	GCCAACGACCAGATCAAGAC (SEQ ID NO: 42)
Cry34 Reverse Primer	GCCGTTGATGGAGTAGTAGATGG (SEQ ID NO: 43)
Cry34 Probe	CCGAATCCAACGGCTTCA (SEQ ID NO: 44)
Cry35 Forward Primer	CCTCATCCGCCTCACCG (SEQ ID NO: 45)
Cry35 Reverse Primer	GGTAGTCCTTGAGCTTGGTGTC (SEQ ID NO: 46)
Cry35 Probe	CAGCAATGGAACCTGACGT (SEQ ID NO: 47)
PAT Forward Primer	ACAAGAGTGGATTGATGATCTAGAGAGGT (SEQ ID NO: 48)
PAT Reverse Primer	CTTTGATGCCTATGTGACACGTAAACAGT (SEQ ID NO: 49)
PAT Probe	GGTGTTGTGGCTGGTATTGCTTACGCTGG (SEQ ID NO: 50)

For *Z. mays* samples transformed with the pDAB108719 and pDAB108720 binary vectors, a coding sequence-specific DNA fragment is amplified with one TAQMAN® primer/probe set containing a probe labeled with FAM fluorescent dye, and invertase is amplified with a second TAQMAN® primer/probe set containing a probe labeled with HEX fluorescence (Table 7). The PCR reaction mixture is prepared as set forth in Table 8, and the gene-specific DNA fragments are amplified according to the conditions set forth in Table 9. Copy number and zygosity of the samples are determined by measuring the relative intensity of fluorescence specific for the coding sequence to fluorescence specific for the reference gene, invertase, as compared to known copy number standards.

Standards are created by diluting the vector (pDAB108719 or pDAB108720) into *Z. mays* B104 genomic DNA (gDNA) to obtain standards with a known relationship of vector:gDNA. For example, samples having one, two, and four cop(ies) of vector DNA per one copy of the *Z. mays* B104 gDNA are prepared. One and two copy dilutions of the vector mixed with the *Z. mays* B104 gDNA standard are validated against a control *Z. mays* event that is known to be hemizygous, and a control *Z. mays* event that is known to be homozygous (*Z. mays* event 278; See PCT International Patent Publication No. WO 2011/022469 A2). A TAQMAN® biplex assay which utilizes oligonucleotides specific to the coding sequence gene and oligonucleotides specific to the endogenous *Z. mays* reference gene, invertase, is performed by amplifying and detecting a gene-specific DNA fragment for coding sequence with one TAQMAN® primer/probe set containing a probe labeled with FAM fluorescent dye, and by amplifying and detecting a gene-specific DNA fragment for invertase with a second TAQMAN® primer/probe set containing a probe labeled with HEX fluorescence. According to Table 7, the coding sequence TAQMAN® reaction mixture is prepared as set forth in Table 8 and the specific fragments are amplified according to the conditions set forth in Table 9.

Table 8. TAQMAN® PCR reaction mixture.

Number of Reactions	µl each	Final Concentration
H_2O	0.5 µL	-
PVP (10%)	0.1 µL	0.1%
ROCHE 2X Master Mix	5.0 µL	1X
Coding sequence Forward Primer (10 µM)	0.4 µL	0.4 µM
Coding sequence Reverse Primer (10 µM)	0.4 µL	0.4 µM
Coding sequence Probe UPL#125 (5 µM)	0.4 µL	0.2 µM
Invertase Forward Primer (10 µM)	0.4 µL	0.4 µM
Invertase Reverse Primer (10 µM)	0.4 µL	0.4 µM
Invertase Probe (5µM)	0.4 µL	0.2 µM
Template DNA	2.0 µL	-
Total reaction volume	10 µL	-

The level of fluorescence generated for each reaction is analyzed using the Roche LightCycler 480™ Thermocycler according to the manufacturer's directions. The FAM fluorescent moiety is excited at an optical density of 465/510 nm, and the HEX fluorescent moiety is excited at an optical density of 533/580 nm. The copy number can be determined by comparison of Target/Reference values for unknown samples (output by the LightCycler 480™) to Target/Reference values of four known copy number standards (for example, Null, 1-Copy (hemi), 2-Copy (homo), and 4-Copy).

Table 9. Thermocycler conditions for PCR amplification.

PCR Steps	Temp (°C)	Time	No. of cycles
Step-1	95	10 minutes	1
Step-2	95	10 seconds	40
	59	35 seconds	
	72	1 second	
Step-3	40	11 seconds	1

Results from the transgene copy number analysis of transgenic plants obtained *via* transformation with a bidirectional SCBV promoter constructs (pDAB108719 and pDAB108720), and of transgenic plants obtained *via* transformation with a control unidirectional ZmUbi1 promoter PhiYFP construct (pDAB105748) are summarized in Table 10. Only plants with 1-2 copies of the all transgenes are transferred to the greenhouse for further expression analyses.

Table 10. Transgene copy number estimation of the transgenic plants obtained from bidirectional promoter and control constructs.

Construct	Number of Embryos Transformed	Number of Positive Events	1-2 Copies of all genes
pDAB108719	250	78	13
pDAB108720	225	57	13
pDAB105748	32	8	2

EXAMPLE 15: Stable PhiYFP Expression in Maize T0 Plants

Stable PhiYFP Expression in *Zea mays* T_0 Plants Driven by bidirectional SCBV Promoter: *Zea mays* embryos transformed with the pDAB108719 binary vector containing the AAD1-2A-PhiYFP gene expression cassette can be observed. The bi-directional SCBV promoter can drive robust expression of the PhiYFP both in shoot and root tissues. The results confirm that the Min-UbiP1 minimal promoter element of the bi-directional SCBV promoter is capable of expressing a reporter gene, for example PhiYFP or YFP that is bicistronically fused with aad1 using a 2A sequence. The levels of expression of the PhiYFP protein is similar to *Z. mays* embryos transformed with a control binary vector which contains the uni-directional Zm Ubi1 promoter driving the *PhiYFP* coding sequence (pDAB105748). Expression of PhiYFP is not detected in the negative control plants which are not transformed with a binary construct and do not contain the *PhiYFP* coding sequences.

PhiYFP expression in leaf and root tissues of *Zea mays* T0 plants transgenic to pDAB108720 binary vector which contains the PhiYFP-2A-AAD1 gene expression cassette can be observed. The bi-directional SCBV promoter can drive robust expression of PhiYFP. The results confirm that the Min-UbiP1 minimal promoter element of the bi-directional SCBV promoter is capable of expressing a reporter gene, for example PhiYFP or YFP fused to aad1 with a 2A sequence or 2A-like sequence.

EXAMPLE 16: Cry34, Cry35, and AAD1 Protein Analysis

Plants are sampled into columns 1-10 of a matrix box in 1.5mL conical tubes to which 1 steel bead is added followed by PBST+0.5% BSA (0.6mL). The box is then bead beated for sample grinding in a Geno Grinder for 5 minutes at 1500 rpm then centrifuged at 3700 rpm for 7 minutes at 4°C.

Cry34/35 ELISA assay: In a separate, 96 deep well plate, a sample of the extract is diluted 1:200 in PBST + 1% blotto. Two volumes of 25 µL of the diluted sample are then transferred to separate 96- well plates that have been arrayed with anti-Cry34 and anti-Cry35 (Meso Scale Discovery). In the 11 and 12 columns of each plate standard concentrations of Cry34 and Cry35 in PBST+1% blotto are

added (25 µL). The plates are then incubated while shaking at room temperature for one hour. The plates are then washed with PBST (3x300 µL). Then 25 µL of a solution of SulfoTAG conjugated anti-Cry34 and anti-Cry35 is added to each well and incubated with shaking at room temperature for one hour. The plates are then washed with PBST (3x300 µL). A volume of 150 µL Read Buffer T (Meso Scale Discovery) is then added and the plate is immediately read on a SECTOR® 6000 reader. Concentrations of proteins in the sample can be calculated using the standard curve for the respective protein generated from the same plate.

AAD-1 ELISA assay: In a separate, 96 deep well plate, a sample of the extract is diluted 1:20 in PBST + 0.5% BSA. Two volumes of 200 µL of the diluted sample are then transferred to separate 96 well plates that have been coated with anti-AAD1 (provided by Acadia Bioscience LLC). In the 11 and 12 columns of each plate standard concentrations of AAD1 in PBST + 0.5% BSA are added (200 µL). A volume of 50 µL of biotinylated anti-AAD1 is then added to each well and the plates are incubated while shaking at room temperature for one hour. The plates are then washed with PBST (5x300 µL). Then 100 µL of a steptavidin-alkaline phosphate conjugate solution is added to each well and incubated with shaking at room temperature for 30 minutes. The plates are then washed with PBST (5x300 µL). A volume of 100 µL substrate (p-nitrophenylphosphate, PNPP) is then added and incubated with shaking at room temperature for 45 minutes. The plates are then read at A405 on a SpectraMax M5 plate reader (Molecular Devices). Concentrations of proteins in the sample can be calculated using the standard curve generated from the same plate.

EXAMPLE 17: Protein Analysis of Maize T0 Plants

Protein analysis of maize T0 plants driven by the bi-directional *Zea mays* SCBV Promoter construct (pDAB108719) is performed in this example.

Table 11. Cry34/Cry35/AAD1 expression in T0 maize pDAB108719 transgenic plants

Plant ID	Cry34 ng/cm^2	Cry35 ng/cm^2	|AAD1 ng/cm^2|
108719[2]-102.001	56	0	2
108719[3]-058.001	20	0	3
108719[3]-061.002	25	0	3
108719[3]-057.001	37	0	1
108719[3]-064.001	20	0	5
108719[1]-009.001	31	0	3
108719[1]-013.001	15	0	8
108719[1]-014.001	31	0	4
108719[1]-016.001	27	2	2
108719[1]-020.001	20	10	5
108719[2]-096.001	20	12	7
108719[2]-101.001	21	4	3

Representative ELISA analysis of 12 transgenic T0 maize plants obtained from *Zea mays* embryos transformed with pDAB108719 that contains Cry34-2A-Cry35 gene expression cassette is summarized in Table 11. Bi-directional SCBV promoter can drive robust expression of both Cry34 and Cry35 coding sequences in leaf. These observations show that the single SCBV bidirectional promoter in construct pDAB108719 can express multiple genes (*e.g.*, Cry34, Cry35, and AAD1).

Protein analysis of maize T0 plants driven by the bi-directional *Zea mays* Ubiquitin1 Promoter construct (pDAB108720): Representative ELISA analysis of 9 transgenic T0 maize plants obtained from *Zea mays* embryos transformed with pDAB108720 that contains the Cry34-2A-Cry35 gene expression cassette is summarized in Table 12. Bi-directional SCBV promoter can drive robust expression of both Cry34 and Cry35 coding sequences in leaf.

Table 12. Cry34/Cry35/AAD1 expression in T0 maize pDAB108720 transgenic plants

Plant ID	Cry34 ng/cm^2	Cry35 ng/cm^2	|AAD1 ng/cm^2|
108720[1]-017.001	19	24	10
108720[1]-024.001	21	0	9
108720[1]-027.001	20	2	8
108720[1]-032.001	32	12	8
108720[2]-085.001	16	0	8
108720[2]-086.001	30	0	5
108720[2]-088.001	0	26	4
108720[2]-092.001	0	0	13
108720[2]-105.001	26	0	2

EXAMPLE 18: Transgene Stacking: Synthetic Bidirectional Promoters (T1 data)

Gene expression of T1 plants driven by the bi-directional promoter constructs: ten to twelve single copy events per construct are selected for analysis except that the control construct pDAB108716 has only one event. Five plants/events for the V6 stage are tested and three plants/events for the V10-12 and/R3 stages are tested. Protein assays are performed using LCMS or ELISA.

The constructs used in this example are shown in FIG. 30. pDAB108708 (SCBV bidirectional (-200)) and pDAB108709 (SCBV bidirectional (-90)) are constructs with representative bidirectional promoter of the present invention in addition to constructs with maize Ubi1 bidirectional promoter (pDAB108706 [ZMUbi bidirectional (-200)) and pDAB108707 (ZMUbi bidirectional (-90))]; pDAB101556 (ZmUbi1-YFP control), pDAB108715 (SCBV without minimal promoter), and pDAB108716 (ZMUbi1 without minimal promoter) serve as control constructs with uni-directional promoters.

Exemplary expression results (V6) from the seven constructs for YFP protein (LCMS) in ng/cm^2 are shown in FIG. 31A. Exemplary relative expression results (V6) from the seven constructs for YFP RNA are shown in FIG. 31B.

Exemplary expression results (V6) from the seven constructs for GUS protein (LCMS) in ng/cm^2 are shown in FIG. 32A. Exemplary relative expression results (V6) from the seven constructs for GUS RNA are shown in FIG. 32B.

Exemplary expression results (V6) from the seven constructs for AAD1 protein (LCMS) in ng/cm^2 are shown in FIG. 33A. Exemplary relative expression results (V6) from the seven constructs for AAD1 RNA are shown in FIG. 33B.

A statistical analysis of expression results (V6) from the seven constructs for YFP protein (LCMS) in ng/cm^2 is shown in FIG. 34A. A statistical analysis of relative expression results (V6) from the seven constructs for YFP RNA is shown in FIG. 34B. The mean values and statistical results are listed.

A statistical analysis of expression results (V6) from the seven constructs for GUS protein (LCMS) in ng/cm^2 is shown in FIG. 35A. A statistical analysis of relative expression results (V6) from the seven constructs for GUS RNA is shown in FIG. 35B. The mean values and statistical results are listed.

A statistical analysis of expression results (V6) from the seven constructs for AAD1 protein (LCMS) in ng/cm^2 is shown in FIG. 36A. A statistical analysis of relative expression results (V6) from the seven constructs for AAD1 RNA is shown in FIG. 36B. The mean values and statistical results are listed.

FIGS. 37A, 37B, and 37C show exemplary expression results (V10) from the seven constructs for YFP, AAD1, and GUS protein (LCMS) in ng/cm^2, respectively.

FIGS. 38A, 38B, and 38C show statistical analysis of expression results (V10) from the seven constructs for YFP, GUS, and AAD1 protein (LCMS) in ng/cm^2, respectively. The mean values and statistical results are listed.

FIGS. 39A, 39B, and 39C show exemplary expression results (R3) from the seven constructs for YFP, GUS, and AAD1 protein (LCMS) in ng/cm^2, respectively.

FIGS. 40A, 40B, and 40C show statistical analysis of expression results (R3) from the seven constructs for YFP, GUS, and AAD1 protein (LCMS) in ng/cm^2, respectively. The mean values and statistical results are listed.

The results show that both SCBV bidirectional promoters of the present invention and maize Ubi1 bidirectional promoters can drive robust expression of GUS and YFP. The YFP expression from Maize Ubi1 bidirectional promoter is similar to unidirectional maize Ubi1 driven YFP. The YFP expression from SCBV bidirectional

promoter is significantly higher than unidirectional maize Ubi1 driven YFP or Maize Ubi1 bidirectional promoter. However, this difference becomes less significant at V10 stage. The results also suggest that bidirectional transcription has non-significant effect on GUS expression (GUS expression compared to the constructs lacking minimal promoter without YFP expression). SCBV bidirectional promoters also provide significantly higher GUS expression compared to maize Ubi1 bidirectional promoters.

EXAMPLE 19: A Combination of Bidirectional Promoter and 2A Bicistronic Sequence to Drive Four Transgenes from One Single Promoter (T1 data)

Gene expression of T1 plants driven by the bi-directional promoter constructs: ten to twelve single copy events per construct are selected for analysis except that the control constructs have four or five events per construct. Five plants/events for the V6 stage are tested and three plants/events for the V10-12 and/R3 stages are tested. Protein assays are performed using LCMS or ELISA.

pDAB108719 and pDAB108720 are shown in FIG. 19. pDAB105748 and pDAB105818 are shown in FIG. 20. Additional multi-transgene constructs using Ubi1 promoter, including pDAB108717 and pDAB108718 are shown in FIG. 41.

Exemplary relative expression results (V6) of Cry34 RNA from six constructs pDAB105748 (ZMUbi1-YFP), pDAB105818 (ZMUbi1-Cry34/ZMUbi1-Cry35/ ZMUbi1-AAD1), pDAB108717 (YFP/AAD-1-ZMUbi1 bidirectional-Cry34-Cry35), pDAB108718 (AAD1/YFP-ZMUbi1 bidirectinal-Cry34-Cry35), pDAB108719 (YFP/AAD1-SCBV bidirectional-Cry34-Cry35), and pDAB108720 (AAD1/YFP – SCBV bidirectional-Cry34-Cry35) are shown in FIG. 42A. Exemplary relative expression results (V6) of Cry34 protein (LCMS) from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 are shown in FIG. 42B.

Exemplary relative expression results (V6) of AAD1 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 are shown in FIG. 43A. Exemplary relative expression results (V6) of AAD1 protein (LCMS) from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 are shown in FIG. 43B.

Exemplary relative expression results (V6) of YFP RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 are shown in FIG. 44A. Exemplary relative expression results (V6) of YFP protein (LCMS) from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 are shown in FIG. 44B.

Exemplary relative expression results (V6) of Cry35 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 are shown in FIG. 45A. Exemplary relative expression results (V6) of Cry35 protein (ELISA) from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 are shown in FIG. 45B.

FIG. 46 shows exemplary relative expression results (V6) of PAT RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

A statistical analysis of expression results (V6) of Cry34 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 is shown in FIG. 47A. A statistical analysis of expression results (V6) of Cry34 protein from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 is shown in FIG. 47B. The mean values and statistical results are listed.

A statistical analysis of expression results (V6) of AAD1 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 is shown in FIG. 48A. A statistical analysis of expression results (V6) of AAD1 protein from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 is shown in FIG. 48B. The mean values and statistical results are listed.

A statistical analysis of expression results (V6) of YFP RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 is shown in FIG. 49A. A statistical analysis of expression results (V6) of YFP protein from the same six constructs pDAB105748, pDAB105818,

pDAB108717, pDAB108718, pDAB108719, and pDAB108720 is shown in FIG. 49B. The mean values and statistical results are listed.

A statistical analysis of expression results (V6) of Cry35 RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 in FIG. 50A. A statistical analysis of expression results (V6) of Cry35 protein from the same six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720 is shown in FIG. 50B. The mean values and statistical results are listed.

FIG. 51 shows a statistical analysis of expression results (V6) of PAT RNA from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIGS. 52A, 52B, 52C, and 52D show exemplary protein expression results (V10) of YFP, AAD1, Cry34, and Cry35 respectively from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

FIGS. 53A, 53B, 53C, and 53D show statistical analysis of protein expression results (V10) of YFP, AAD1, Cry34, and Cry35 respectively from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIGS. 54A, 54B, 54C, and 54D show exemplary protein expression results (R3) of YFP, AAD1, Cry34, and Cry35 respectively from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720.

FIGS. 55A, 55B, 55C, and 55D show statistical analysis of protein expression results (R3) of YFP, AAD1, Cry34, and Cry35 respectively from the six constructs pDAB105748, pDAB105818, pDAB108717, pDAB108718, pDAB108719, and pDAB108720. The mean values and statistical results are listed.

FIG. 56 shows exemplary results of Western blot for protein expression of Cry34, Cry35, and AAD1 from pDAB108718, pDAB108717, pDAB108719, and pDAB108720.

The results show that all four transgenes in the single promoter-driven constructs are functional with good expression levels. Three genes

(Cry34/Cry35/AAD1) in Ubi1 bidirectional stack show robust expression levels as similar to expression levels provided by the single Ubi1-driven gene stack (DExT).

While a number of exemplary aspects and embodiments have been discussed above, those of skill in the art will recognize certain modifications, permutations, additions and sub-combinations thereof. It is therefore intended that the following appended claims and claims hereafter introduced are interpreted to include all such modifications, permutations, additions and sub-combinations as are within their true spirit and scope.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 55118-38 Seq 29-04-14 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

CLAIMS:

1. A synthetic polynucleotide comprising

(a) a minimal core promoter element from an Ubiquitin-1 gene of *Zea mays* wherein the minimal core promoter element comprises SEQ ID NO: 1 or its complement;

(b) a functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter; and

(c) an intron from an alcohol dehydrogenase gene;

wherein the functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter and the intron from the alcohol dehydrogenase gene comprise SEQ ID NO: 6 or its complement.

2. The synthetic polynucleotide of claim 1, wherein the minimal core promoter element from a *Zea mays* Ubiquitin-1 gene and the functional promoter nucleotide sequence from a Sugar Cane Bacilliform Virus promoter are in reverse complementary orientation with respect to each other in the polynucleotide.

3. The synthetic polynucleotide of claim 2, wherein the synthetic polynucleotide comprises an exon from an Ubiquitin-1 gene, an intron from an Ubiquitin-1 gene, and the intron from the alcohol dehydrogenase gene.

4. The synthetic polynucleotide of claim 1, comprising SEQ ID NO: 5 or its complement.

5. A nucleic acid construct for expressing multiple genes in plant cells and/or tissues comprising

(a) a bi-directional promoter, wherein the bi-directional promoter comprises a functional promoter nucleotide sequence from Sugar Cane Bacilliform Virus (SCBV) promoter; and

(b) two gene expression cassettes on opposite ends of the bi-directional promoter; wherein at least one of the gene expression cassettes comprises two or more genes linked via a translation switch.

6. The nucleic acid construct of claim 5, wherein the bi-directional promoter further comprises at least one enhancer.

7. The nucleic acid construct of claim 5, wherein the nucleic acid construct further comprises a binary vector for *Agrobacterium*-mediated transformation.

8. The nucleic acid construct of claim 5, wherein the bi-directional promoter further comprises an element selected from the group consisting of an upstream regulatory sequence (URS), an enhancer element, an exon, an intron, a transcription start site, a TATA box, a heat shock consensus element, and combinations thereof.

9. The nucleic acid construct of claim 5, wherein the bi-directional promoter further comprises a minimal core promoter element from an Ubiquitin-1 gene of *Zea mays* or *Zea luxurians*.

10. The nucleic acid construct of claim 9, wherein the minimal core promoter element comprises a polynucleotide sequence at least 75% identical to SEQ ID NO: 1 or its complement.

11. The nucleic acid construct of claim 9, wherein the bi-directional promoter further comprises an exon from an Ubiquitin-1 gene and/or an intron from an Ubiquitin gene.

12. The nucleic acid construct of claim 9, wherein the bi-directional promoter further comprises an upstream regulatory sequence from an Ubiquitin gene or an upstream regulatory sequence from the Sugar Cane Bacilliform Virus (SCBV) promoter.

13. The nucleic acid construct of claim 5, wherein the bi-directional promoter comprises a polynucleotide of SEQ ID NO: 5 or its complement.

14. The nucleic acid construct of claim 5, wherein the bi-directional promoter comprises a polynucleotide of SEQ ID NO: 6 or its complement.

15. The nucleic acid construct of claim 5, wherein both the gene expression cassettes comprise two or more genes linked via the translation switch.

16. The nucleic acid construct of claim 5, wherein the translation switch is selected from the group consisting of an internal ribosome entry site (IRES), an alternative splicing site, a polynucleotide sequence coding a 2A peptide, a polynucleotide sequence coding a 2A-like peptide, a polynucleotide sequence coding an intein, a polynucleotide sequence coding a protease cleavage site, and combinations thereof.

17. The nucleic acid construct of claim 5, wherein a gene upstream of the translational switch does not comprise a translation stop codon.

18. The nucleic acid construct of claim 5, wherein the nucleic acid construct enables expression of at least four genes.

19. The nucleic acid construct of claim 5 wherein the nucleic acid construct enables expression of genes between three and twenty.

20. The nucleic acid construct of claim 19, wherein the nucleic acid construct enables expression of genes between four and eight.

21. The nucleic acid construct of claim 5, wherein expression of genes from the bi-directional promoter is at least four-fold higher as compared to a uni-directional promoter.

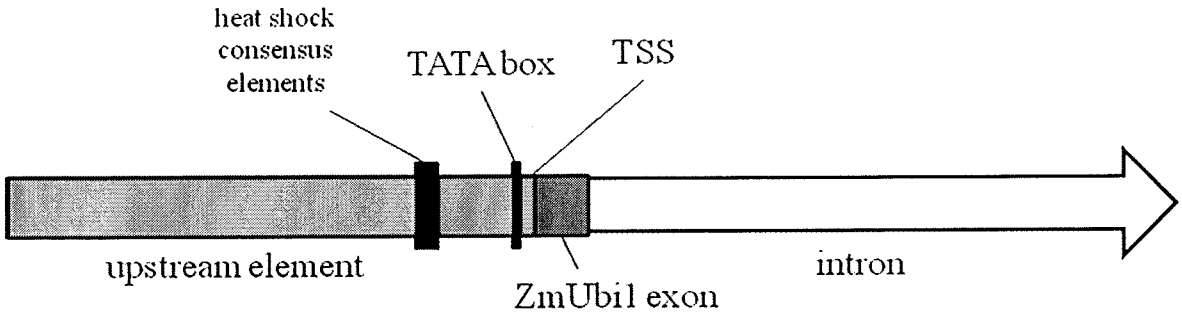
22. The nucleic acid construct of claim 5, wherein expression of genes from the bi-directional promoter is from three to ten fold higher as compared to a uni-directional promoter.

23. A method for generating a transgenic plant, comprising transforming a plant cell with the nucleic acid construct of claim 5.

24. A method for generating a transgenic cell, comprising transforming the cell with the nucleic acid construct of claim 5.

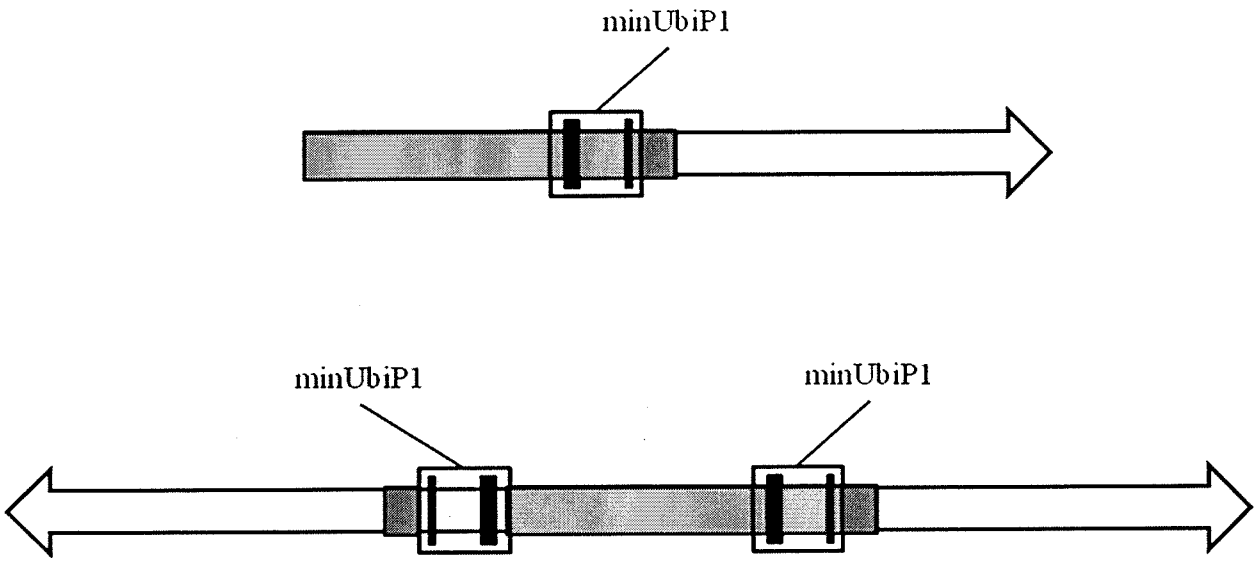
25. A plant cell comprising the nucleic acid construct of claim 5.

26. The plant cell of claim 25, wherein the nucleic acid construct is stably transformed into the plant cell.



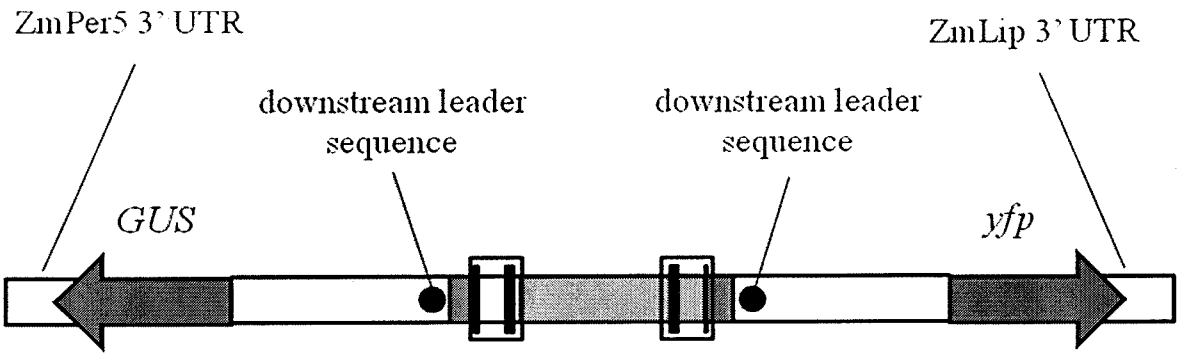
maize Ubi1 promoter

FIG. 1



synthetic bidirectional Ubi1 promoter

FIG. 2



bidirectional GUS and yfp expression cassettes

FIG. 3

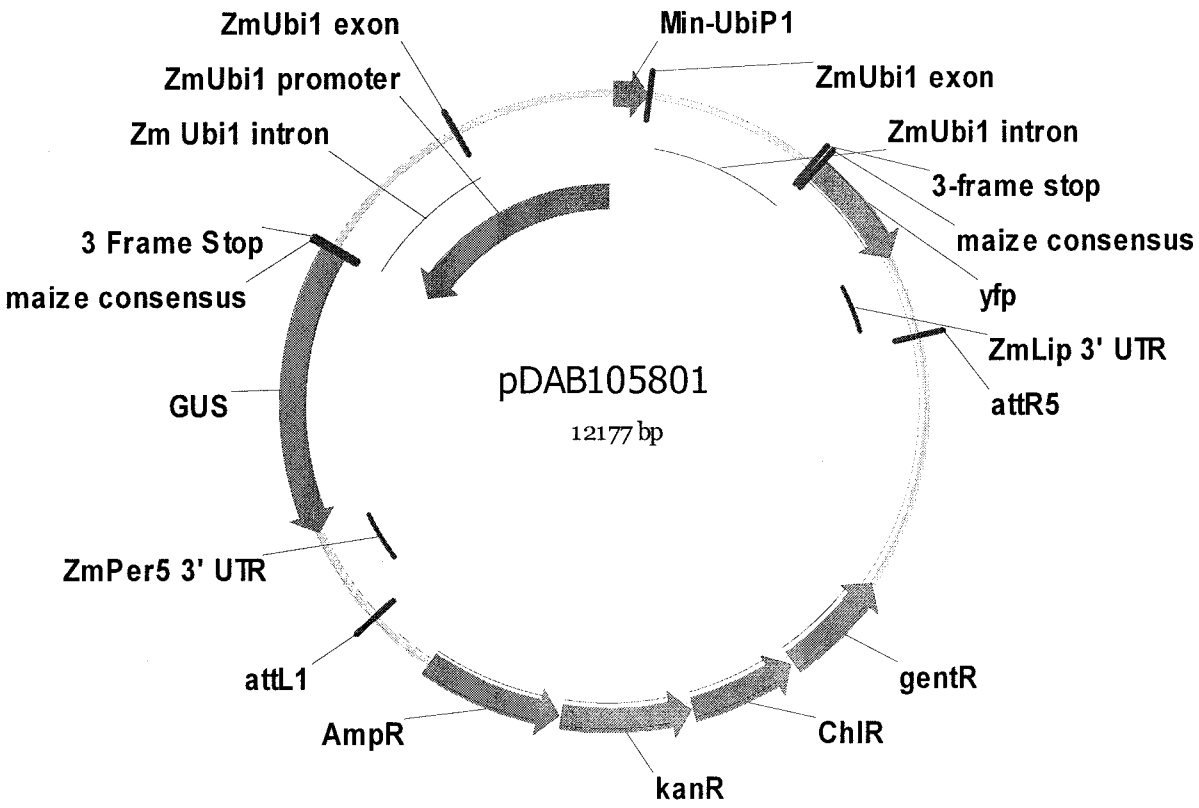
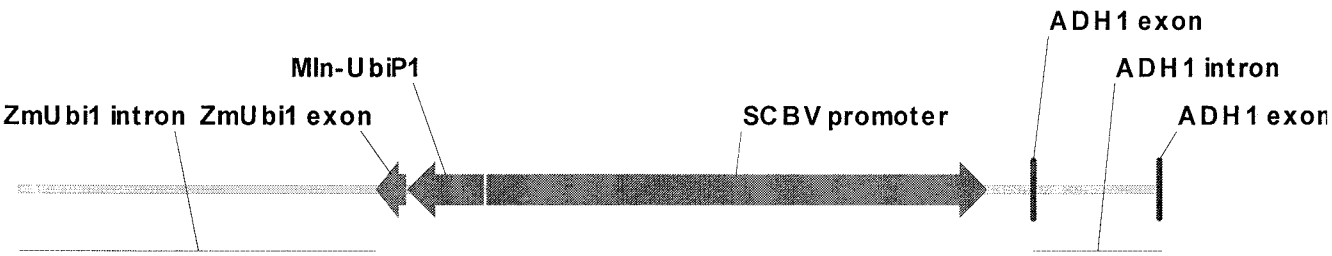


FIG. 4



108708-bidirectional SCBV (promoter only)

FIG. 5

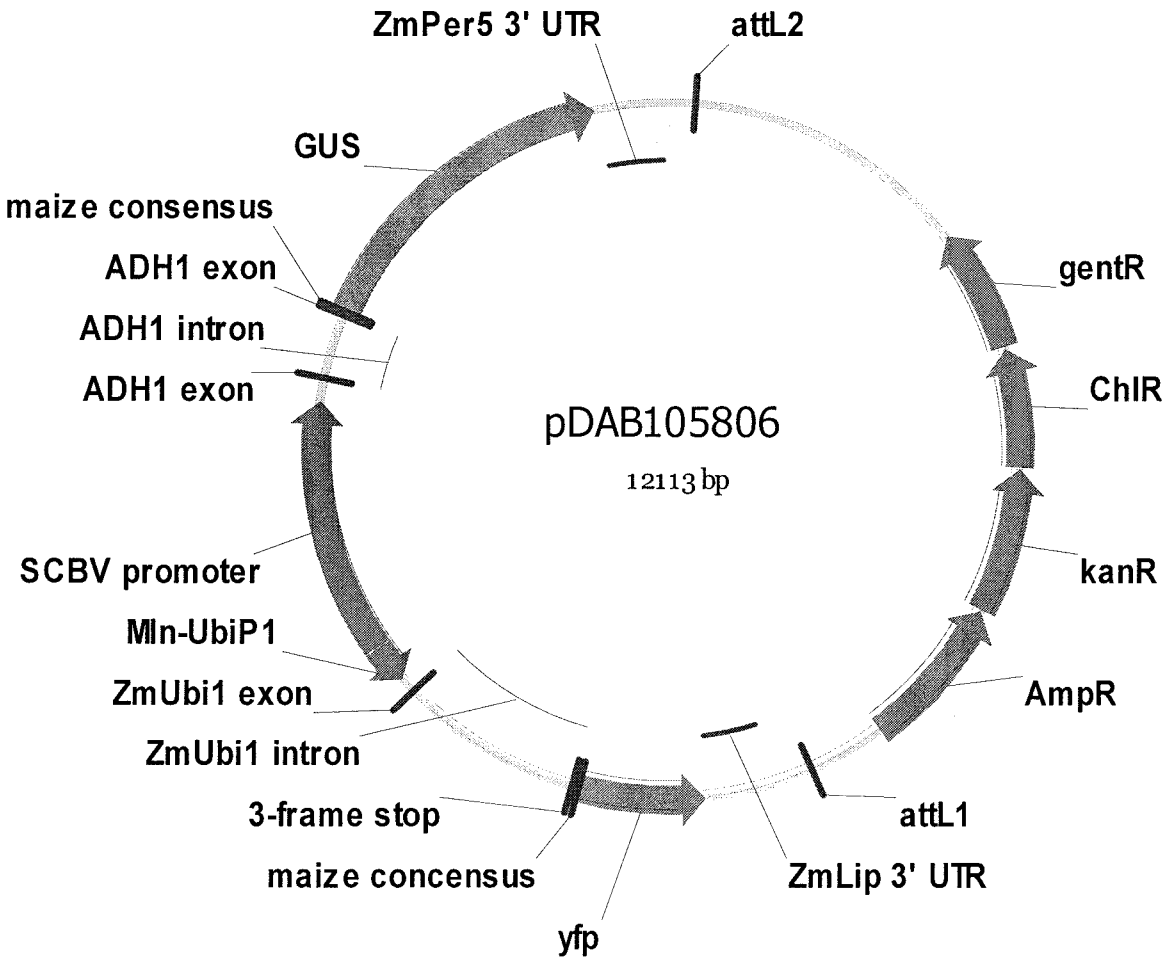
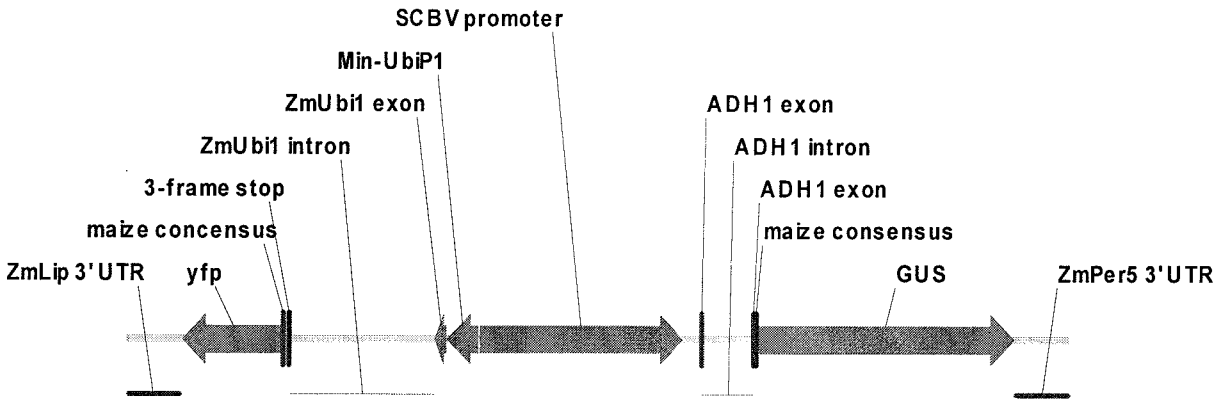


FIG. 6



GUS and yfp Expression Cassettes

FIG. 7

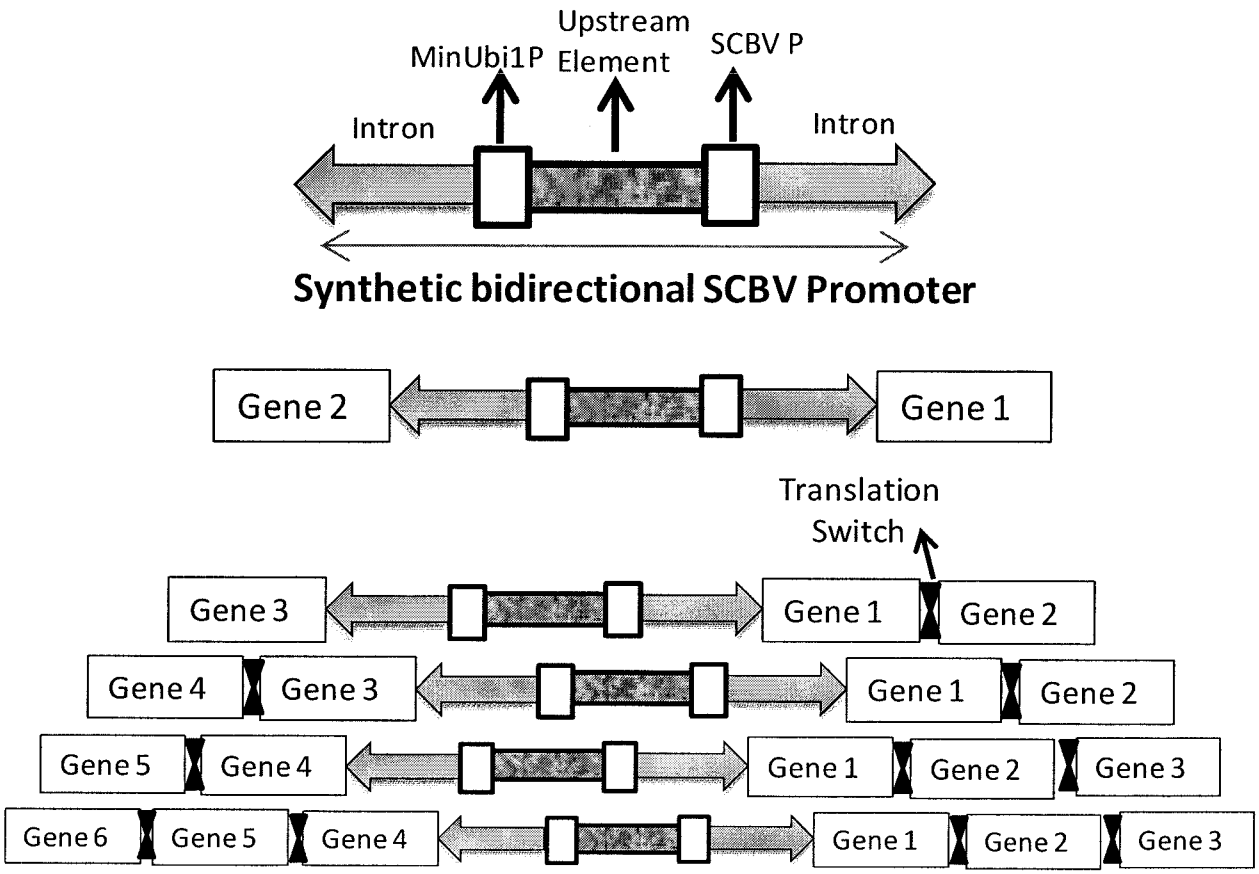
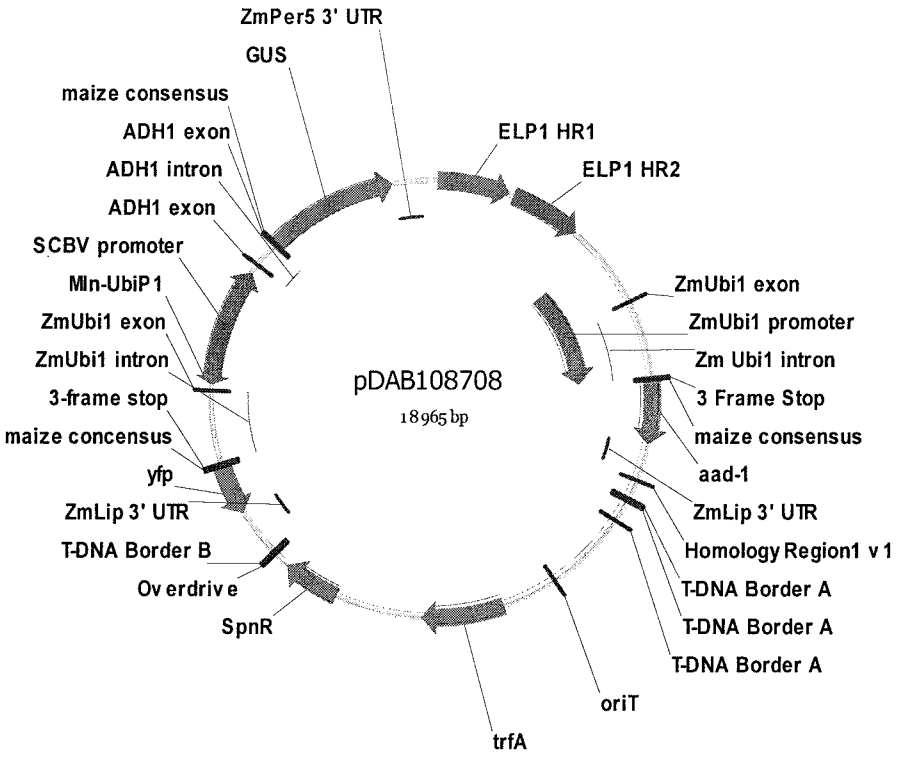


FIG. 8


ZmPer5 3' UTR
GUS
maize consensus
ADH1 exon
ADH1 intron
ADH1 exon
SCBV promoter
Mln-UbiP1
ZmUbi1 exon
ZmUbi1 intron
3-frame stop
maize concensus
yfp
ZmLip 3' UTR
T-DNA Border B
Overdrive
SpnR
trfA
oriT
T-DNA Border A
T-DNA Border A
T-DNA Border A
Homology Region1 v1
ZmLip 3' UTR
aad-1
maize consensus
3 Frame Stop
Zm Ubi1 intron
ZmUbi1 promoter
ZmUbi1 exon
ELP1 HR2
ELP1 HR1
pDAB108708
18965bp

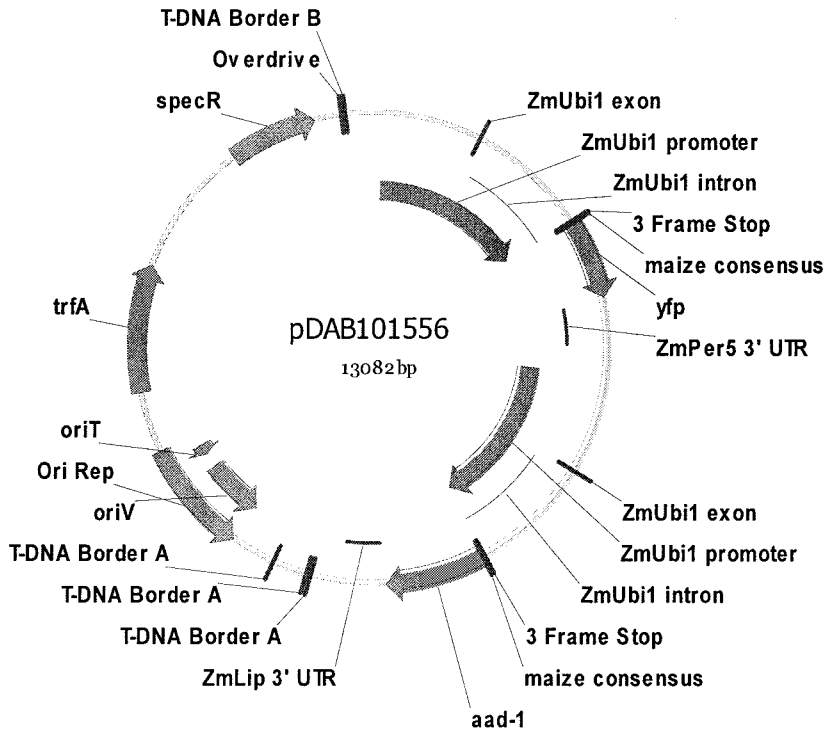

T-DNA Border B
Overdrive
specR
ZmUbi1 exon
ZmUbi1 promoter
ZmUbi1 intron
3 Frame Stop
maize consensus
yfp
ZmPer5 3' UTR
trfA
oriT
Ori Rep
oriV
T-DNA Border A
T-DNA Border A
T-DNA Border A
ZmLip 3' UTR
aad-1
maize consensus
3 Frame Stop
ZmUbi1 intron
ZmUbi1 promoter
ZmUbi1 exon
pDAB101556
13082bp

FIG. 9

SEQ ID NO: 1 shows a 215 bp region of a *Zea mays* Ubiquitin 1 minimal core promoter (minUbi1P):

CTGGACCCCTCTCGAGAGTTCCGCTCCACCGTTGGACTTGCTCCGCTGTCGGCATCCAG
AAATTGCGTGGCGGAGCGGCAGACGTGAGCCGGCACGGCAGGCGGCCTCCTCCTCCT
CTCACGGCACCGGCAGCTACGGGGGATTCCTTTCCCACCGCTCCTTCGCTTTCCCTTCC
TCGCCCGCCGTAATAAATAGACACCCCCTCCACACCCTCT

FIG. 10A

SEQ ID NO: 2 shows the reverse complement of a polynucleotide comprising a *Z. mays* minUbi1P minimal core promoter (underlined); a *Z. mays* Ubi1 leader (ZmUbi1 exon; bold font); and a *Z. mays* Ubi1 intron (lower case):

ctgcagaagtaacaccaaacaacagggtgagcatcgacaaaagaaacagtaccaagcaaataaatagcgtatgaaggcagggctaaaaaaat
ccacatatagctgctgcatatgccatcatccaagtatatcaagatcgaaataattataaaacatacttgtttattataatagataggtactcaaggttag
agcatatgaatagatgctgcatatgccatcatgtatatgcatcagtaaaacccacatcaacatgtatacctatcctagatcgatatttccatccatctta
aactcgtaactatgaagatgtatgacacacacatacagttccaaaattaataaatacaccaggtagtttgaaacagtattctactccgatctagaacg
aatgaacgaccgcccaaccacaccacatcatcacaaccaagcgaacaaaaagcatctctgtatatgcatcagtaaaacccgcatcaacatgtata
cctatcctagatcgatatttccatccatcatcttcaattcgtaactatgaatatgtatggcacacacatacagatccaaaattaataaatccaccaggta
gtttgaaacagaattctactccgatctagaacgaccgcccaaccagaccacatcatcacaaccaagacaaaaaaaagcatgaaaagatgaccc
gacaaacaagtgcacggcatatattgaaataaaggaaaagggcaaaccaaaccctatgcaacgaaacaaaaaaaatcatgaaatcgatcccgt
ctgcggaacggctagagccatcccaggattccccaaagagaaacactggcaagttagcaatcagaacgtgtctgacgtacaggtcgcatccgt
gtacgaacgctagcagcacggatctaacacaaacacggatctaacacaaacatgaacagaagtagaactaccgggccctaaccatgcatgga
ccggaacgccgatctagagaaggtagagagggggggggggggaggacgagcggcgtac**CTTGAAGCGGAGGTGCC**
GACGGGTGGATTTGGGGGAGATCTGGTTGTGTGTGTGCGCTCCGAACAACAC
GAGGTTGGGGAAGGTACCAAGAGGGTGTGGAGGGGGTGTCTATTTATTACGGCGGGC
GAGGAAGGGAAAGCGAAGGAGCGGTGGGAAAGGAATCCCCCGTAGCTGCCGGTGCC
GTGAGAGGAGGAGGAGGCCGCCTGCCGTGCCGGCTCACGTCTGCCGCTCCGCCACGC
AATTTCTGGATGCCGACAGCGGAGCAAGTCCAACGGTGGAGCGGAACTCTCGAGAGG
GGTCCAG

FIG. 10B

SEQ ID NO: 3 shows an exemplary synthetic Ubi1 bidirectional promoter, wherein the reverse complement of a first minUbi1P, and a second minUbi1P, are underlined:

CTGCAGAAGTAACACCAAACAACAGGGTGAGCATCGACAAAAGAAACAGTACCAAG
CAAATAAATAGCGTATGAAGGCAGGGCTAAAAAAATCCACATATAGCTGCTGCATAT
GCCATCATCCAAGTATATCAAGATCGAAATAATTATAAAACATACTTGTTTATTATAA
TAGATAGGTACTCAAGGTTAGAGCATATGAATAGATGCTGCATATGCCATCATGTATA
TGCATCAGTAAAACCCACATCAACATGTATACCTATCCTAGATCGATATTTCCATCCAT
CTTAAACTCGTAACTATGAAGATGTATGACACACACATACAGTTCCAAAATTAATAAA
TACACCAGGTAGTTTGAAACAGTATTCTACTCCGATCTAGAACGAATGAACGACCGCC
CAACCACACCACATCATCACAACCAAGCGAACAAAAAGCATCTCTGTATATGCATCAG
TAAAACCCGCATCAACATGTATACCTATCCTAGATCGATATTTCCATCCATCATCTTCA
ATTCGTAACTATGAATATGTATGGCACACACATACAGATCCAAAATTAATAAATCCAC
CAGGTAGTTTGAAACAGAATTCTACTCCGATCTAGAACGACCGCCCAACCAGACCACA
TCATCACAACCAAGACAAAAAAAAGCATGAAAAGATGACCCGACAAACAAGTGCAC
GGCATATATTGAAATAAAGGAAAAGGGCAAACCAAACCCTATGCAACGAAACAAAAA
AAATCATGAAATCGATCCCGTCTGCGGAACGGCTAGAGCCATCCCAGGATTCCCCAAA
GAGAAACACTGGCAAGTTAGCAATCAGAACGTGTCTGACGTACAGGTCGCATCCGTGT
ACGAACGCTAGCAGCACGGATCTAACACAAACACGGATCTAACACAAACATGAACAG
AAGTAGAACTACCGGGCCCTAACCATGCATGGACCGGAACGCCGATCTAGAGAAGGT
AGAGAGGGGGGGGGGGGGGAGGACGAGCGGCGTACCTTGAAGCGGAGGTGCCGACG
GGTGGATTTGGGGGAGATCTGGTTGTGTGTGTGCGCTCCGAACAACACGAGGTTGG
GGAGGTACCAAGAGGGTGTGGAGGGGGTGTCTATTTATTACGGCGGGCGAGGAAGGG
AAAGCGAAGGAGCGGTGGGAAAGGAATCCCCCGTAGCTGCCGGTGCCGTGAGAGGA
GGAGGAGGCCGCCTGCCGTGCCGGCTCACGTCTGCCGCTCCGCCACGCAATTTCTGGA
TGCCGACAGCGGAGCAAGTCCAACGGTGGAGCGGAACTCTCGAGAGGGGTCCAGCCG
CGGAGTGTGCAGCGTGACCCGGTCGTGCCCCTCTCTAGAGATAATGAGCATTGCATGT
CTAAGTTATAAAAAATTACCACATATTTTTTTTGTCACACTTGTTTGAAGTGCAGTTTA
TCTATCTTTATACATATATTTAAACTTTACTCTACGAATAATATAATCTATAGTACTAC
AATAATATCAGTGTTTTAGAGAATCATATAAATGAACAGTTAGACATGGTCTAAAGGA
CAATTGAGTATTTTGACAACAGGACTCTACAGTTTTATCTTTTTAGTGTGCATGTGTTC
TCCTTTTTTTTTTGCAAATAGCTTCACCTATATAATACTTCATCCATTTTATTAGTACATC
CATTTAGGGTTTAGGGTTAATGGTTTTTATAGACTAATTTTTTTTAGTACATCTATTTTAT
TCTATTTTAGCCTCTAAATTAAGAAAACTAAAACTCTATTTTAGTTTTTTTATTTAATAG
TTTAGATATAAAATAGAATAAAATAAAGTGACTAAAAATTAAACAAATACCCTTTAAG
AAATTAAAAAAACTAAGGAAACATTTTTCTTGTTTCGAGTAGATAATGCCAGCCTGTT
AAACGCCGTCGACGAGTCTAACGGACACCAACCAGCGAACCAGCAGCGTCGCGTCGG
GCCAAGCGAAGCAGACGGCACGGCATCTCTGTCGCTGCCTCTGGACCCCTCTCGAGAG
TTCCGCTCCACCGTTGGACTTGCTCCGCTGTCGGCATCCAGAAATTGCGTGGCGGAGC
GGCAGACGTGAGCCGGCACGGCAGGCGGCCTCCTCCTCCTCTCACGGCACCGGCAGCT
ACGGGGGATTCCTTTCCCACCGCTCCTTCGCTTTCCCTTCCTCGCCCGCCGTAATAAAT
AGACACCCCCTCCACACCCTCTTTCCCCAACCTCGTGTTGTTC

FIG. 11 (page 1 of 2)

GGAGCGCACACACACACAACCAGATCTCCCCCAAATCCACCCGTCGGCACCTCCGCTT
CAAGGTACGCCGCTCGTCCTCCCCCCCCCCCCCCCCTCTCTACCTTCTCTAGATCGGCGT
TCCGGTCCATGCATGGTTAGGGCCCGGTAGTTCTACTTCTGTTCATGTTTGTGTTAGAT
CCGTGTTTGTGTTAGATCCGTGCTGCTAGCGTTCGTACACGGATGCGACCTGTACGTCA
GACACGTTCTGATTGCTAACTTGCCAGTGTTTCTCTTTGGGGAATCCTGGGATGGCTCT
AGCCGTTCCGCAGACGGGATCGATTTCATGATTTTTTTTGTTTCGTTGCATAGGGTTTG
GTTTGCCCTTTTCCTTTATTTCAATATATGCCGTGCACTTGTTTGTCGGGTCATCTTTTC
ATGCTTTTTTTTGTCTTGGTTGTGATGATGTGGTCTGGTTGGGCGGTCGTTCTAGATCG
GAGTAGAATTCTGTTTCAAACTACCTGGTGGATTTATTAATTTTGGATCTGTATGTGTG
TGCCATACATATTCATAGTTACGAATTGAAGATGATGGATGGAAATATCGATCTAGGA
TAGGTATACATGTTGATGCGGGTTTTACTGATGCATATACAGAGATGCTTTTTGTTCGC
TTGGTTGTGATGATGTGGTGTGGTTGGGCGGTCGTTCATTCGTTCTAGATCGGAGTAGA
ATACTGTTTCAAACTACCTGGTGTATTTATTAATTTTGGAACTGTATGTGTGTGTCATA
CATCTTCATAGTTACGAGTTTAAGATGGATGGAAATATCGATCTAGGATAGGTATACA
TGTTGATGTGGGTTTTACTGATGCATATACATGATGGCATATGCAGCATCTATTCATAT
GCTCTAACCTTGAGTACCTATCTATTATAATAAACAAGTATGTTTTATAATTATTTCGA
TCTTGATATACTTGGATGATGGCATATGCAGCAGCTATATGTGGATTTTTTTAGCCCTG
CCTTCATACGCTATTTATTTGCTTGGTACTGTTTCTTTTGTCGATGCTCACCCTGTTGTT
TGGTGTTACTTCTGCAG

FIG. 11 (page 2 of 2)

SEQ ID NO: 4 shows an exemplary nucleic acid comprising *YFP* and *GUS* gene expression cassettes driven by a synthetic Ubi1 bidirectional promoter.

AGCACTTAAAGATCTTTAGAAGAAAGCAAAGCATTTATTAATACATAACAATGTCCAG
GTAGCCCAGCTGAATTACAATACGCAACTGCTCATAATAATTCAACAAACCCAAGTAG
TACACAACATCCAGAAGCAAATAAAGCCCATACGTACCAAAGCCTACACAAGCAGCA
ACACTCACTGCCAGTGCCGGTGGGTCTTTAAAGCACACGGGCCTTGACCACGCGATCC
ACCTTGAAACAAACTTGGTAAAATTAAAGCAAACCAGAAGCACACACACGCCAACGC
AACGCTTCTGATCGCGCGCCCAAGGCCCGGCCGGCCAGAACGTACGACGGACACGCA
CACGCTGCGACCGAGCTCTAGGTGATTAAGCTAACTACTCAAAGGTAGGTCTTGCGAC
AGTCAACAGCTCTGACAGTTTCTTTCAAGGACATGTTGTCTCTGTGGTCTGTCACATCT
TTGGAAAGTTTCACATGGTAAGACATGTGATGATACTCTGGAACATGAACTGGACCTC
CACCAATGGGAGTGTTCATCTGGGTGTGGTCAGCCACTATGAAGTCGCCTTTGCTGCC
AGTAATCTCATGACAGATCTTGAAGGCTGACTTGAGACCGTGGTTGGCTTGGTCACCC
CAGATGTAGAGGCAGTGGGGAGTGAAGTTGAACTCCAAGTTCTTTCCCAACACATGAC
CATCTTTCTTGAAGCCTTGACCATTGAGTTTGACCCTATTGTAGACAGACCCATTCTCA
AAGGTGACTTCAGCCCTAGTCTTGAAGTTGCCATCTCCTTCAAAGGTGATTGTGCGCTC
TTGCACATAGCCATCTGGCATACAGGACTTGTAGAAGTCCTTCAACTCTGGACCATAC
TTGGCAAAGCACTGTGCTCCATAGGTGAGAGTGGTGACAAGTGTGCTCCAAGGCACA
GGAACATCACCAGTTGTGCAGATGAACTGTGCATCAACCTTTCCCACTGAGGCATCTC
CGTAGCCTTTCCCACGTATGCTAAAGGTGTGGCCATCAACATTCCCTTCCATCTCCACA
ACGTAAGGAATCTTCCCATGAAAGAGAAGTGCTCCAGATGCCATGGTGTCGTGTGGAT
CCGGTACACACGTGCCTAGGACCGGTTCAACTAACTACTGCAGAAGTAACACCAAAC
AACAGGGTGAGCATCGACAAAAGAAACAGTACCAAGCAAATAAATAGCGTATGAAG
GCAGGGCTAAAAAAATCCACATATAGCTGCTGCATATGCCATCATCCAAGTATATCAA
GATCGAAATAATTATAAAACATACTTGTTTATTATAATAGATAGGTACTCAAGGTTAG
AGCATATGAATAGATGCTGCATATGCCATCATGTATATGCATCAGTAAAACCCACATC
AACATGTATACCTATCCTAGATCGATATTTCCATCCATCTTAAACTCGTAACTATGAAG
ATGTATGACACACACATACAGTTCCAAAATTAATAAATACACCAGGTAGTTTGAAACA
GTATTCTACTCCGATCTAGAACGAATGAACGACCGCCCAACCACACCACATCATCACA
ACCAAGCGAACAAAAAGCATCTCTGTATATGCATCAGTAAAACCCGCATCAACATGTA
TACCTATCCTAGATCGATATTTCCATCCATCATCTTCAATTCGTAACTATGAATATGTA
TGGCACACACATACAGATCCAAAATTAATAAATCCACCAGGTAGTTTGAAACAGAATT
CTACTCCGATCTAGAACGACCGCCCAACCAGACCACATCATCACAACCAAGACAAAA
AAAAGCATGAAAAGATGACCCGACAAACAAGTGCACGGCATATATTGAAATAAAGGA
AAAGGGCAAACCAAACCCTATGCAACGAAACAAAAAAAATCATGAAATCGATCCCGT
CTGCGGAACGGCTAGAGCCATCCCAGGATTCCCCAAAGAGAAACACTGGCAAGTTAG
CAATCAGAACGTGTCTGACGTACAGGTCGCATCCGTGTACGAACGCTAGCAGCACGG
ATCTAACACAAACACGGATCTAACACAAACATGAACAGAAGTAGAACTACCGGGCCC
TAACCATGCATGGACCGGAACGCCGATCTAGAGAAGGTAGAGAGGGGGGGGGGGGG
GAGGACGAGCGGCGTACCTTGAAGCGGAGGTGCCGACGGGTGGATTTGGGGGAGATC
TGGTTGTGTGTGTGTGCGCTCCGAACAACACGAGGTTGGGGAGGTACCAAGAGGGTGT
GGAGGGGGTGTCTATTTATTACGGCGGGCGAGGAAGGGAAAGCGAAGGAGCGGTGGG
AAAGGAATCCCCCGTAGCTGCCGGTGCCGTGAGAGGAGGAGGAGGCCGCCTGCCGTG
CCGGCTCACGTCTGCCGCTCCGCCACGCAATTTCTGGATGCCGACAGCGGAGCAAGTC
CAACGGTGGAGCGGAACTCTCGAGAGGGGTCCAGCCGCGGAGTGTGCAGCGTGACCC
GGTCGTGCCCCTCTCTAGAGATAATGAGCATTGCATGTCTAAGTTATAAAAAATTACC
ACATATTTTTTTTGTCACACTTGTTTGAAGTGCAG

FIG. 12 (page 1 of 3)

TTTATCTATCTTTATACATATATTTAAACTTTACTCTACGAATAATATAATCTATAGTAC
TACAATAATATCAGTGTTTTAGAGAATCATATAAATGAACAGTTAGACATGGTCTAAA
GGACAATTGAGTATTTTGACAACAGGACTCTACAGTTTTATCTTTTTAGTGTGCATGTG
TTCTCCTTTTTTTTTGCAAATAGCTTCACCTATATAATACTTCATCCATTTTATTAGTAC
ATCCATTTAGGGTTTAGGGTTAATGGTTTTTATAGACTAATTTTTTTAGTACATCTATTT
TATTCTATTTTAGCCTCTAAATTAAGAAAACTAAAACTCTATTTTAGTTTTTTTATTTAA
TAGTTTAGATATAAAATAGAATAAAATAAAGTGACTAAAAATTAAACAAATACCCTTT
AAGAAATTAAAAAAACTAAGGAAACATTTTTCTTGTTTCGAGTAGATAATGCCAGCCT
GTTAAACGCCGTCGACGAGTCTAACGGACACCAACCAGCGAACCAGCAGCGTCGCGT
CGGGCCAAGCGAAGCAGACGGCACGGCATCTCTGTCGCTGCCTCTGGACCCCTCTCGA
GAGTTCCGCTCCACCGTTGGACTTGCTCCGCTGTCGGCATCCAGAAATTGCGTGGCGG
AGCGGCAGACGTGAGCCGGCACGGCAGGCGGCCTCCTCCTCCTCTCACGGCACCGGC
AGCTACGGGGGATTCCTTTCCCACCGCTCCTTCGCTTTCCCTTCCTCGCCCGCCGTAAT
AAATAGACACCCCCTCCACACCCTCTTTCCCCAACCTCGTGTTGTTCGGAGCGCACAC
ACACACAACCAGATCTCCCCCAAATCCACCCGTCGGCACCTCCGCTTCAAGGTACGCC
GCTCGTCCTCCCCCCCCCCCCCCCCTCTCTACCTTCTCTAGATCGGCGTTCCGGTCCATG
CATGGTTAGGGCCCGGTAGTTCTACTTCTGTTCATGTTTGTGTTAGATCCGTGTTTGTG
TTAGATCCGTGCTGCTAGCGTTCGTACACGGATGCGACCTGTACGTCAGACACGTTCT
GATTGCTAACTTGCCAGTGTTTCTCTTTGGGGAATCCTGGGATGGCTCTAGCCGTTCCG
CAGACGGGATCGATTTCATGATTTTTTTTGTTTCGTTGCATAGGGTTTGGTTTGCCCTTT
TCCTTTATTTCAATATATGCCGTGCACTTGTTTGTCGGGTCATCTTTTCATGCTTTTTTTT
GTCTTGGTTGTGATGATGTGGTCTGGTTGGGCGGTCGTTCTAGATCGGAGTAGAATTCT
GTTTCAAACTACCTGGTGGATTTATTAATTTTGGATCTGTATGTGTGTGCCATACATAT
TCATAGTTACGAATTGAAGATGATGGATGGAAATATCGATCTAGGATAGGTATACATG
TTGATGCGGGTTTTACTGATGCATATACAGAGATGCTTTTTGTTCGCTTGGTTGTGATG
ATGTGGTGTGGTTGGGCGGTCGTTCATTCGTTCTAGATCGGAGTAGAATACTGTTTCAA
ACTACCTGGTGTATTTATTAATTTTGGAACTGTATGTGTGTGTCATACATCTTCATAGT
TACGAGTTTAAGATGGATGGAAATATCGATCTAGGATAGGTATACATGTTGATGTGGG
TTTTACTGATGCATATACATGATGGCATATGCAGCATCTATTCATATGCTCTAACCTTG
AGTACCTATCTATTATAATAAACAAGTATGTTTTATAATTATTTCGATCTTGATATACT
TGGATGATGGCATATGCAGCAGCTATATGTGGATTTTTTTAGCCCTGCCTTCATACGCT
ATTTATTTGCTTGGTACTGTTTCTTTTGTCGATGCTCACCCTGTTGTTTGGTGTTACTTCT
GCAGGTACAGTAGTTAGTTGAGGTACAGCGGCCGCAGGGCACCATGGTCCGTCCTGTA
GAAACCCCAACCCGTGAAATCAAAAAACTCGACGGCCTGTGGGCATTCAGTCTGGATC
GCGAAAACTGTGGAATTGATCAGCGTTGGTGGGAAAGCGCGTTACAAGAAAGCCGGG
CAATTGCTGTGCCAGGCAGTTTTAACGATCAGTTCGCCGATGCAGATATTCGTAATTAT
GCGGGCAACGTCTGGTATCAGCGCGAAGTCTTTATACCGAAAGGTTGGGCAGGCCAG
CGTATCGTGCTGCGTTTCGATGCGGTCACTCATTACGGCAAAGTGTGGGTCAATAATC
AGGAAGTGATGGAGCATCAGGGCGGCTATACGCCATTTGAAGCCGATGTCACGCCGT
ATGTTATTGCCGGGAAAAGTGTACGTATCACCGTTTGTGTGAACAACGAACTGAACTG
GCAGACTATCCCGCCGGGAATGGTGATTACCGACGAAAACGGCAAGAAAAAGCAGTC
TTACTTCCATGATTTCTTTAACTATGCCGGAATCCATCGCAGCGTAATGCTCTACACCA
CGCCGAACACCTGGGTGGACGATATCACCGTGGTGACGCATGTCGCGCAAGACTGTA
ACCACGCGTCTGTTGACTGGCAGGTGGTGGCCAATGGTGATGTCAGCGTTGAACTGCG
TGATGCGGATCAACAGGTGGTTGCAACTGGACAAGGCACTAGCGGGACTTTGCAAGT
GGTGAAT

FIG. 12 (page 2 of 3)

CCGCACCTCTGGCAACCGGGTGAAGGTTATCTCTATGAACTGTGCGTCACAGCCAAAA
GCCAGACAGAGTGTGATATCTACCCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTGAA
GGGCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCATG
AAGATGCGGACTTGCGTGGCAAAGGATTCGATAACGTGCTGATGGTGCACGACCACG
CATTAATGGACTGGATTGGGGCCAACTCCTACCGTACCTCGCATTACCCTTACGCTGA
AGAGATGCTCGACTGGGCAGATGAACATGGCATCGTGGTGATTGATGAAACTGCTGCT
GTCGGCTTTAACCTCTCTTTAGGCATTGGTTTCGAAGCGGGCAACAAGCCGAAAGAAC
TGTACAGCGAAGAGGCAGTCAACGGGGAAACTCAGCAAGCGCACTTACAGGCGATTA
AAGAGCTGATAGCGCGTGACAAAAACCACCCAAGCGTGGTGATGTGGAGTATTGCCA
ACGAACCGGATACCCGTCCGCAAGGTGCACGGGAATATTTCGCGCCACTGGCGGAAG
CAACGCGTAAACTCGACCCGACGCGTCCGATCACCTGCGTCAATGTAATGTTCTGCGA
CGCTCACACCGATACCATCAGCGATCTCTTTGATGTGCTGTGCCTGAACCGTTATTACG
GATGGTATGTCCAAAGCGGCGATTTGGAAACGGCAGAGAAGGTACTGGAAAAAGAAC
TTCTGGCCTGGCAGGAGAAACTGCATCAGCCGATTATCATCACCGAATACGGCGTGGA
TACGTTAGCCGGGCTGCACTCAATGTACACCGACATGTGGAGTGAAGAGTATCAGTGT
GCATGGCTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTCGTCGGTGAAC
AGGTATGGAATTTCGCCGATTTTGCGACCTCGCAAGGCATATTGCGCGTTGGCGGTAA
CAAGAAAGGGATCTTCACTCGCGACCGCAAACCGAAGTCGGCGGCTTTTCTGCTGCAA
AAACGCTGGACTGGCATGAACTTCGGTGAAAAACCGCAGCAGGGAGGCAAACAATGA
GACGTCCGGTAACCTTTAAACTGAGGGCACTGAAGTCGCTTGATGTGCTGAATTGTTT
GTGATGTTGGTGGCGTATTTTGTTTAAATAAGTAAGCATGGCTGTGATTTTATCATATG
ATCGATCTTTGGGGTTTTATTTAACACATTGTAAAATGTGTATCTATTAATAACTCAAT
GTATAAGATGTGTTCATTCTTCGGTTGCCATAGATCTGCTTATTTGACCTGTGATGTTTT
GACTCCAAAAACCAAAATCACAACTCAATAAACTCATGGAATATGTCCACCTGTTTCT
TGAAGAGTTCATCTACCATTCCAGTTGGCATTTATCAGTGTTGCAGCGGCGCTGTGCTT
TGTAACATAACAATTGTTACGGCATATATCCAA

FIG. 12 (page 3 of 3)

SEQ ID NO: 5 shows an exemplary SCBV bidirectional promoter comprising a minUbi1P minimal core promoter, wherein the reverse complement of the minUbi1P is underlined:

CTGCAGAAGTAACACCAAACAACAGGGTGAGCATCGACAAAAGAAACAGTACCAAG
CAAATAAATAGCGTATGAAGGCAGGGCTAAAAAAATCCACATATAGCTGCTGCATAT
GCCATCATCCAAGTATATCAAGATCGAAATAATTATAAAACATACTTGTTTATTATAA
TAGATAGGTACTCAAGGTTAGAGCATATGAATAGATGCTGCATATGCCATCATGTATA
TGCATCAGTAAAACCCACATCAACATGTATACCTATCCTAGATCGATATTTCCATCCAT
CTTAAACTCGTAACTATGAAGATGTATGACACACACATACAGTTCCAAAATTAATAAA
TACACCAGGTAGTTTGAAACAGTATTCTACTCCGATCTAGAACGAATGAACGACCGCC
CAACCACACCACATCATCACAACCAAGCGAACAAAAAGCATCTCTGTATATGCATCAG
TAAAACCCGCATCAACATGTATACCTATCCTAGATCGATATTTCCATCCATCATCTTCA
ATTCGTAACTATGAATATGTATGGCACACACATACAGATCCAAAATTAATAAATCCAC
CAGGTAGTTTGAAACAGAATTCTACTCCGATCTAGAACGACCGCCCAACCAGACCACA
TCATCACAACCAAGACAAAAAAAAAGCATGAAAAGATGACCCGACAAACAAGTGCAC
GGCATATATTGAAATAAAGGAAAAGGGCAAACCAAACCCTATGCAACGAAACAAAAA
AAATCATGAAATCGATCCCGTCTGCGGAACGGCTAGAGCCATCCCAGGATTCCCCAAA
GAGAAACACTGGCAAGTTAGCAATCAGAACGTGTCTGACGTACAGGTCGCATCCGTGT
ACGAACGCTAGCAGCACGGATCTAACACAAACACGGATCTAACACAAACATGAACAG
AAGTAGAACTACCGGGCCCTAACCATGCATGGACCGGAACGCCGATCTAGAGAAGGT
AGAGAGGGGGGGGGGGGGGAGGACGAGCGGCGTACCTTGAAGCGGAGGTGCCGACG
GGTGGATTTGGGGGAGATCTGGTTGTGTGTGTGTGCGCTCCGAACAACACGAGGTTGG
GGAGGTACCAAGAGGGTGTGGAGGGGGTGTCTATTTATTACGGCGGGCGAGGAAGGG
AAAGCGAAGGAGCGGTGGGAAAGGAATCCCCCGTAGCTGCCGGTGCCGTGAGAGGA
GGAGGAGGCCGCCTGCCGTGCCGGCTCACGTCTGCCGCTCCGCCACGCAATTTCTGGA
TGCCGACAGCGGAGCAAGTCCAACGGTGGAGCGGAACTCTCGAGAGGGGTCCAGCCG
CGGAGTATCGGAAGTTGAAGACAAAGAAGGTCTTAAATCCTGGCTAGCAACACTGAA
CTATGCCAGAAACCACATCAAAGCATATCGGCAAGCTTCTTGGCCCATTATATCCAAA
GACCTCAGAGAAAGGTGAGCGAAGGCTCAATTCAGAAGATTGGAAGCTGATCAATAG
GATCAAGACAATGGTGAGAACGCTTCCAAATCTCACTATTCCACCAGAAGATGCATAC
ATTATCATTGAAACAGATGCATGTGCAACTGGATGGGGAGCAGTATGCAAGTGGAAG
AAAAACAAGGCAGACCCAAGAAATACAGAGCAAATCTGTAGGTATGCCAGTGGAAAA
TTTGATAAGCCAAAAGGAACCTGTGATGCAGAAATCTATGGGGTTATGAATGGCTTAG
AAAAGATGAGATTGTTCTACTTGGACAAAAGAGAGATCACAGTCAGAACTGACAGTA
GTGCAATCGAAAGGTTCTACAACAAGAGTGCTGAACACAAGCCTTCTGAGATCAGAT
GGATCAGGTT

FIG. 13 (page 1 of 2)

CATGGACTACATCACTGGTGCAGGACCAGAGATAGTCATTGAACACATAAAAGGGAA
GAGCAATGGTTTAGCTGACATCTTGTCCAGGCTCAAAGCCAAATTAGCTCAGAATGAA
CCAACGGAAGAGATGATCCTGCTTACACAAGCCATAAGGGAAGTAATTCCTTATCCAG
ATCATCCATACACTGAGCAACTCAGAGAATGGGGAAACAAAATTCTGGATCCATTCCC
CACATTCAAGAAGGACATGTTCGAAAGAACAGAGCAAGCTTTTATGCTAACAGAGGA
ACCAGTTCTACTCTGTGCATGCAGGAAGCCTGCAATTCAGTTAGTGTCCAGAACATCT
GCCAACCCAGGAAGGAAATTCTTCAAGTGCGCAATGAACAAATGCCATTGCTGGTACT
GGGCAGATCTCATTGAAGAACACATTCAAGACAGAATTGATGAATTTCTCAAGAATCT
TGAAGTTCTGAAGACCGGTGGCGTGCAAACAATGGAGGAGGAACTTATGAAGGAAGT
CACCAAGCTGAAGATAGAAGAGCAGGAGTTCGAGGAATACCAGGCCACACCAAGGG
CTATGTCGCCAGTAGCCGCAGAAGATGTGCTAGATCTCCAAGACGTAAGCAATGACG
ATTGAGGAGGCATTGACGTCAGGGATGACCGCAGCGGAGAGTACTGGGCCCATTCAG
TGGATGCTCCACTGAGTTGTATTATTGTGTGCTTTTCGGACAAGTGTGCTGTCCACTTT
CTTTTGGCACCTGTGCCACTTTATTCCTTGTCTGCCACGATGCCTTTGCTTAGCTTGTAA
GCAAGGATCGCAGTGCGTGTGTGACACCACCCCCCTTCCGACGCTCTGCCTATATAAG
GCACCGTCTGTAAGCTCTTACGATCATCGGTAGTTCACCAAGGCCCGGGGTCGGATCT
AGCTGAAGGCTCGACAAGGCAGTCCACGGAGGAGCTGATATTTGGTGGACAAGCTGT
GGATAGGAGCAACCCTATCCCTAATATACCAGCACCACCAAGTCAGGGCAATCCCCA
GATCACCCCAGCAGATTCGAAGAAGGTACAGTACACACACATGTATATATGTATGATG
TATCCCTTCGATCGAAGGCATGCCTTGGTATAATCACTGAGTAGTCATTTTATTACTTT
GTTTTGACAAGTCAGTAGTTCATCCATTTGTCCCATTTTTTCAGCTTGGAAGTTTGGTT
GCACTGGCCTTGGTCTAATAACTGAGTAGTCATTTTATTACGTTGTTTCGACAAGTCAG
TAGCTCATCCATCTGTCCCATTTTTTCAGCTAGGAAGTTTGGTTGCACTGGCCTTGGAC
TAATAACTGATTAGTCATTTTATTACATTGTTTCGACAAGTCAGTAGCTCATCCATCTG
TCCCATTTTTCAGCTAGGAAGTTC

FIG. 13 (page 2 of 2)

SEQ ID NO: 6 shows a SCBV promoter containing ADH1 exon 6 (underlined), intron 6 (lower case font), and exon 7 (bold font).

ATCGGAAGTTGAAGACAAAGAAGGTCTTAAATCCTGGCTAGCAACACTGAACTATGC
CAGAAACCACATCAAAGCATATCGGCAAGCTTCTTGGCCCATTATATCCAAAGACCTC
AGAGAAAGGTGAGCGAAGGCTCAATTCAGAAGATTGGAAGCTGATCAATAGGATCAA
GACAATGGTGAGAACGCTTCCAAATCTCACTATTCCACCAGAAGATGCATACATTATC
ATTGAAACAGATGCATGTGCAACTGGATGGGGAGCAGTATGCAAGTGGAAGAAAAAC
AAGGCAGACCCAAGAAATACAGAGCAAATCTGTAGGTATGCCAGTGGAAAATTTGAT
AAGCCAAAAGGAACCTGTGATGCAGAAATCTATGGGGTTATGAATGGCTTAGAAAAG
ATGAGATTGTTCTACTTGGACAAAAGAGAGATCACAGTCAGAACTGACAGTAGTGCA
ATCGAAAGGTTCTACAACAAGAGTGCTGAACACAAGCCTTCTGAGATCAGATGGATC
AGGTTCATGGACTACATCACTGGTGCAGGACCAGAGATAGTCATTGAACACATAAAA
GGGAAGAGCAATGGTTTAGCTGACATCTTGTCCAGGCTCAAAGCCAAATTAGCTCAGA
ATGAACCAACGGAAGAGATGATCCTGCTTACACAAGCCATAAGGGAAGTAATTCCTT
ATCCAGATCATCCATACACTGAGCAACTCAGAGAATGGGGAAACAAAATTCTGGATC
CATTCCCCACATTCAAGAAGGACATGTTCGAAAGAACAGAGCAAGCTTTTATGCTAAC
AGAGGAACCAGTTCTACTCTGTGCATGCAGGAAGCCTGCAATTCAGTTAGTGTCCAGA
ACATCTGCCAACCCAGGAAGGAAATTCTTCAAGTGCGCAATGAACAAATGCCATTGCT
GGTACTGGGCAGATCTCATTGAAGAACACATTCAAGACAGAATTGATGAATTTCTCAA
GAATCTTGAAGTTCTGAAGACCGGTGGCGTGCAAACAATGGAGGAGGAACTTATGAA
GGAAGTCACCAAGCTGAAGATAGAAGAGCAGGAGTTCGAGGAATACCAGGCCACACC
AAGGGCTATGTCGCCAGTAGCCGCAGAAGATGTGCTAGATCTCCAAGACGTAAGCAA
TGACGATTGAGGAGGCATTGACGTCAGGGATGACCGCAGCGGAGAGTACTGGGCCCA
TTCAGTGGATGCTCCACTGAGTTGTATTATTGTGTGCTTTTCGGACAAGTGTGCTGTCC
ACTTTCTTTTGGCACCTGTGCCACTTTATTCCTTGTCTGCCACGATGCCTTTGCTTAGCT
TGTAAGCAAGGATCGCAGTGCGTGTGTGACACCACCCCCCTTCCGACGCTCTGCCTAT
ATAAGGCACCGTCTGTAAGCTCTTACGATCATCGGTAGTTCACCAAGGCCCGGGGTCG
GATCTAGCTGAAGGCTCGACAAGGCAGTCCACGGAGGAGCTGATATTTGGTGGACAA
GCTGTGGATAGGAGCAACCCTATCCCTAATATACCAGCACCACCAAGTCAGGGCAATC
CCCAGATCA<u>CCCCAGCAGATTCGAAGAAG</u>gtacagtacacacacatgtatatatgtatgatgtatcccttcgatcgaa
ggcatgccttggtataatcactgagtagtcattttattactttgttttgacaagtcagtagttcatccatttgtcccatttttttcagcttggaagtttggttgc
actggccttggtctaataactgagtagtcattttattacgttgtttcgacaagtcagtagctcatccatctgtcccatttttttcagctaggaagtttggttg
cactggccttggactaataactgattagtcattttattacattgtttcgacaagtcagtagctcatccatctgtcccatttttcag**CTAGGAAGT**
TC

FIG. 14

SEQ ID NO: 7 shows a nucleic acid comprising *YFP* and *GUS* gene expression cassettes driven by an exemplary SCBV bidirectional promoter.

AGCACTTAAAGATCTTTAGAAGAAAGCAAAGCATTTATTAATACATAACAATGTCCAG
GTAGCCCAGCTGAATTACAATACGCAACTGCTCATAATAATTCAACAAACCCAAGTAG
TACACAACATCCAGAAGCAAATAAAGCCCATACGTACCAAAGCCTACACAAGCAGCA
ACACTCACTGCCAGTGCCGGTGGGTCTTTAAAGCACACGGGCCTTGACCACGCGATCC
ACCTTGAAACAAACTTGGTAAAATTAAAGCAAACCAGAAGCACACACACGCCAACGC
AACGCTTCTGATCGCGCGCCCAAGGCCCGGCCGGCCAGAACGTACGACGGACACGCA
CACGCTGCGACCGAGCTCTAGGTGATTAAGCTAACTACTCAAAGGTAGGTCTTGCGAC
AGTCAACAGCTCTGACAGTTTCTTTCAAGGACATGTTGTCTCTGTGGTCTGTCACATCT
TTGGAAAGTTTCACATGGTAAGACATGTGATGATACTCTGGAACATGAACTGGACCTC
CACCAATGGGAGTGTTCATCTGGGTGTGGTCAGCCACTATGAAGTCGCCTTTGCTGCC
AGTAATCTCATGACAGATCTTGAAGGCTGACTTGAGACCGTGGTTGGCTTGGTCACCC
CAGATGTAGAGGCAGTGGGGAGTGAAGTTGAACTCCAAGTTCTTTCCCAACACATGAC
CATCTTTCTTGAAGCCTTGACCATTGAGTTTGACCCTATTGTAGACAGACCCATTCTCA
AAGGTGACTTCAGCCCTAGTCTTGAAGTTGCCATCTCCTTCAAAGGTGATTGTGCGCTC
TTGCACATAGCCATCTGGCATACAGGACTTGTAGAAGTCCTTCAACTCTGGACCATAC
TTGGCAAAGCACTGTGCTCCATAGGTGAGAGTGGTGACAAGTGTGCTCCAAGGCACA
GGAACATCACCAGTTGTGCAGATGAACTGTGCATCAACCTTTCCCACTGAGGCATCTC
CGTAGCCTTTCCCACGTATGCTAAAGGTGTGGCCATCAACATTCCCTTCCATCTCCACA
ACGTAAGGAATCTTCCCATGAAAGAGAAGTGCTCCAGATGCCATGGTGTCGTGTGGAT
CCGGTACACACGTGCCTAGGACCGGTTCAACTAACTACTGCAGAAGTAACACCAAAC
AACAGGGTGAGCATCGACAAAAGAAACAGTACCAAGCAAATAAATAGCGTATGAAG
GCAGGGCTAAAAAAATCCACATATAGCTGCTGCATATGCCATCATCCAAGTATATCAA
GATCGAAATAATTATAAAACATACTTGTTTATTATAATAGATAGGTACTCAAGGTTAG
AGCATATGAATAGATGCTGCATATGCCATCATGTATATGCATCAGTAAAACCCACATC
AACATGTATACCTATCCTAGATCGATATTTCCATCCATCTTAAACTCGTAACTATGAAG
ATGTATGACACACACATACAGTTCCAAAATTAATAAATACACCAGGTAGTTTGAAACA
GTATTCTACTCCGATCTAGAACGAATGAACGACCGCCCAACCACACCACATCATCACA
ACCAAGCGAACAAAAAGCATCTCTGTATATGCATCAGTAAAACCCGCATCAACATGTA
TACCTATCCTAGATCGATATTTCCATCCATCATCTTCAATTCGTAACTATGAATATGTA
TGGCACACACATACAGATCCAAAATTAATAAATCCACCAGGTAGTTTGAAACAGAATT
CTACTCCGATCTAGAACGACCGCCCAACCAGACCACATCATCACAACCAAGACAAAA
AAAAGCATGAAAAGATGACCCGACAAACAAGTGCACGGCATATATTGAAATAAAGGA
AAAGGGCAAACCAAACCCTATGCAACGAAACAAAAAAAATCATGAAATCGATCCCGT
CTGCGGAACGGCTAGAGCCATCCCAGGATTCCCCAAAGAGAAACACTGGCAAGTTAG
CAATCAGAACGTGTCTGACGTACAGGTCGCATCCGTGTACGAACGCTAGCAGCACGG
ATCTAACACAAACACGGATCTAACACAAACATGAACAGAAGTAGAACTACCGGGCCC
TAACCATGCATGGACCGGAACGCCGATCTAGAGAAGGTAGAGAGGGGGGGGGGGGG
GAGGACGAGCGGCGTACCTTGAAGCGGAGGTGCCGACGGGTGGATTTGGGGGAGATC
TGGTTGTGTGTGTGTGCGCTCCGAACAACACGAGGTTGGGGAGGTACCAAGAGGGTGT
GGAGGGGGTGTCTATTTATTACGGCGGGCGAGGAAGGGAAAGCGAAGGAGCGGTGGG
AAAGGAATCCCCCGTAGCTGCCGGTGCCGTGAGAGGAGGAGGAGGCCGCCTGCCGTG
CCGGCTCACGTCTGCCGCTCCGCCACGCAATTTCTGGATGCCGACAGCGGAGCAAGTC
CAACGGTGGAGCGGAACT

FIG. 15 (page 1 of 3)

CTCGAGAGGGGTCCAGCCGCGGAGTATCGGAAGTTGAAGACAAAGAAGGTCTTAAAT
CCTGGCTAGCAACACTGAACTATGCCAGAAACCACATCAAAGCATATCGGCAAGCTTC
TTGGCCCATTATATCCAAAGACCTCAGAGAAAGGTGAGCGAAGGCTCAATTCAGAAG
ATTGGAAGCTGATCAATAGGATCAAGACAATGGTGAGAACGCTTCCAAATCTCACTAT
TCCACCAGAAGATGCATACATTATCATTGAAACAGATGCATGTGCAACTGGATGGGGA
GCAGTATGCAAGTGGAAGAAAAACAAGGCAGACCCAAGAAATACAGAGCAAATCTGT
AGGTATGCCAGTGGAAAATTTGATAAGCCAAAAGGAACCTGTGATGCAGAAATCTAT
GGGGTTATGAATGGCTTAGAAAAGATGAGATTGTTCTACTTGGACAAAAGAGAGATC
ACAGTCAGAACTGACAGTAGTGCAATCGAAAGGTTCTACAACAAGAGTGCTGAACAC
AAGCCTTCTGAGATCAGATGGATCAGGTTCATGGACTACATCACTGGTGCAGGACCAG
AGATAGTCATTGAACACATAAAAGGGAAGAGCAATGGTTTAGCTGACATCTTGTCCAG
GCTCAAAGCCAAATTAGCTCAGAATGAACCAACGGAAGAGATGATCCTGCTTACACA
AGCCATAAGGGAAGTAATTCCTTATCCAGATCATCCATACACTGAGCAACTCAGAGAA
TGGGGAAACAAAATTCTGGATCCATTCCCCACATTCAAGAAGGACATGTTCGAAAGA
ACAGAGCAAGCTTTTATGCTAACAGAGGAACCAGTTCTACTCTGTGCATGCAGGAAGC
CTGCAATTCAGTTAGTGTCCAGAACATCTGCCAACCCAGGAAGGAAATTCTTCAAGTG
CGCAATGAACAAATGCCATTGCTGGTACTGGGCAGATCTCATTGAAGAACACATTCAA
GACAGAATTGATGAATTTCTCAAGAATCTTGAAGTTCTGAAGACCGGTGGCGTGCAAA
CAATGGAGGAGGAACTTATGAAGGAAGTCACCAAGCTGAAGATAGAAGAGCAGGAG
TTCGAGGAATACCAGGCCACACCAAGGGCTATGTCGCCAGTAGCCGCAGAAGATGTG
CTAGATCTCCAAGACGTAAGCAATGACGATTGAGGAGGCATTGACGTCAGGGATGAC
CGCAGCGGAGAGTACTGGGCCCATTCAGTGGATGCTCCACTGAGTTGTATTATTGTGT
GCTTTTCGGACAAGTGTGCTGTCCACTTTCTTTTGGCACCTGTGCCACTTTATTCCTTGT
CTGCCACGATGCCTTTGCTTAGCTTGTAAGCAAGGATCGCAGTGCGTGTGTGACACCA
CCCCCCTTCCGACGCTCTGCCTATATAAGGCACCGTCTGTAAGCTCTTACGATCATCGG
TAGTTCACCAAGGCCCGGGGTCGGATCTAGCTGAAGGCTCGACAAGGCAGTCCACGG
AGGAGCTGATATTTGGTGGACAAGCTGTGGATAGGAGCAACCCTATCCCTAATATACC
AGCACCACCAAGTCAGGGCAATCCCCAGATCACCCCAGCAGATTCGAAGAAGGTACA
GTACACACACATGTATATATGTATGATGTATCCCTTCGATCGAAGGCATGCCTTGGTAT
AATCACTGAGTAGTCATTTTATTACTTTGTTTTGACAAGTCAGTAGTTCATCCATTTGT
CCCATTTTTTCAGCTTGGAAGTTTGGTTGCACTGGCCTTGGTCTAATAACTGAGTAGTC
ATTTTATTACGTTGTTTCGACAAGTCAGTAGCTCATCCATCTGTCCCATTTTTTCAGCTA
GGAAGTTTGGTTGCACTGGCCTTGGACTAATAACTGATTAGTCATTTTATTACATTGTT
TCGACAAGTCAGTAGCTCATCCATCTGTCCCATTTTTCAGCTAGGAAGTTCGCGGCCGC
AGGGCACCATGGTCCGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAAACTCGACG
GCCTGTGGGCATTCAGTCTGGATCGCGAAAACTGTGGAATTGATCAGCGTTGGTGGGA
AAGCGCGTTACAAGAAAGCCGGGCAATTGCTGTGCCAGGCAGTTTTAACGATCAGTTC
GCCGATGCAGATATTCGTAATTATGCGGGCAACGTCTGGTATCAGCGCGAAGTCTTTA
TACCGAAAGGTTGGGCAGGCCAGCGTATCGTGCTGCGTTTCGATGCGGTCACTCATTA
CGGCAAAGTGTGGGTCAATAATCAGGAAGTGATGGAGCATCAGGGCGGCTATACGCC
ATTTGAAGCCGATGTCACGCCGTATGTTATTGCCGGGAAAAGTGTACGTATCACCGTT
TGTGTGAACAACGAACTGAACTGGCAGACTATCCCGCCGGGAATGGTGATTACCGAC
GAAAACGGCAAGAAAAAGCAGTCTTACTTCCATGATTTCTTTAACTATGCCGGAATCC
ATCGCAGCGTAATGCTCTACACCACGCCGAACACCTGGGTGGACGATATCACCGTGGT
GACGCATGTCGCGCAAGACTGTAACCACG

FIG. 15 (page 2 of 3)

CGTCTGTTGACTGGCAGGTGGTGGCCAATGGTGATGTCAGCGTTGAACTGCGTGATGC
GGATCAACAGGTGGTTGCAACTGGACAAGGCACTAGCGGGACTTTGCAAGTGGTGAA
TCCGCACCTCTGGCAACCGGGTGAAGGTTATCTCTATGAACTGTGCGTCACAGCCAAA
AGCCAGACAGAGTGTGATATCTACCCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTGA
AGGGCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCAT
GAAGATGCGGACTTGCGTGGCAAAGGATTCGATAACGTGCTGATGGTGCACGACCAC
GCATTAATGGACTGGATTGGGGCCAACTCCTACCGTACCTCGCATTACCCTTACGCTG
AAGAGATGCTCGACTGGGCAGATGAACATGGCATCGTGGTGATTGATGAAACTGCTG
CTGTCGGCTTTAACCTCTCTTTAGGCATTGGTTTCGAAGCGGGCAACAAGCCGAAAGA
ACTGTACAGCGAAGAGGCAGTCAACGGGGAAACTCAGCAAGCGCACTTACAGGCGAT
TAAAGAGCTGATAGCGCGTGACAAAAACCACCCAAGCGTGGTGATGTGGAGTATTGC
CAACGAACCGGATACCCGTCCGCAAGGTGCACGGGAATATTTCGCGCCACTGGCGGA
AGCAACGCGTAAACTCGACCCGACGCGTCCGATCACCTGCGTCAATGTAATGTTCTGC
GACGCTCACACCGATACCATCAGCGATCTCTTTGATGTGCTGTGCCTGAACCGTTATTA
CGGATGGTATGTCCAAAGCGGCGATTTGGAAACGGCAGAGAAGGTACTGGAAAAAGA
ACTTCTGGCCTGGCAGGAGAAACTGCATCAGCCGATTATCATCACCGAATACGGCGTG
GATACGTTAGCCGGGCTGCACTCAATGTACACCGACATGTGGAGTGAAGAGTATCAGT
GTGCATGGCTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTCGTCGGTGA
ACAGGTATGGAATTTCGCCGATTTTGCGACCTCGCAAGGCATATTGCGCGTTGGCGGT
AACAAGAAAGGGATCTTCACTCGCGACCGCAAACCGAAGTCGGCGGCTTTTCTGCTGC
AAAAACGCTGGACTGGCATGAACTTCGGTGAAAAACCGCAGCAGGGAGGCAAACAAT
GAGACGTCCGGTAACCTTTAAACTGAGGGCACTGAAGTCGCTTGATGTGCTGAATTGT
TTGTGATGTTGGTGGCGTATTTTGTTTAAATAAGTAAGCATGGCTGTGATTTTATCATA
TGATCGATCTTTGGGGTTTTATTTAACACATTGTAAAATGTGTATCTATTAATAACTCA
ATGTATAAGATGTGTTCATTCTTCGGTTGCCATAGATCTGCTTATTTGACCTGTGATGT
TTTGACTCCAAAAACCAAAATCACAACTCAATAAACTCATGGAATATGTCCACCTGTT
TCTTGAAGAGTTCATCTACCATTCCAGTTGGCATTTATCAGTGTTGCAGCGGCGCTGTG
CTTTGTAACATAACAATTGTTACGGCATATATCCAA

FIG. 15 (page 3 of 3)

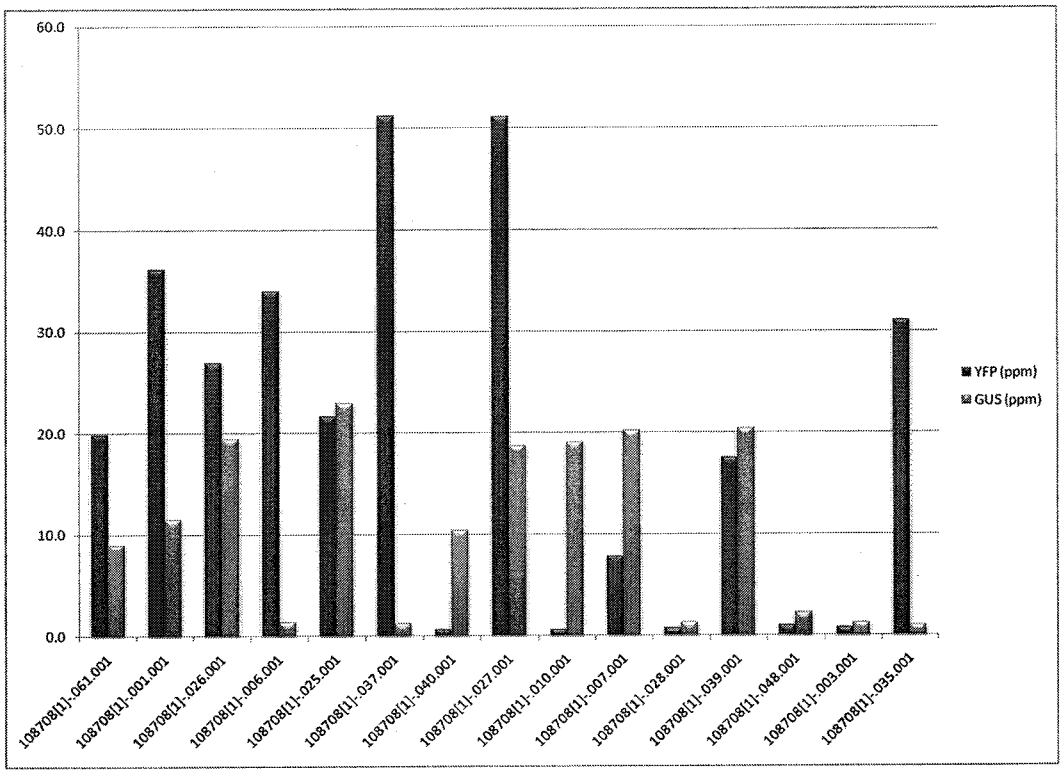

60.0
50.0
40.0
30.0
20.0
10.0
0.0
YFP (ppm)
GUS (ppm)
108708[1]-061.001
108708[1]-001.001
108708[1]-026.001
108708[1]-006.001
108708[1]-025.001
108708[1]-037.001
108708[1]-040.001
108708[1]-027.001
108708[1]-010.001
108708[1]-007.001
108708[1]-028.001
108708[1]-039.001
108708[1]-048.001
108708[1]-003.001
108708[1]-035.001

FIG. 16

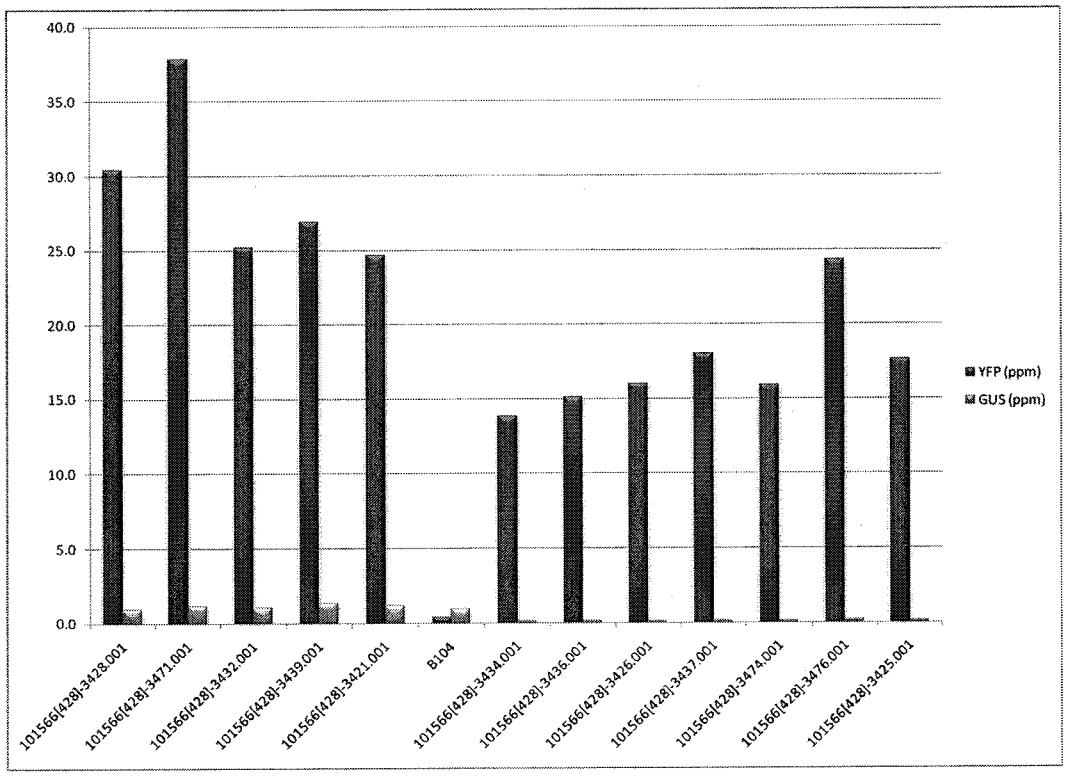

40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
YFP (ppm)
GUS (ppm)
101566[428]-3428.001
101566[428]-3471.001
101566[428]-3432.001
101566[428]-3439.001
101566[428]-3421.001
B104
101566[428]-3434.001
101566[428]-3436.001
101566[428]-3426.001
101566[428]-3437.001
101566[428]-3474.001
101566[428]-3476.001
101566[428]-3425.001

FIG. 17

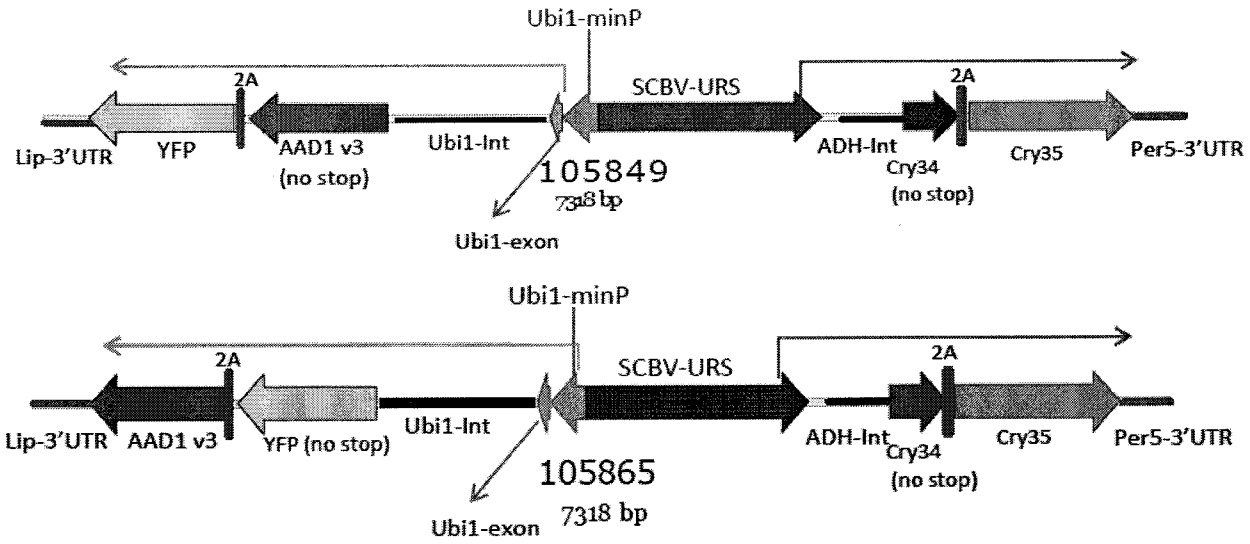

Ubi1-minP
2A
SCBV-URS
2A
Lip-3'UTR
YFP
AAD1 v3
(no stop)
Ubi1-Int
ADH-Int
Cry34
(no stop)
Cry35
Per5-3'UTR
105849
7318 bp
Ubi1-exon
Ubi1-minP
2A
SCBV-URS
2A
Lip-3'UTR
AAD1 v3
YFP (no stop)
Ubi1-Int
ADH-Int
Cry34
(no stop)
Cry35
Per5-3'UTR
105865
7318 bp
Ubi1-exon

FIG. 18

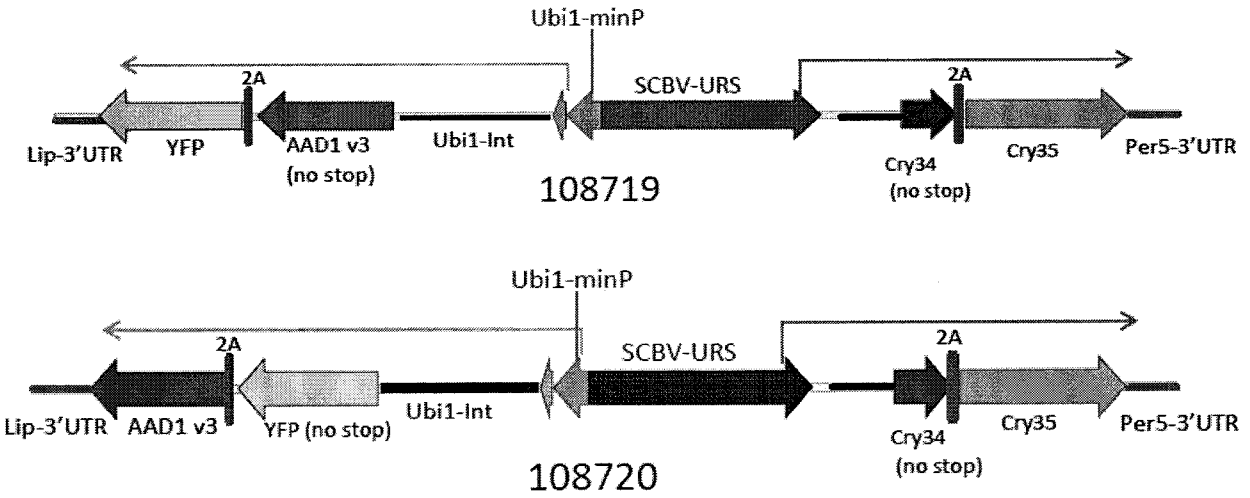
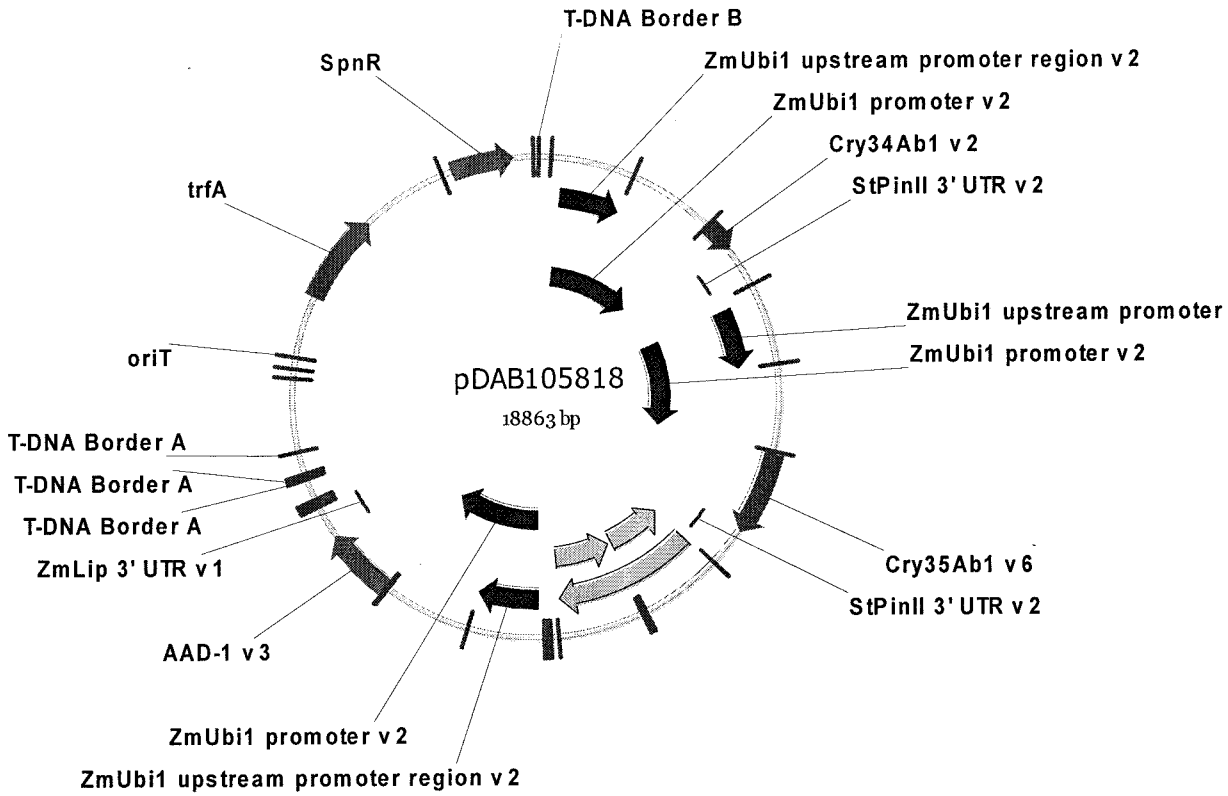

Ubi1-minP
2A
SCBV-URS
2A
Lip-3'UTR
YFP
AAD1 v3
(no stop)
Ubi1-Int
Cry34
(no stop)
Cry35
Per5-3'UTR
108719
Ubi1-minP
2A
SCBV-URS
2A
Lip-3'UTR
AAD1 v3
YFP (no stop)
Ubi1-Int
Cry34
(no stop)
Cry35
Per5-3'UTR
108720

FIG. 19


T-DNA Border B
SpnR
ZmUbi1 upstream promoter region v2
ZmUbi1 promoter v2
Cry34Ab1 v2
StPinII 3' UTR v2
trfA
ZmUbi1 upstream promoter
ZmUbi1 promoter v2
oriT
pDAB105818
18863 bp
T-DNA Border A
T-DNA Border A
T-DNA Border A
ZmLip 3' UTR v1
Cry35Ab1 v6
StPinII 3' UTR v2
AAD-1 v3
ZmUbi1 promoter v2
ZmUbi1 upstream promoter region v2

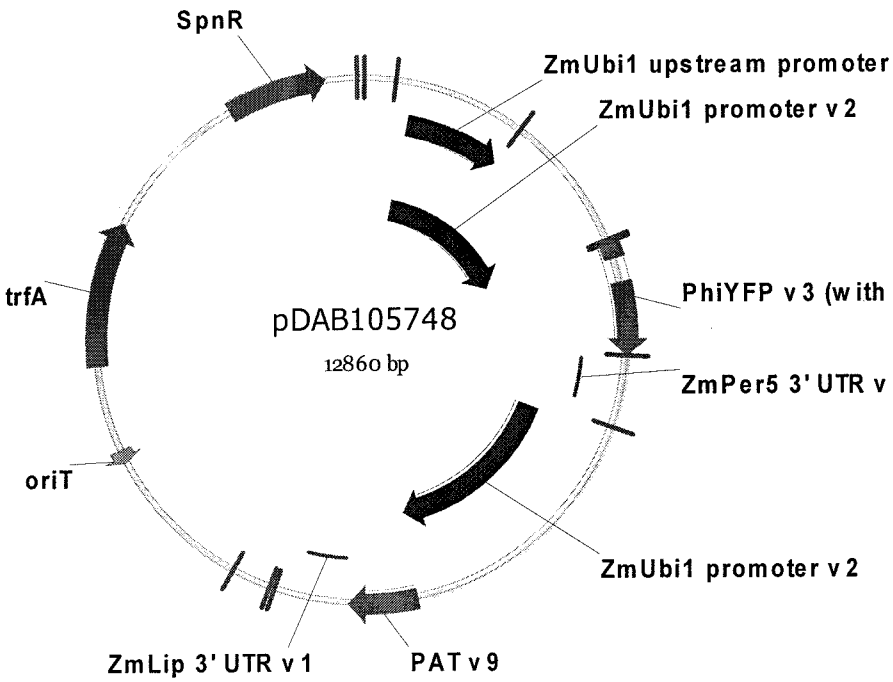

SpnR
ZmUbi1 upstream promoter
ZmUbi1 promoter v2
trfA
pDAB105748
12860 bp
PhiYFP v3 (with
ZmPer5 3' UTR v
oriT
ZmUbi1 promoter v2
ZmLip 3' UTR v1
PAT v9

FIG. 20

SEQ ID NO: 16
CTGGACCCCTCTCGAGTGTTCCGCTTCACCGTTGGACTTGCTACGCTGTCAGCATCGA
GATGTTGCGTGGCGGAGCGGCAGACTTGAGCCGTCACGGCAGGCGGCCTCCTCCTCC
TCTCACGGCATCTGTAGCTACGGGGGATTCCTTTCGCACCGCTCGTTCGCTTTCCCTT
CCTCGTCTGCCGAAATAATGTTACACCCCCTCCACAGCCTCT

SEQ ID NO: 17
CTGGACCCCTCTCGAGAGTTCCGCTCCACCGTTGGACTAGCTCTGCTGTCGGCATCCA
GAAAATGCTTGGCAGTGCGGCAGACGTGAGCCGGCACGGCAGGGGGCCTCCTCCTG
CTCTCACGGCACATGAAGCTACGGGTGATAGCTTGCCCACCGCTCCAACGCTTTCCC
TTACTCTCACGCCGTAATAAATAGACACCCCTTCCACAACCTCT

SEQ ID NO: 18
CTGGACCTCTCTCGAGAGTTGCGCTCCACCGATGGACTTGCTCCGCTGTCGGCGTCC
ATAATTTGCGTGGCGGAGCGGCAGACGGGAGCCGGCACGGCAGGGAGCCTCGTCCT
CCTCTCACGGCACCTGCAACTACGGGGGATTCCTATCCCACCGCTCCTTCGCTTTCAC
TTCTTCGCCCTCCTTAATAAGTAGACACCCCATCCGAGCCCTCT

SEQ ID NO: 19
CAAGACCCCTCTCGAGAGTTCCGCACCACCGTTGGACGTGCTCCGCTATCTGCATCC
AGAAATTGCGTGGCGGAACGGTAAACGTGAGCCGTCACGGCAGGCGGCCTCCTCCT
CCTCTCACGACACCGGCAGCTACGGGGGATACCTGTCACACAGCTCCTTCGCTTTTCT
TTCCTCGCCCGCCGTAATATGTATACACTCCCTCCGCACCCTCT

SEQ ID NO: 20
CTGGACCCCTCTCGAGGGTTCCGTTCCACCGTTGGTCTTGGTCCGCTGTCGGGATCCA
GAAATAGCGTGGCGGAGCGGCAGACGTGATCCGGCACGGCATGCGGCCTCCTAGTC
CTATCACAGCACCGGCAGCTATGGGAGATTCCATTCCCACCGCTCCTGCGCTTTCACT
GGCTGGCCCGCCGTGATAGATAGACACCCCCTCCACACCCTCT

FIG. 21A

SEQ ID NO: 21
GTTGGCTTCTCTTGTGAGTTCTGCTTCACGGATGGACTTGGTCAACGGACGGCATCCA
GAATTTGCGTGGCGTAGCGGCGGACGTGATCCGGCGCGGCAGGCGGCTTCCTCCTCC
TCTCACTTAAGCGACAGCTACAGGGGATTCCTTTCCCACCGCTCCTTCGCTTGCCGTA
CCTCGCCCGCCGTAATAAATAGACACCCCTTCCACTCCCTCT

SEQ ID NO: 22
CTGGATCCCTCTCGAGAGTGCGGCTCCGACGTTGGACTTGCTCCGAAGTCGGCATCC
AAAAATTGCGTGGTGGAGAGGCAGACTTGAGCCGGCACGGCAGGAGGCCTCGTCCT
ACTCGCACGGTATCGGCAGCAACGGGAGAATCCTTGCACTCTGCTCCTTCGCTGTAC
CTTCCTCGCCCGCTGATATTGATAGACACCCCCTGCATACCCTCT

SEQ ID NO: 23
ATGGACCCTTCTCGAGTGTTCGGCTCCACCGTTAGACTTGCTCCACGATCGACATCA
AGAAATTGCGAGACGGAGCTACAAACGTAAGAAATCTCGGTAGGGGGCCTCCTCCT
CCTCTCACGGCACCGGCAGCTACGGGGGATTCCTGTCCCACCTCTCCTTCACGTTCCC
TACCTCGCCCGCCATAATTAATAAGCACCCCCTCCGCACCCTCT

SEQ ID NO: 24
CTGGACCCCTCTAAAGAGTTCCACGCCACCGTTATAATGGCTCCGCTGTCGGCATCC
AGAAATTACTTGGCGGATCAGCAGACGTGAGCCAGCATGGCTGGCGGCCTCCTCCTC
CTCTCACGATGCCGTCAGCTACGGGGGATTCCTTTCCCAACGCTCCTTCGCTTTCCTA
TGCGCGCCTGCCGGATTAAATAGGCAGCTTCTCGTCACCCTCT

SEQ ID NO: 25
CAAGACACCTCTCGATTGTTCCGCTTCACCGTTGGACTTTCTCCTCAGTCGGCATACA
GAAATTGCTTGGCGAAGCGGCAGACATGAGCCGGCACGACATGCGTCCTCATTCTCC
TCTCATGGCACCGGCAGTTACTGGTGAATCCTATCGCACCGCTCCTTCGCTGTCCCTT
AATCGCCCGCCGAAAATAATTGACACCCCATCCACACCCTCT

FIG. 21B

SEQ ID NO: 26
GAGGACCCCTCTCGTGTGTATCGCTCCACCTTTGGAGTTGGTCCACTATCGGCGTACA
GAAAATTCGTTGCGAAGCGGCAGACGTGAGCCTACACGGCAGTCGGCCTCTACCTCC
TGACAAGGCACGTGCAGCTACAGATGATGCCTTTCCCACCACTCCTTCGCGTTCCTTT
CCTCGCCATCAGTAATGAATGGACACGTCCTCCAGACTCTCT

SEQ ID NO: 27
CTGAACCCATCTCGAGTATGCCGCACGATCGATTGACATGCTCCACTGGCAGCATCC
AGAAATTGCATTGGGGAGCATCAGGCGTGAGCCTGCACGGCAGGCGGACTATTCCT
CCTCGCGCGGCACCGGCAACTACGGGGGATGCTTGACCGACCGCTCCATCGATTTCC
CAATCTCGCTTGCCGTATTAAATAGATAACCCCTTCACACCCTCT

SEQ ID NO: 28
CTGGACTCCTTACGGGAGATCCGCTCCACCGTTGGACTAGCTCCGTTTTCGGCTTCAA
TAAAGGGCGTGGGGGAGCGGCAGTCGGGGGCAGGCACGGCAGTGGTCCTCATCCAT
ATCTCACGGGGCCGGCAGTTGAGGGGGATTCCTGTCCCACCTCACCTACTCTTTCCCT
ACCTCGTCTGCCATATTAAATAGTCACCCCCTCCACAACCTTT

SEQ ID NO: 29
TTGGACCCCTCTCGAAAGTTAGGCTCCGCCGTTGGACTGGTTTCGCGGTCATCAATC
AGGAATTGCGGGGCGGAGGGTCAGACGTGTGCCGGCACAGCAGGTGGCCTCCTCAT
CGTCACAAGGCACTGGCAACTACGGGTGATTCATTTCCTTCAGCACCTACGCTTACC
CTGCCACGCCCTCCGTATTATAATGACACCCCCTCCACACCTTAT

SEQ ID NO: 30
CTGGACCCCACGCGGGGTTTTCGTTCCTCCGTTGGGATAGCTCCGGTGTCAGCATAC
AGAGAATATATGTCGGAGCGGAAGACGTGAGCCGACACGGCGGGCTGCCGCCTCCT
CCTGTCACGACACCGGCAGGTACGGGGGATTCCGTTCCCGCCGCACAGTCACTTTCG
CTTCCTTGCCGGTCGTATTAAATAGACACCGTGTCCACAGCCTCT

FIG. 21C

SEQ ID NO: 31
CTTGAGCCCACTCTAGAGTTCCGTTTCACCGAATGACTAGCTCCGCTGTCGGTATCCA
TTAAGTGGGAGGCAGAACGTCATATGAGAGTCGGCACGGGAGGCGTTCGCCACGTC
CGCGCACTACAGCGGGAGCTGCGGAATATACCTGTCCCAATGCTGCTACGCTTTCCC
TTCCGCGCCCACCGTAGAAAAATGACAGTCCCTTCACACCCTCT

SEQ ID NO: 32
TAGGAGGCCTCTCGAAAGGTCCGGAACTCCGTAGGACGTGCTCCGCTGACAGCATCC
AGGAATATCATGGGGGAGCTGCAGACGAGAGCCTGGACGACAAGGGGTCACCTCGG
CCGCTGACAGCTGCGGCAGCAACGGAGTATGCTTTTCTCACCGCTCCGGCGCTTTCC
CTTCGACGCAGGCCAGAATAAGTAGACATCAGCGCCACACCCTCT

SEQ ID NO: 33
CTTGTCTCCACTCTGATGTTCCGCTCCAACATTTGATTTGCTCCTCTGTAGGCATACA
GTTATTGGGGGACTGATCGGCAGACGTGAGCCAGCACTGCAAACGGCCAACTCCTCC
TCTCTCGACTAAGGGATTAATTAAGGATACCTTACCCGCGGCTCCTTCTCTTTCCCTA
CCTAGCCCGCCTTATTAAATAGAGACCGCCTCCACAGCCGCT

SEQ ID NO: 34
CTGTACCCTTCACAAGGGTTACACGCTACCGATGGACTTGCACCACTGTGGGGTTCC
AATAATTGCGTGGCTGGGCGTCAGACATATTCCGGCATGGCAAGCGGCCTGCTCCTC
CTCTGGGAGCACCGGCAACAATGGGGGATTCCAAGCCCGCAGGTCCTTCGTTTTACC
GTCCTCGCCCGCCGTAGTATGTAGGCATCCCAGAGACTACCTCT

SEQ ID NO: 35
CAGGAACCCTAACGAGGGTTCCGCACGACCAAATGACTTGATCTTCTGTCGGCATCC
AGAAATGGGGTGTCAGAGCGGCATGCGTGAGCCGGCGGGGCGTGCGGCCTCATGCT
GCTCTCGCGGGACTAGGAGTTACGGGGGATACCTGTATTGCCGCTCCGACACTGTAC
CATCCTCTCCCGCCGGAGTATAGAGACACCCCCTCGACGCCATAT

FIG. 21D

SEQ ID NO: 36
CTGTGCTCCTGTATGGGGTTCAACTCCACCGTGAAATTTGCGCCTCTGTCGTCATCCA
GAAATTGCGTGGTTGATCTGCTGACGTTAAAGGGCTCTGCAGGCGGCTTCCTTCGGC
TATGAAGGTACTGGCGTCTGCAAGTGATGCTTTTGCTAACTCGCCTTCGATGTCCCTT
CCTCGCGTGCTTTAATAGGTTGTCAGCCGCTCCAGACCATTT

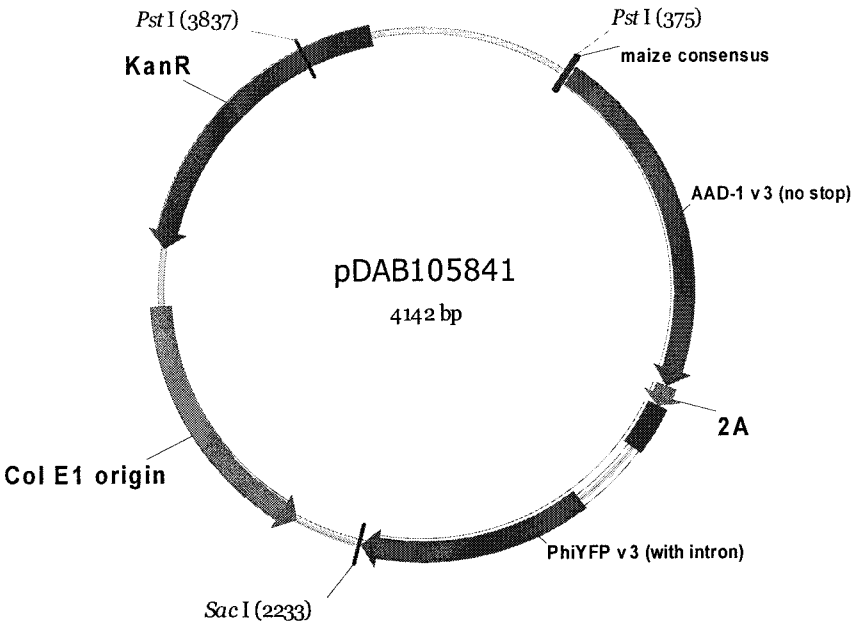
SEQ ID NO: 37
CTGGTCCCATCGCTAGTGGTACGCTCCACCGGTGGAGTAGCTCAGATGTCTGAAGGG
TGGAATTTAGAGGTGGAGAGACAGACGTGAGCTAGAGCGGCATGGGACCTGGTCCA
CCGCTCGAGGCAATGGCAACGACTGTTGAAACCTTGCCCACCACTCCTGCAATTTTC
CATCCTCACCGGCCGGAATGAATTAAAACCCACGTCACAACCTCT

SEQ ID NO: 38
CGTGACAGGGCTCGGGTGTTCGGCTCCATCGTAGTGCATGCGCCGATGTAAGTATAC
AAGAAGTACGTGGCTTGGCGTCTGACGAGGGCCGTCAAGGCAGGCGGCCTCCTTCTA
AGCTTACGGCGCCGGCAGGTTCGTAGGTTACCTTACACTCAACTCATAGTCTATCTAT
TACTCGTACTGCGTTATAAATTGTCACCCCCTCCACACCCTCT

SEQ ID NO: 39
AGGAACGCTTCTCGATGGTTGCGCACATAGGAGGGACTTGATAGTCGGTGGAAATCT
AAGAATTGCATATCAGATCTGCAGACGTTAGCCGACATGGCTAGCAGACTACTCCGC
TTCACACGTCAGCGAAAGCGACGGAGGATTTCTTGCCAACGGCGCCTTCGCGAACCC
TTCCTCGCCCGTCGGAAGAAAGATACTCCCCTTGCACACCCTCT

SEQ ID NO: 40
CTTGACTTGGCTCGAGAGTTCTGCGCTTCCATTGTAGTTGCAGCGATGTCGGAGTCCG
AGGGTTGCGTGGCGGTGCGGCAGACGTGGGCAGATACGACTGTATGCCAGCACCTA
AACATACGGTACCAGAAGCTGCGGTGGATACCTTTCCCGACGCATATACGTTTTCCG
TGCCTCTCACGCCGTAGTAAATAAACTCCCCCTCCTGTTCCTTT

FIG. 21E


PstI (3837)
KanR
PstI (375)
maize consensus
AAD-1 v3 (no stop)
pDAB105841
4142 bp
2A
Col E1 origin
PhiYFP v3 (with intron)
SacI (2233)

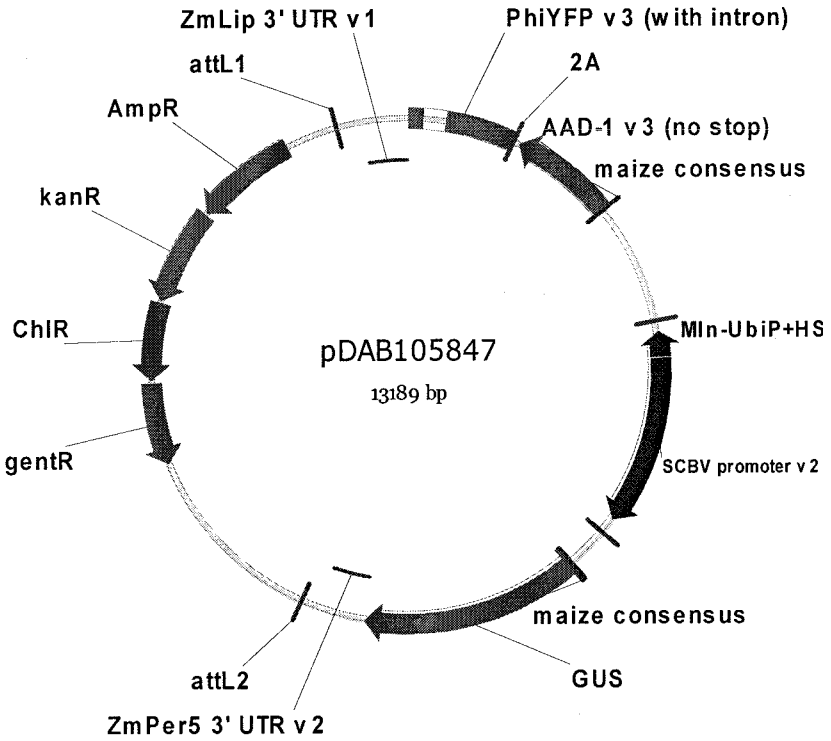
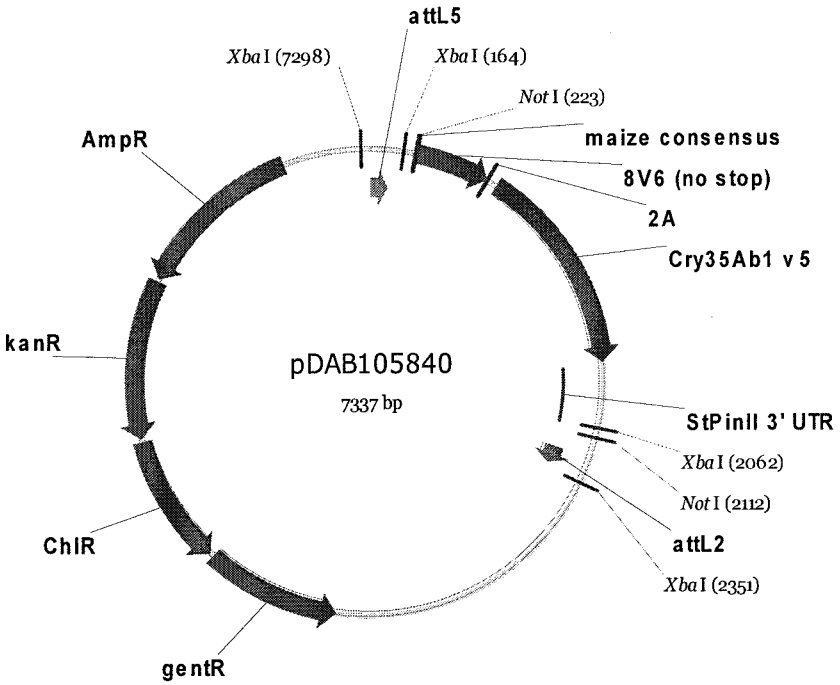

ZmLip 3' UTR v1
PhiYFP v3 (with intron)
attL1
2A
AmpR
AAD-1 v3 (no stop)
maize consensus
kanR
ChlR
pDAB105847
13189 bp
MIn-UbiP+HS
gentR
SCBV promoter v2
maize consensus
attL2
GUS
ZmPer5 3' UTR v2

FIG. 22


attL5
XbaI (7298)
XbaI (164)
NotI (223)
maize consensus
8V6 (no stop)
2A
Cry35Ab1 v 5
AmpR
kanR
pDAB105840
7337 bp
StPinII 3' UTR
XbaI (2062)
NotI (2112)
attL2
XbaI (2351)
ChlR
gentR

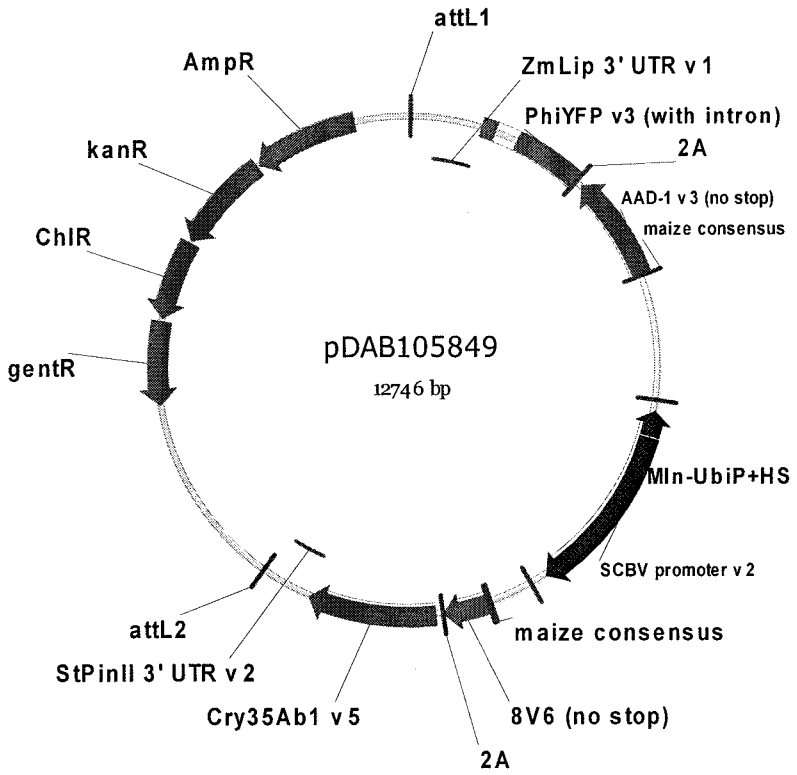

attL1
AmpR
ZmLip 3' UTR v 1
PhiYFP v3 (with intron)
2A
kanR
AAD-1 v 3 (no stop)
maize consensus
ChlR
pDAB105849
12746 bp
gentR
MIn-UbiP+HS
SCBV promoter v 2
attL2
maize consensus
StPinII 3' UTR v 2
Cry35Ab1 v 5
8V6 (no stop)
2A

FIG. 23

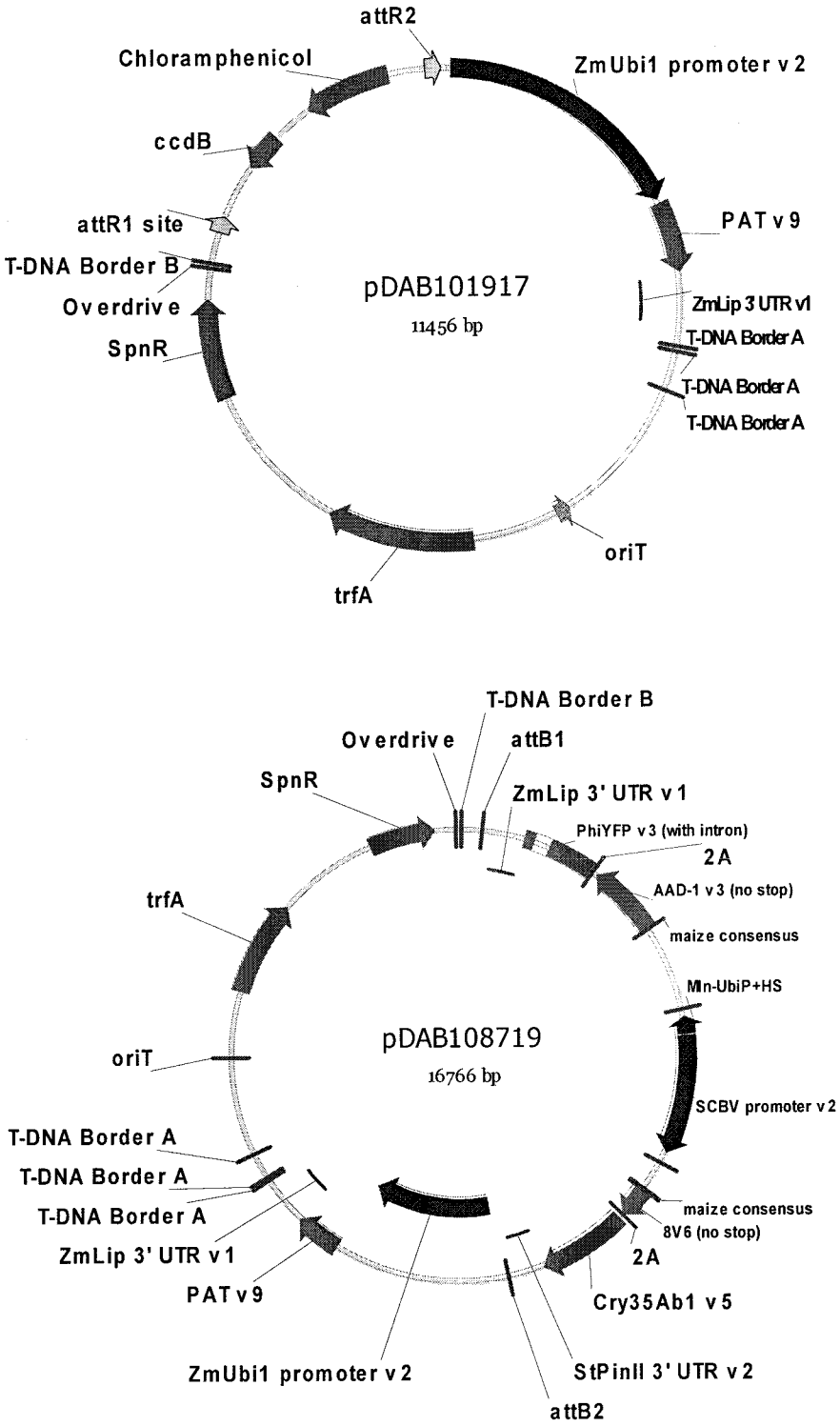

attR2
Chloramphenicol
ZmUbi1 promoter v2
ccdB
PAT v9
attR1 site
T-DNA Border B
pDAB101917
11456 bp
ZmLip 3 UTR v1
Overdrive
T-DNA Border A
SpnR
T-DNA Border A
T-DNA Border A
oriT
trfA
T-DNA Border B
Overdrive
attB1
SpnR
ZmLip 3' UTR v1
PhiYFP v3 (with intron)
2A
AAD-1 v3 (no stop)
trfA
maize consensus
Mn-UbiP+HS
pDAB108719
16766 bp
oriT
SCBV promoter v2
T-DNA Border A
T-DNA Border A
maize consensus
T-DNA Border A
8V6 (no stop)
ZmLip 3' UTR v1
2A
PAT v9
Cry35Ab1 v5
ZmUbi1 promoter v2
StPinII 3' UTR v2
attB2

FIG. 24

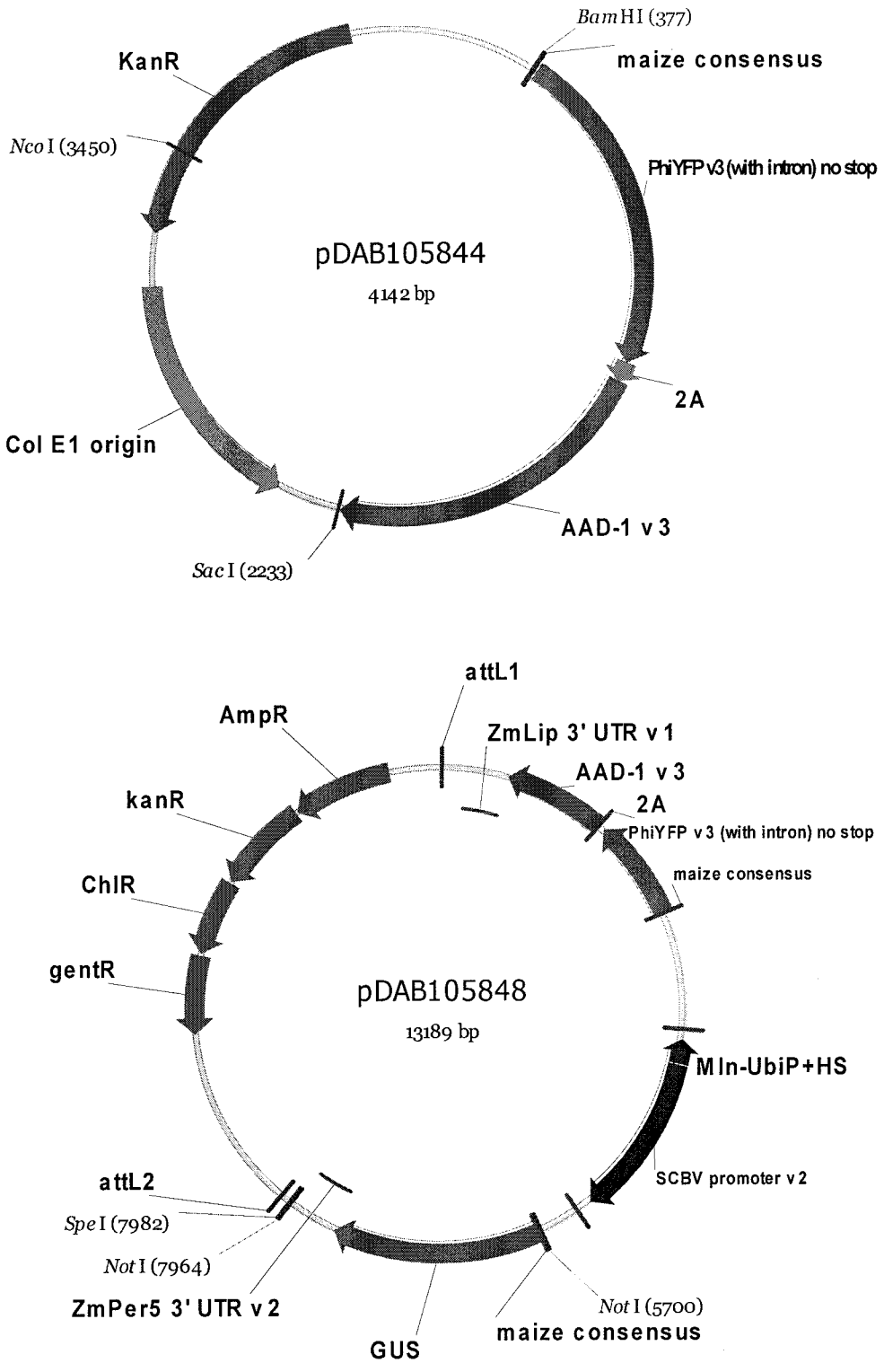
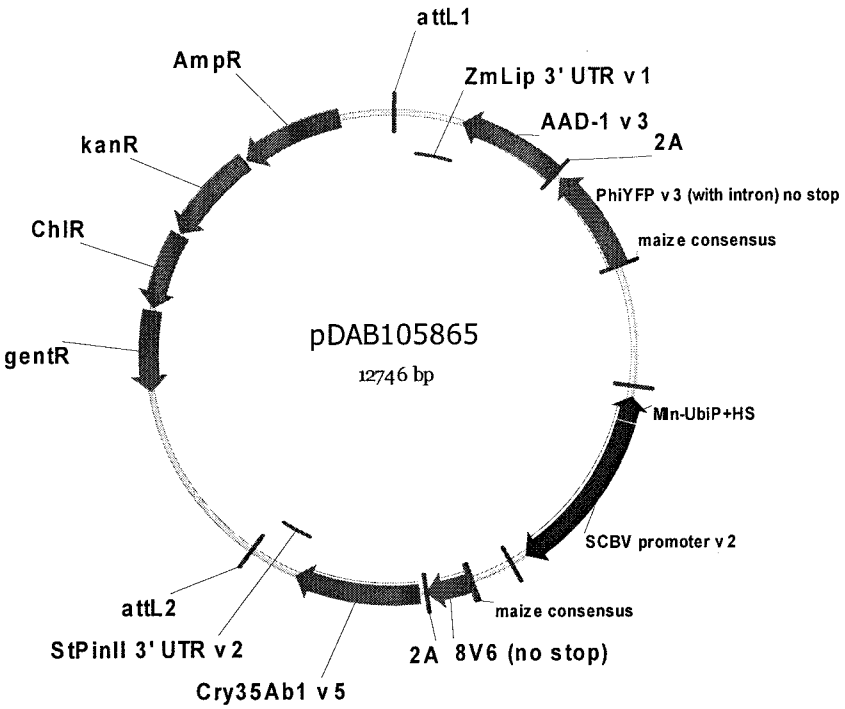

BamHI (377)
maize consensus
PhiYFP v3 (with intron) no stop
2A
AAD-1 v3
SacI (2233)
Col E1 origin
NcoI (3450)
KanR
pDAB105844
4142 bp
attL1
ZmLip 3' UTR v1
AAD-1 v3
2A
PhiYFP v3 (with intron) no stop
maize consensus
MIn-UbiP+HS
SCBV promoter v2
NotI (5700)
maize consensus
GUS
ZmPer5 3' UTR v2
NotI (7964)
SpeI (7982)
attL2
gentR
ChlR
kanR
AmpR
pDAB105848
13189 bp

FIG. 25


attL1
AmpR
ZmLip 3' UTR v1
AAD-1 v3
2A
kanR
PhiYFP v3 (with intron) no stop
maize consensus
ChlR
pDAB105865
12746 bp
gentR
Mln-UbiP+HS
SCBV promoter v2
attL2
maize consensus
StPinII 3' UTR v2
2A
8V6 (no stop)
Cry35Ab1 v5

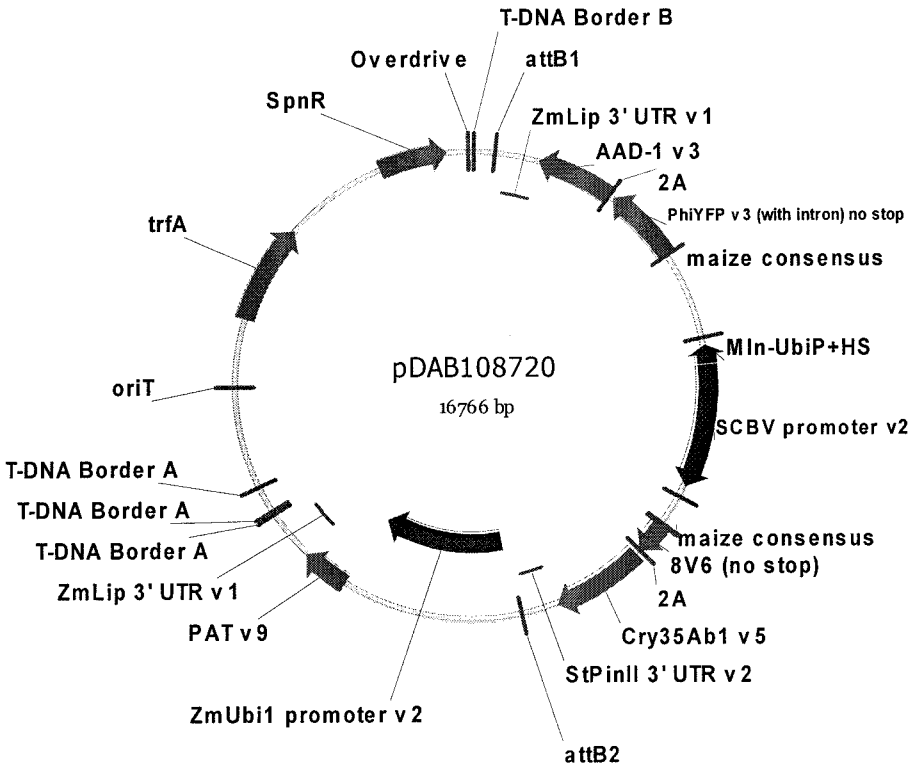

T-DNA Border B
Overdrive
attB1
SpnR
ZmLip 3' UTR v1
AAD-1 v3
2A
PhiYFP v3 (with intron) no stop
trfA
maize consensus
Mln-UbiP+HS
pDAB108720
16766 bp
oriT
SCBV promoter v2
T-DNA Border A
T-DNA Border A
maize consensus
8V6 (no stop)
T-DNA Border A
2A
ZmLip 3' UTR v1
Cry35Ab1 v5
PAT v9
StPinII 3' UTR v2
ZmUbi1 promoter v2
attB2

FIG. 26

```
   1    AATTACAACG GTATATATCC TGCCAGTCAG CATCATCACA CCAAAAGTTA GGCCCGAATA
  61    GTTTGAAATT AGAAAGCTCG CAATTGAGGT CTACAGGCCA AATTCGCTCT TAGCCGTACA
 121    ATATTACTCA CCAGATCCTA ACCGGTGTGA TCATGGGCCG CGATTAAAAA TCTCAATTAT
 181    ATTTGGTCTA ATTTAGTTTG GTATTGAGTA AAACAAATTC GGCGCCATGC CCGGGCAAGC
 241    GGCCGCACAA GTTTGTACAA AAAAGCAGGC TGAGTATTCA CTACAGTAGT GCATCGATGG
 301    AGTCATCACG CAGACTATCT CAGCATGTGC GTAGCACGTC TAGACCTAGG TAGGTTAATT
 361    AAGCTTGCAT GCCGGAGGAA ATATGAATTC AGCACTTAAA GATCTTTAGA AGAAAGCAAA
                                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                             ZmLip 3' UTR v1
 421    GCATTTATTA ATACATAACA ATGTCCAGGT AGCCCAGCTG AATTACAATA CGCAACTGCT
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          ZmLip 3' UTR v1
 481    CATAATAATT CAACAAACCC AAGTAGTACA CAACATCCAG AAGCAAATAA AGCCCATACG
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          ZmLip 3' UTR v1
 541    TACCAAAGCC TACACAAGCA GCAACACTCA CTGCCAGTGC CGGTGGGTCT TTAAAGCACA
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          ZmLip 3' UTR v1
 601    CGGGCCTTGA CCACGCGATC CACCTTGAAA CAAACTTGGT AAAATTAAAG CAAACCAGAA
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          ZmLip 3' UTR v1
 661    GCACACACAC GCCAACGCAA CGCTTCTGAT CGCGCGCCCA AGGCCCGGCC GGCCAGAACG
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          ZmLip 3' UTR v1
 721    TACGACGGAC ACGCACACGC TGCGACCGAG CTCTCAAAGG TAGGTCTTGC GACAGTCAAC
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          ZmLip 3' UTR v1                    PhiYFP v3 (with intron)
 781    AGCTCTGACA GTTTCTTTCA AGCTCATGTT GTCTCTGTGG TCTGTCACAT CTTTGGAAAG
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                        PhiYFP v3 (with intron)
 841    TTTCACATGG TAAGACATAT GATGATACTC TGGAACATGA ACTGGACCTC CACCAATGGG
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                        PhiYFP v3 (with intron)
 901    AGTGTTCATC TGGGTGTGGT CAGCCACTAT GAAGTCGCCT TTGCTGCCAG TAATCTCATG
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                        PhiYFP v3 (with intron)
 961    ACATATCTTG AAGGCTGACT TGAGACCGTG GTTGGCTTGG TCTCCCCAGA TGTAGAGGCA
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                        PhiYFP v3 (with intron)
1021    GTGGGGAGTG AAGTTGAACT CCAAGTTCTT TCCCAACACG TGACCATCTT TCTTGAAGCC
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                        PhiYFP v3 (with intron)
1081    TTGACCATTG AGTTTGACCC TATTGTAGAC AGACCCATTC TCAAAGGTGA CTTCAGCCCT
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                        PhiYFP v3 (with intron)
1141    AGTCTTGAAG TTGCCATCTC CTTCAAAGGT GATTGTGCGC TCTTGCACAT AGCCATCTGG
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                        PhiYFP v3 (with intron)
1201    CATACAGGAC TTGTAGAAGT CCTTCAACTC TGGACCATAC TTGGCAAAGC ACTGTGCTCC
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

FIG. 27A

```
                    PhiYFP v3 (with intron)
1261    ATAGGTGAGA GTGGTGACAA GTGTGCTCCA AGGCACAGGA ACATCTCCGG TAGTACAGAT
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                    PhiYFP v3 (with intron)
1321    GAATTGTGCA TCAACCTGCA CATCACCATG TTTTGGTCAT ATATTAGAAA AGTTATAAAT
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                    PhiYFP v3 (with intron)
1381    TAAAATATAC ACACTTATAA ACTACAGAAA AGCAATAGCT ATATACTACA TTCTTTTATT
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                    PhiYFP v3 (with intron)
1441    TTGAAAAAAA TACTTGAAAT ACTATATTAC TACTAATTAG TGATAATTAT TATATATATA
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                    PhiYFP v3 (with intron)
1501    TCAAAGGTAG AAGCAGAAAC ATACCTTTCC CACTGAGGCA TCTCCGTAGC CTTTCCCACG
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                    PhiYFP v3 (with intron)
1561    TATGCTAAAG GTGTGGCCAT CAACATTCCC TTCCATCTCC ACAACGTAAG GAATCTTCCC
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                    PhiYFP v3 (with intron)
1621    ATGAAAGAGA AGTGCTCCAG ATGACATAGG GCCGGGATTC TCCTCCACGT CACCGCATGT
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    PhiYFP v3 (with intron)
1681    TAGAAGACTT CCTCTGCCCT CGCGGGCAGG CCTAACTCCA CCAACTGTGG TGCGAGTCAA
                             ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                        AAD-1 v3 (no stop)
1741    GTATCTGAAC TTGCCAGCAT AGTCAGGAAC AGCACGGTGC ATGGTGCACA AGTTGTCCCA
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          AAD-1 v3 (no stop)
1801    GACAAGGACT TGGTCTTTCT TCCACCTCAC ACGGCAAGTG AAGTCAAATC TGGTGGCATG
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          AAD-1 v3 (no stop)
1861    CTCATAGAGG AACTGAAGCA ATGGCTTTGA TTCTGCATCT GTCATGCCCT CAATTCTCTG
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          AAD-1 v3 (no stop)
1921    ACAGTAGACT TGATTCACAT AAAGGCCTTT CCTTCCAGAG CCAGGATGAG TCACAACCAA
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          AAD-1 v3 (no stop)
1981    GGGATGGACT GTCTCTCTGT CACCAGCATC AACATCCATC ACCTTGACTG AGGTGTTGCT
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          AAD-1 v3 (no stop)
2041    GAAGCGACGG TTCTGTGCTT GGTAGAGGGA ACCGAACACA CGTGTGGCAG AGTGCACAAC
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          AAD-1 v3 (no stop)
2101    GTTGAGCCCT TCGATGGTGG CTTGCATGGT TGGAGACAAG GTCTCCCAAG CTGTGTACAT
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          AAD-1 v3 (no stop)
2161    TGAAAGGAAC CCAGTGTCTC CGCCATGCTC AGGAACATCT ATGGCCCTCA TCACAACAGC
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          AAD-1 v3 (no stop)
2221    AGCTGGAGGT GCATCAAGGA AAGTGGAGTC TGTGTGCCAG TCATCACCAA TCACCCTTCC
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

FIG. 27B

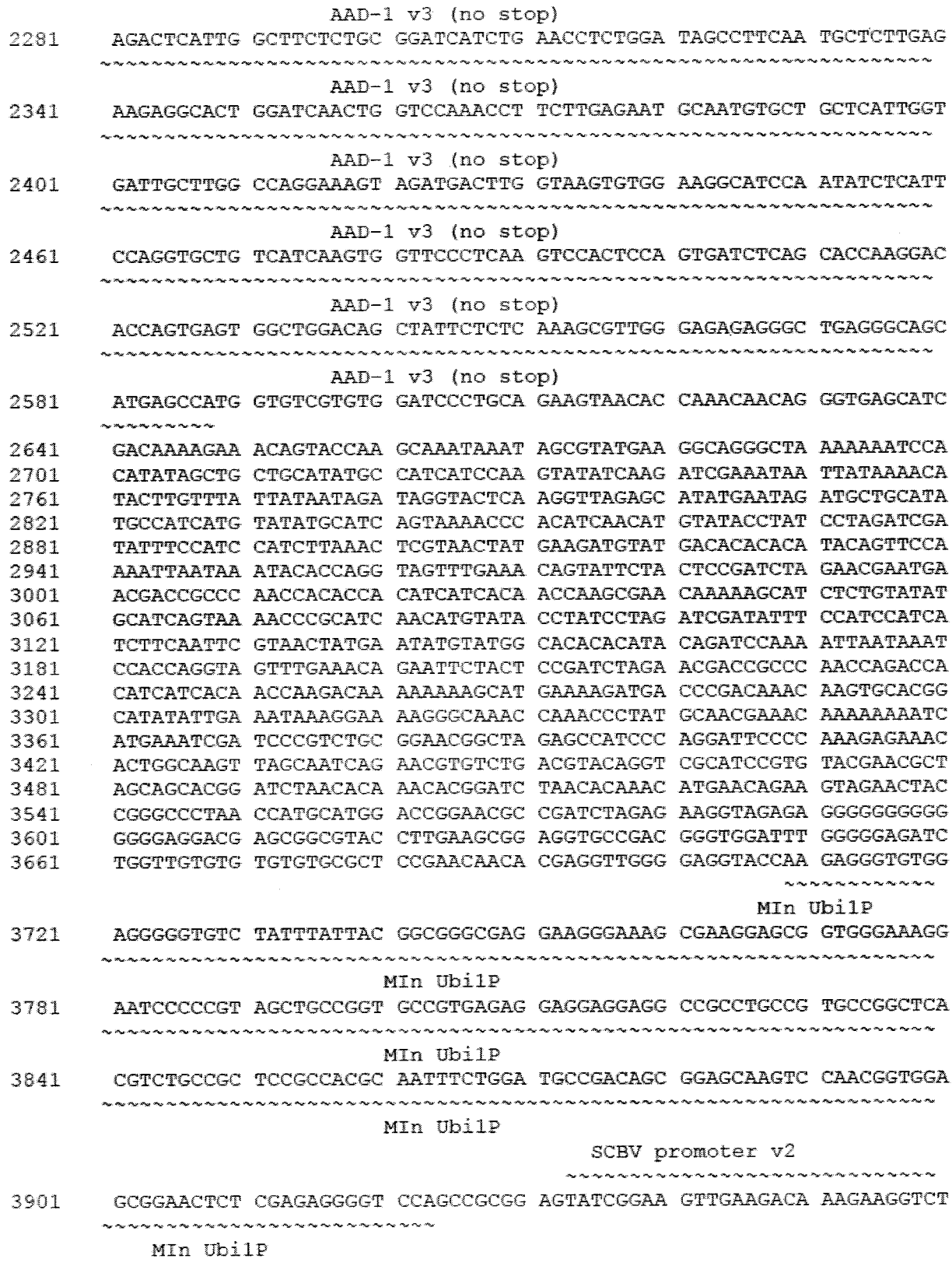

AAD-1 v3 (no stop)
2281 AGACTCATTG GCTTCTCTGC GGATCATCTG AACCTCTGGA TAGCCTTCAA TGCTCTTGAG
AAD-1 v3 (no stop)
2341 AAGAGGCACT GGATCAACTG GTCCAAACCT TCTTGAGAAT GCAATGTGCT GCTCATTGGT
AAD-1 v3 (no stop)
2401 GATTGCTTGG CCAGGAAAGT AGATGACTTG GTAAGTGTGG AAGGCATCCA ATATCTCATT
AAD-1 v3 (no stop)
2461 CCAGGTGCTG TCATCAAGTG GTTCCCTCAA GTCCACTCCA GTGATCTCAG CACCAAGGAC
AAD-1 v3 (no stop)
2521 ACCAGTGAGT GGCTGGACAG CTATTCTCTC AAAGCGTTGG GAGAGAGGGC TGAGGGCAGC
AAD-1 v3 (no stop)
2581 ATGAGCCATG GTGTCGTGTG GATCCCTGCA GAAGTAACAC CAAACAACAG GGTGAGCATC
2641 GACAAAAGAA ACAGTACCAA GCAAATAAAT AGCGTATGAA GGCAGGGCTA AAAAAATCCA
2701 CATATAGCTG CTGCATATGC CATCATCCAA GTATATCAAG ATCGAAATAA TTATAAAACA
2761 TACTTGTTTA TTATAATAGA TAGGTACTCA AGGTTAGAGC ATATGAATAG ATGCTGCATA
2821 TGCCATCATG TATATGCATC AGTAAAACCC ACATCAACAT GTATACCTAT CCTAGATCGA
2881 TATTTCCATC CATCTTAAAC TCGTAACTAT GAAGATGTAT GACACACACA TACAGTTCCA
2941 AAATTAATAA ATACACCAGG TAGTTTGAAA CAGTATTCTA CTCCGATCTA GAACGAATGA
3001 ACGACCGCCC AACCACACCA CATCATCACA ACCAAGCGAA CAAAAAGCAT CTCTGTATAT
3061 GCATCAGTAA AACCCGCATC AACATGTATA CCTATCCTAG ATCGATATTT CCATCCATCA
3121 TCTTCAATTC GTAACTATGA ATATGTATGG CACACACATA CAGATCCAAA ATTAATAAAT
3181 CCACCAGGTA GTTTGAAACA GAATTCTACT CCGATCTAGA ACGACCGCCC AACCAGACCA
3241 CATCATCACA ACCAAGACAA AAAAAAGCAT GAAAAGATGA CCCGACAAAC AAGTGCACGG
3301 CATATATTGA AATAAAGGAA AAGGGCAAAC CAAACCCTAT GCAACGAAAC AAAAAAAATC
3361 ATGAAATCGA TCCCGTCTGC GGAACGGCTA GAGCCATCCC AGGATTCCCC AAAGAGAAAC
3421 ACTGGCAAGT TAGCAATCAG AACGTGTCTG ACGTACAGGT CGCATCCGTG TACGAACGCT
3481 AGCAGCACGG ATCTAACACA AACACGGATC TAACACAAAC ATGAACAGAA GTAGAACTAC
3541 CGGGCCCTAA CCATGCATGG ACCGGAACGC CGATCTAGAG AAGGTAGAGA GGGGGGGGGG
3601 GGGGAGGACG AGCGGCGTAC CTTGAAGCGG AGGTGCCGAC GGGTGGATTT GGGGGAGATC
3661 TGGTTGTGTG TGTGTGCGCT CCGAACAACA CGAGGTTGGG GAGGTACCAA GAGGGTGTGG
MIn Ubi1P
3721 AGGGGGTGTC TATTTATTAC GGCGGGCGAG GAAGGGAAAG CGAAGGAGCG GTGGGAAAGG
MIn Ubi1P
3781 AATCCCCCGT AGCTGCCGGT GCCGTGAGAG GAGGAGGAGG CCGCCTGCCG TGCCGGCTCA
MIn Ubi1P
3841 CGTCTGCCGC TCCGCCACGC AATTTCTGGA TGCCGACAGC GGAGCAAGTC CAACGGTGGA
MIn Ubi1P
SCBV promoter v2
3901 GCGGAACTCT CGAGAGGGGT CCAGCCGCGG AGTATCGGAA GTTGAAGACA AAGAAGGTCT
MIn Ubi1P

FIG. 27C

```
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3961   TAAATCCTGG CTAGCAACAC TGAACTATGC CAGAAACCAC ATCAAAGCAT ATCGGCAAGC
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4021   TTCTTGGCCC ATTATATCCA AAGACCTCAG AGAAAGGTGA GCGAAGGCTC AATTCAGAAG
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4081   ATTGGAAGCT GATCAATAGG ATCAAGACAA TGGTGAGAAC GCTTCCAAAT CTCACTATTC
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4141   CACCAGAAGA TGCATACATT ATCATTGAAA CAGATGCATG TGCAACTGGA TGGGGAGCAG
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4201   TATGCAAGTG GAAGAAAAAC AAGGCAGACC CAAGAAATAC AGAGCAAATC TGTAGGTATG
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4261   CCAGTGGAAA ATTTGATAAG CCAAAAGGAA CCTGTGATGC AGAAATCTAT GGGGTTATGA
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4321   ATGGCTTAGA AAAGATGAGA TTGTTCTACT TGGACAAAAG AGAGATCACA GTCAGAACTG
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4381   ACAGTAGTGC AATCGAAAGG TTCTACAACA AGAGTGCTGA ACACAAGCCT TCTGAGATCA
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4441   GATGGATCAG GTTCATGGAC TACATCACTG GTGCAGGACC AGAGATAGTC ATTGAACACA
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4501   TAAAAGGGAA GAGCAATGGT TTAGCTGACA TCTTGTCCAG GCTCAAAGCC AAATTAGCTC
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4561   AGAATGAACC AACGGAAGAG ATGATCCTGC TTACACAAGC CATAAGGGAA GTAATTCCTT
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4621   ATCCAGATCA TCCATACACT GAGCAACTCA GAGAATGGGG AAACAAAATT CTGGATCCAT
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4681   TCCCCACATT CAAGAAGGAC ATGTTCGAAA GAACAGAGCA AGCTTTTATG CTAACAGAGG
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4741   AACCAGTTCT ACTCTGTGCA TGCAGGAAGC CTGCAATTCA GTTAGTGTCC AGAACATCTG
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4801   CCAACCCAGG AAGGAAATTC TTCAAGTGCG CAATGAACAA ATGCCATTGC TGGTACTGGG
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4861   CAGATCTCAT TGAAGAACAC ATTCAAGACA GAATTGATGA ATTTCTCAAG AATCTTGAAG
                    SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4921   TTCTGAAGAC CGGTGGCGTG CAAACAATGG AGGAGGAACT TATGAAGGAA GTCACCAAGC
```

FIG. 27D

```
                          SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4981   TGAAGATAGA AGAGCAGGAG TTCGAGGAAT ACCAGGCCAC ACCAAGGGCT ATGTCGCCAG
                          SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5041   TAGCCGCAGA AGATGTGCTA GATCTCCAAG ACGTAAGCAA TGACGATTGA GGAGGCATTG
                          SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5101   ACGTCAGGGA TGACCGCAGC GGAGAGTACT GGGCCCATTC AGTGGATGCT CCACTGAGTT
                          SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5161   GTATTATTGT GTGCTTTTCG GACAAGTGTG CTGTCCACTT TCTTTTGGCA CCTGTGCCAC
                          SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5221   TTTATTCCTT GTCTGCCACG ATGCCTTTGC TTAGCTTGTA AGCAAGGATC GCAGTGCGTG
                          SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5281   TGTGACACCA CCCCCCTTCC GACGCTCTGC CTATATAAGG CACCGTCTGT AAGCTCTTAC
      SCBV promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~
5341   GATCATCGGT AGTTCACCAA GGCCCGGGGT CGGATCTAGC TGAAGGCTCG ACAAGGCAGT
5401   CCACGGAGGA GCTGATATTT GGTGGACAAG CTGTGGATAG GAGCAACCCT ATCCCTAATA
5461   TACCAGCACC ACCAAGTCAG GGCAATCCCC AGATCACCCC AGCAGATTCG AAGAAGGTAC
5521   AGTACACACA CATGTATATA TGTATGATGT ATCCCTTCGA TCGAAGGCAT GCCTTGGTAT
5581   AATCACTGAG TAGTCATTTT ATTACTTTGT TTTGACAAGT CAGTAGTTCA TCCATTTGTC
5641   CCATTTTTTC AGCTTGGAAG TTTGGTTGCA CTGGCCTTGG TCTAATAACT GAGTAGTCAT
5701   TTTATTACGT TGTTTCGACA AGTCAGTAGC TCATCCATCT GTCCCATTTT TTCAGCTAGG
5761   AAGTTTGGTT GCACTGGCCT TGGACTAATA ACTGATTAGT CATTTTATTA CATTGTTTCG
5821   ACAAGTCAGT AGCTCATCCA TCTGTCCCAT TTTTCAGCTA GGAAGTTCGC GGCCGCACAC
                              8V6 (no stop)
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5881   GACACCATGT CCGCCCGCGA GGTGCACATC GACGTGAACA ACAAGACCGG CCACACCCTC
                            8V6 (no stop)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5941   CAGCTGGAGG ACAAGACCAA GCTCGACGGC GGCAGGTGGC GCACCTCCCC GACCAACGTG
                            8V6 (no stop)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6001   GCCAACGACC AGATCAAGAC CTTCGTGGCC GAATCCAACG GCTTCATGAC CGGCACCGAG
                            8V6 (no stop)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6061   GGCACCATCT ACTACTCCAT CAACGGCGAG GCCGAGATCA GCCTCTACTT CGACAACCCG
                            8V6 (no stop)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6121   TTCGCCGGCT CCAACAAATA CGACGGCCAC TCCAACAAGT CCCAGTACGA GATCATCACC
                            8V6 (no stop)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6181   CAGGGCGGCT CCGGCAACCA GTCCCACGTG ACCTACACCA TCCAGACCAC CTCCTCCCGC
     8V6 (no stop)
       ~~~~~~~~~~~~~~~~~
6241   TACGGCCACA AGTCCGAGGG CAGAGGAAGT CTTCTAACAT GCGGTGACGT GGAGGAGAAT
```

FIG. 27E

```
                                  Cry35Ab1 v5
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6301    CCCGGCCCTA TGCTCGACAC CAACAAGGTG TACGAGATCA GCAACCACGC CAACGGCCTC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6361    TACGCCGCCA CCTACCTCTC CCTCGACGAC TCCGGCGTGT CCCTCATGAA CAAGAACGAC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6421    GACGACATCG ACGACTACAA CCTCAAGTGG TTCCTCTTCC CGATCGACGA CGACCAGTAC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6481    ATCATCACCT CCTACGCCGC CAACAACTGC AAGGTGTGGA ACGTGAACAA CGACAAGATC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6541    AACGTGTCCA CCTACTCCTC CACCAACTCC ATCCAGAAGT GGCAGATCAA GGCCAACGGC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6601    TCCTCCTACG TGATCCAGTC CGACAACGGC AAGGTGCTCA CCGCCGGCAC CGGCCAGGCC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6661    CTCGGCCTCA TCCGCCTCAC CGACGAGTCC TCCAACAACC CGAACCAGCA GTGGAACCTG
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6721    ACGTCCGTGC AGACCATCCA GCTCCCGCAG AAGCCGATCA TCGACACCAA GCTCAAGGAC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6781    TACCCGAAGT ACTCCCCGAC CGGCAACATC GACAACGGCA CCTCCCCGCA GCTCATGGGC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6841    TGGACCCTCG TGCCGTGCAT CATGGTGAAC GACCCGAACA TCGACAAGAA CACCCAGATC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6901    AAGACCACCC CGTACTACAT CCTCAAGAAG TACCAGTACT GGCAGAGGGC CGTGGGCTCC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6961    AACGTCGCGC TCCGCCCGCA CGAGAAGAAG TCCTACACCT ACGAGTGGGG CACCGAGATC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7021    GACCAGAAGA CCACCATCAT CAACACCCTC GGCTTCCAGA TCAACATCGA CAGCGGCATG
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7081    AAGTTCGACA TCCCGGAGGT GGGCGGCGGT ACCGACGAGA TCAAGACCCA GCTCAACGAG
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7141    GAGCTCAAGA TCGAGTACTC CCACGAGACG AAGATCATGG AGAAGTACCA GGAGCAGTCC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7201    GAGATCGACA ACCCGACCGA CCAGTCCATG AACTCCATCG GCTTCCTCAC CATCACCTCC
                                  Cry35Ab1 v5
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7261    CTGGAGCTCT ACCGCTACAA CGGCTCCGAG ATCCGCATCA TGCAGATCCA GACCTCCGAC
```

FIG. 27F

```
                          Cry35Ab1 v5
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7321     AACGACACCT ACAACGTGAC CTCCTACCCG AACCACCAGC AGGCCCTGCT GTGAGTAGTT
                                          StPinII 3' UTR v2
                                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7381     AGCTTAATCA CCTAGAACCT AGACTTGTCC ATCTTCTGGA TTGGCCAACT TAATTAATGT
                          StPinII 3' UTR v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7441     ATGAAATAAA AGGATGCACA CATAGTGACA TGCTAATCAC TATAATGTGG GCATCAAAGT
                          StPinII 3' UTR v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7501     TGTGTGTTAT GTGTAATTAC TAGTTATCTG AATAAAAGAG AAAGAGATCA TCCATATTTC
                          StPinII 3' UTR v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7561     TTATCCTAAA TGAATGTCAC GTGTCTTTAT AATTCTTTGA TGAACCAGAT GCATTTCATT
                          StPinII 3' UTR v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7621     AACCAAATCC ATATACATAT AAATATTAAT CATATATAAT TAATATCAAT TGGGTTAGCA
          StPinII 3' UTR v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7681     AAACAAATCT AGTCTAGGTG TGTTTTGCTC TAGTGCTAGC CTCGAGGTCG ACTCTGATCA
7741     TGGATGCTAC GTCACGGCAG TACAGGACTA TCATCTTGAA AGTCGATTGA GCATCGAAAC
7801     CCAGCTTTCT TGTACAAAGT GGTTGCGGCC GCTTAATTAA ATTTAAATGT TTGGGGATCC
                                          ZmUbi1 promoter v2
                                   ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7861     TCTAGAGTCG ACCTGCAGTG CAGCGTGACC CGGTCGTGCC CCTCTCTAGA GATAATGAGC
                          ZmUbi1 promoter v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7921     ATTGCATGTC TAAGTTATAA AAAATTACCA CATATTTTTT TTGTCACACT TGTTTGAAGT
                          ZmUbi1 promoter v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7981     GCAGTTTATC TATCTTTATA CATATATTTA AACTTTACTC TACGAATAAT ATAATCTATA
                          ZmUbi1 promoter v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8041     GTACTACAAT AATATCAGTG TTTTAGAGAA TCATATAAAT GAACAGTTAG ACATGGTCTA
                          ZmUbi1 promoter v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8101     AAGGACAATT GAGTATTTTG ACAACAGGAC TCTACAGTTT TATCTTTTTA GTGTGCATGT
                          ZmUbi1 promoter v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8161     GTTCTCCTTT TTTTTTGCAA ATAGCTTCAC CTATATAATA CTTCATCCAT TTTATTAGTA
                          ZmUbi1 promoter v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8221     CATCCATTTA GGGTTTAGGG TTAATGGTTT TTATAGACTA ATTTTTTTAG TACATCTATT
                          ZmUbi1 promoter v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8281     TTATTCTATT TTAGCCTCTA AATTAAGAAA ACTAAAACTC TATTTTAGTT TTTTTATTTA
                          ZmUbi1 promoter v2
              ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8341     ATAGTTTAGA TATAAAATAG AATAAAATAA AGTGACTAAA AATTAAACAA ATACCCTTTA
```

FIG. 27G

ZmUbi1 promoter v2

8401 AGAAATTAAA AAAACTAAGG AAACATTTTT CTTGTTTCGA GTAGATAATG CCAGCCTGTT

ZmUbi1 promoter v2

8461 AAACGCCGTC GACGAGTCTA ACGGACACCA ACCAGCGAAC CAGCAGCGTC GCGTCGGGCC

ZmUbi1 promoter v2

8521 AAGCGAAGCA GACGGCACGG CATCTCTGTC GCTGCCTCTG GACCCCTCTC GAGAGTTCCG

ZmUbi1 promoter v2

8581 CTCCACCGTT GGACTTGCTC CGCTGTCGGC ATCCAGAAAT TGCGTGGCGG AGCGGCAGAC

ZmUbi1 promoter v2

8641 GTGAGCCGGC ACGGCAGGCG GCCTCCTCCT CCTCTCACGG CACCGGCAGC TACGGGGGAT

ZmUbi1 promoter v2

8701 TCCTTTCCCA CCGCTCCTTC GCTTTCCCTT CCTCGCCCGC CGTAATAAAT AGACACCCCC

ZmUbi1 promoter v2

8761 TCCACACCCT CTTTCCCCAA CCTCGTGTTG TTCGGAGCGC ACACACACAC AACCAGATCT

ZmUbi1 promoter v2

8821 CCCCCAAATC CACCCGTCGG CACCTCCGCT TCAAGGTACG CCGCTCGTCC TCCCCCCCCC

ZmUbi1 promoter v2

8881 CCCCCCTCTC TACCTTCTCT AGATCGGCGT TCCGGTCCAT GCATGGTTAG GGCCCGGTAG

ZmUbi1 promoter v2

8941 TTCTACTTCT GTTCATGTTT GTGTTAGATC CGTGTTTGTG TTAGATCCGT GCTGCTAGCG

ZmUbi1 promoter v2

9001 TTCGTACACG GATGCGACCT GTACGTCAGA CACGTTCTGA TTGCTAACTT GCCAGTGTTT

ZmUbi1 promoter v2

9061 CTCTTTGGGG AATCCTGGGA TGGCTCTAGC CGTTCCGCAG ACGGGATCGA TTTCATGATT

ZmUbi1 promoter v2

9121 TTTTTTGTTT CGTTGCATAG GGTTTGGTTT GCCCTTTTCC TTTATTTCAA TATATGCCGT

ZmUbi1 promoter v2

9181 GCACTTGTTT GTCGGGTCAT CTTTTCATGC TTTTTTTTGT CTTGGTTGTG ATGATGTGGT

ZmUbi1 promoter v2

9241 CTGGTTGGGC GGTCGTTCTA GATCGGAGTA GAATTCTGTT TCAAACTACC TGGTGGATTT

ZmUbi1 promoter v2

9301 ATTAATTTTG GATCTGTATG TGTGTGCCAT ACATATTCAT AGTTACGAAT TGAAGATGAT

ZmUbi1 promoter v2

9361 GGATGGAAAT ATCGATCTAG GATAGGTATA CATGTTGATG CGGGTTTTAC TGATGCATAT

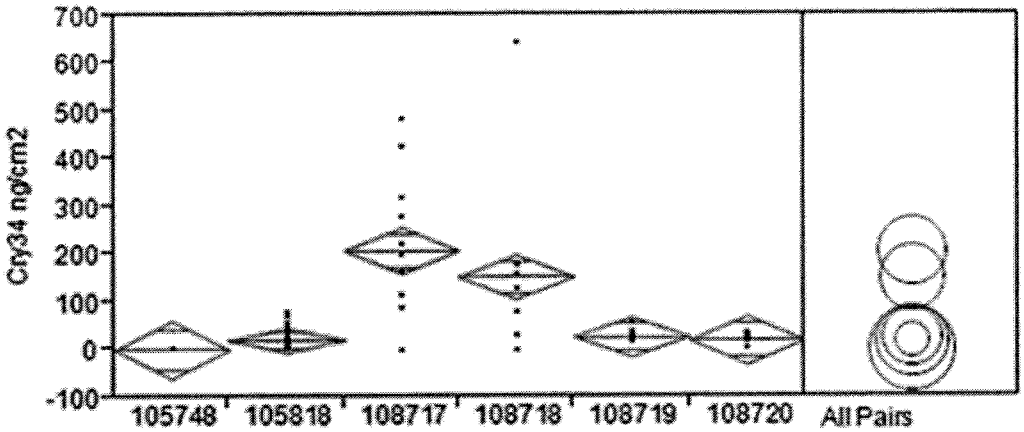
FIG. 27H

```
                        ZmUbi1 promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9421   ACAGAGATGC TTTTTGTTCG CTTGGTTGTG ATGATGTGGT GTGGTTGGGC GGTCGTTCAT
                        ZmUbi1 promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9481   TCGTTCTAGA TCGGAGTAGA ATACTGTTTC AAACTACCTG GTGTATTTAT TAATTTTGGA
                        ZmUbi1 promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9541   ACTGTATGTG TGTGTCATAC ATCTTCATAG TTACGAGTTT AAGATGGATG GAAATATCGA
                        ZmUbi1 promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9601   TCTAGGATAG GTATACATGT TGATGTGGGT TTTACTGATG CATATACATG ATGGCATATG
                        ZmUbi1 promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9661   CAGCATCTAT TCATATGCTC TAACCTTGAG TACCTATCTA TTATAATAAA CAAGTATGTT
                        ZmUbi1 promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9721   TTATAATTAT TTCGATCTTG ATATACTTGG ATGATGGCAT ATGCAGCAGC TATATGTGGA
                        ZmUbi1 promoter v2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9781   TTTTTTTAGC CCTGCCTTCA TACGCTATTT ATTTGCTTGG TACTGTTTCT TTTGTCGATG
        ZmUbi1 promoter v2                                          PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~                              ~
9841   CTCACCCTGT TGTTTGGTGT TACTTCTGCA GGGTACAGTA GTTAGTTGAC ACGACACCAT
                                  PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9901   GTCTCCGGAG AGGAGACCAG TTGAGATTAG GCCAGCTACA GCAGCTGATA TGGCCGCGGT
                                  PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9961   TTGTGATATC GTTAACCATT ACATTGAGAC GTCTACAGTG AACTTTAGGA CAGAGCCACA
                                  PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10021  AACACCACAA GAGTGGATTG ATGATCTAGA GAGGTTGCAA GATAGATACC CTTGGTTGGT
                                  PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10081  TGCTGAGGTT GAGGGTGTTG TGGCTGGTAT TGCTTACGCT GGGCCCTGGA AGGCTAGGAA
                                  PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10141  CGCTTACGAT TGGACAGTTG AGAGTACTGT TTACGTGTCA CATAGGCATC AAAGGTTGGG
                                  PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10201  CCTAGGATCC ACATTGTACA CACATTTGCT TAAGTCTATG GAGGCGCAAG GTTTTAAGTC
                                  PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10261  TGTGGTTGCT GTTATAGGCC TTCCAAACGA TCCATCTGTT AGGTTGCATG AGGCTTTGGG
                                  PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10321  ATACACAGCC CGTGGTACAT TGCGCGCAGC TGGATACAAG CATGGTGGAT GGCATGATGT
                                  PAT v9
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10381  TGGTTTTTGG CAAAGGGATT TTGAGTTGCC AGCTCCTCCA AGGCCAGTTA GGCCAGTTAC
```

FIG. 27I

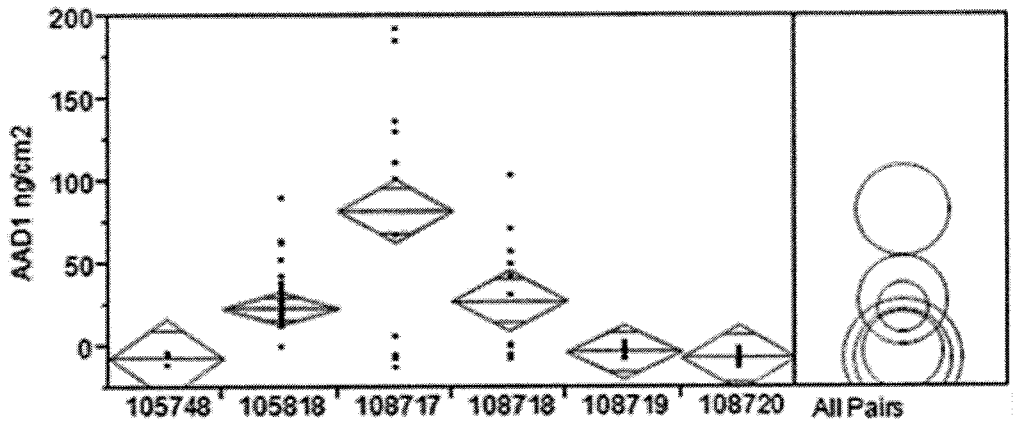
```
        PAT v9                                                                ZmLip 3' UTR v1
         ~~~~~~~~~~                                                            ~~~~~~~~~~~~~~
10441    CCAGATCTGA CTGAGCTTGA GCTTATGAGC TTATGAGCTT AGAGCTCGGT CGCAGCGTGT
                          ZmLip 3' UTR v1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10501    GCGTGTCCGT CGTACGTTCT GGCCGGCCGG GCCTTGGGCG CGCGATCAGA AGCGTTGCGT
                          ZmLip 3' UTR v1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10561    TGGCGTGTGT GTGCTTCTGG TTTGCTTTAA TTTTACCAAG TTTGTTTCAA GGTGGATCGC
                          ZmLip 3' UTR v1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10621    GTGGTCAAGG CCCGTGTGCT TTAAAGACCC ACCGGCACTG GCAGTGAGTG TTGCTGCTTG
                          ZmLip 3' UTR v1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10681    TGTAGGCTTT GGTACGTATG GGCTTTATTT GCTTCTGGAT GTTGTGTACT ACTTGGGTTT
                          ZmLip 3' UTR v1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10741    GTTGAATTAT TATGAGCAGT TGCGTATTGT AATTCAGCTG GGCTACCTGG ACATTGTTAT
                    ZmLip 3' UTR v1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10801    GTATTAATAA ATGCTTTGCT TTCTTCTAAA GATCTTTAAG TGCTTCTAGA GCATGCACAT
10861    AGACACACAC ATCATCTCAT TGATGCTTGG TAATAATTGT CATTAGATTG TTTTTATGCA
10921    TAGATGCACT CGAAATCAGC CAATTTTAGA CAAGTATCAA ACGGATGTGA CTTCAGTACA
10981    TTAAAAACGT CCGCAATGTG TTATTAAGTT GTCTAAGCGT CAATTTGATT TACAATTGAA
11041    TATATCCTGC CCCAGCCAGC CAACAGCTCG ATTTACAATT GAATATATCC TGCCGGCCGG
11101    CCCACGCGTG TCGAGGAATT CTGATCTGGC CCCCATTTGG ACGTGAATGT AGACACGTCG
11161    AAATAAAGAT TTCCGAATTA GAATAATTTG TTTATTGCTT TCGCCTATAA ATACGACGGA
11221    TCGTAATTTG TCGTTTTATC AAAATGTACT TTCATTTTAT AATAACGCTG CGGACATCTA
11281    CATTTTTGAA TTGAAAAAAA ATTGGTAATT ACTCTTTCTT TTTCTCCATA TTGACCATCA
11341    TACTCATTGC TGATCCATGT AGATTTCCCG GACATGAAGC CATTTACAAT TGAATATATC
11401    CTGCCG
```

FIG. 27J



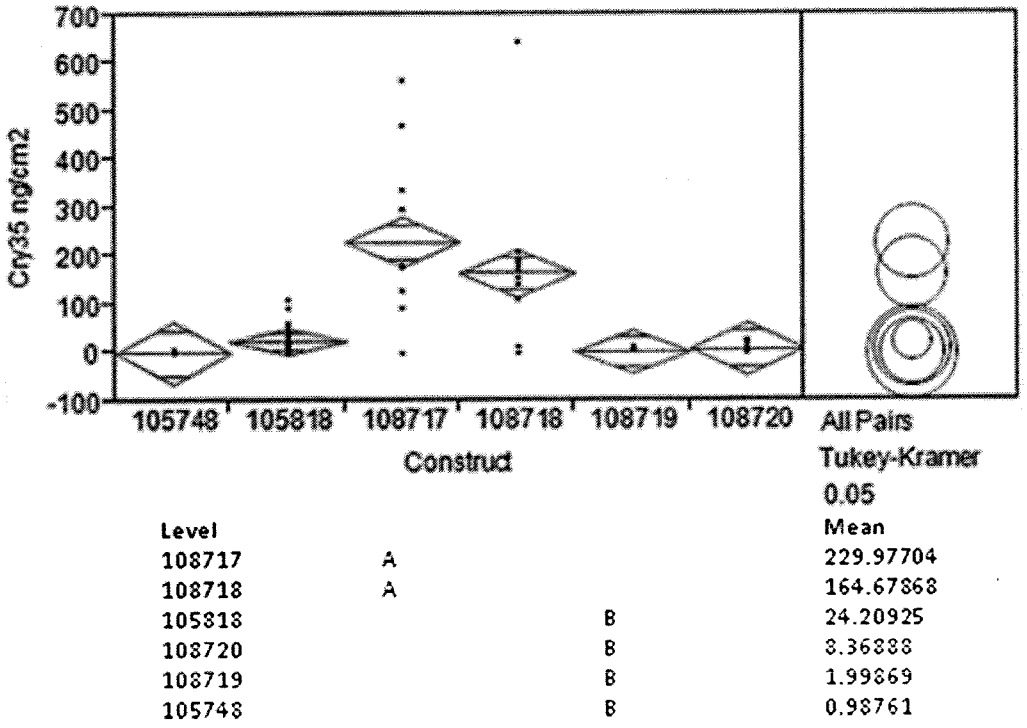
Level			Mean
108717	A		82.50000
108718		B	28.08333
105818		B	23.52500
108719		B	-3.00000
105748		B	-6.50000
108720		B	-6.70000

FIG. 28A



Level			Mean
108717	A		82.50000
108718		B	28.08333
105818		B	23.52500
108719		B	-3.00000
105748		B	-6.50000
108720		B	-6.70000

FIG. 28B



Level			Mean
108717	A		229.97704
108718	A		164.67868
105818		B	24.20925
108720		B	8.36888
108719		B	1.99869
105748		B	0.98761

FIG. 28C

SEQ ID NO: 51: yellow fluorescent protein from Phialidium sp. SL-2003 (PhiYFP; 234 a.a.; GenBank: AAR85349.1):
MSSGALLFHG KIPYVVEMEG NVDGHTFSIR GKGYGDASVG KVDAQFICTT
GDVPVPWSTL VTTLTYGAQC FAKYGPELKD FYKSCMPEGY VQERTITFEG
DGVFKTRAEV TFENGSVYNR VKLNGQGFKK DGHVLGKNLE FNFTPHCLYI
WGDQANHGLK SAFKIMHEIT GSKEDFIVAD HTQMNTPIGG GPVHVPEYHH
ITYHVTLSKD VTDHRDNMSL VETVRAVDCR KTYL

SEQ ID NO: 52: PhiYFPv3; 234 a.a.

MSSGALLFHG KIPYVVEMEG NVDGHTFSIR GKGYGDASVG KVDAQFICTT
GDVPVPWSTL VTTLTYGAQC FAKYGPELKD FYKSCMPDGY VQERTITFEG
DGNFKTRAEV TFENGSVYNR VKLNGQGFKK DGHVLGKNLE FNFTPHCLYI
WGDQANHGLK SAFKICHEIT GSKGDFIVAD HTQMNTPIGG GPVHVPEYHH
MSYHVKLSKD VTDHRDNMSL KETVRAVDCR KTYL

FIG. 29

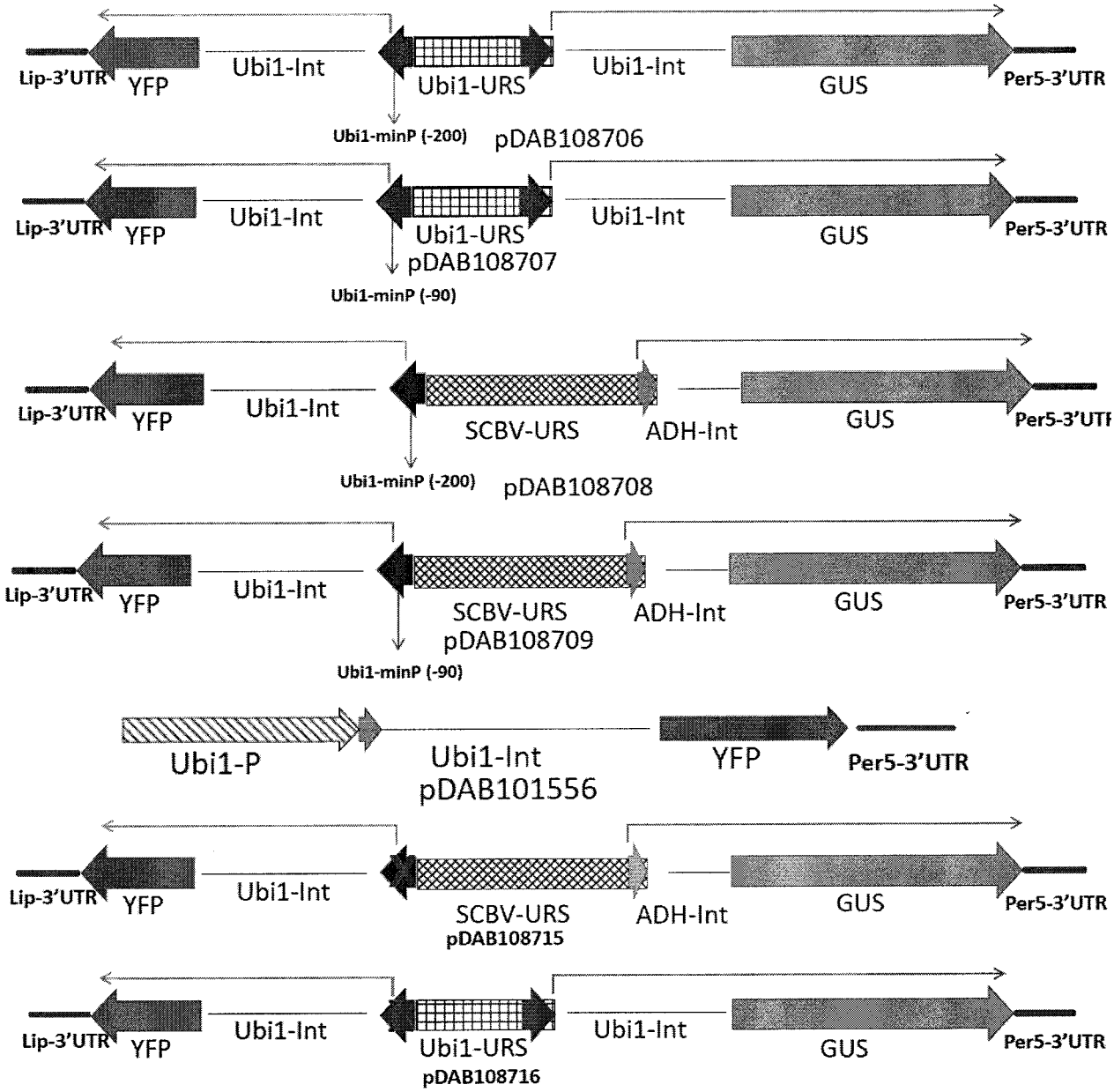

Lip-3'UTR
YFP
Ubi1-Int
Ubi1-URS
Ubi1-Int
GUS
Per5-3'UTR
Ubi1-minP (-200)
pDAB108706
Ubi1-URS
pDAB108707
Ubi1-minP (-90)
SCBV-URS
ADH-Int
pDAB108708
pDAB108709
Ubi1-P
pDAB101556
pDAB108715
pDAB108716

FIG. 30

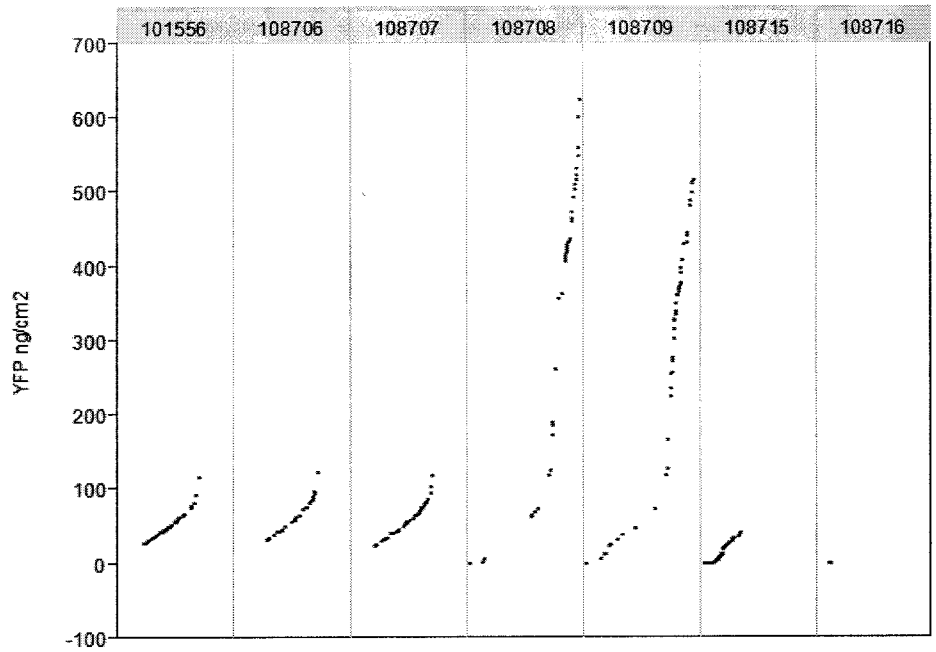


FIG. 31A

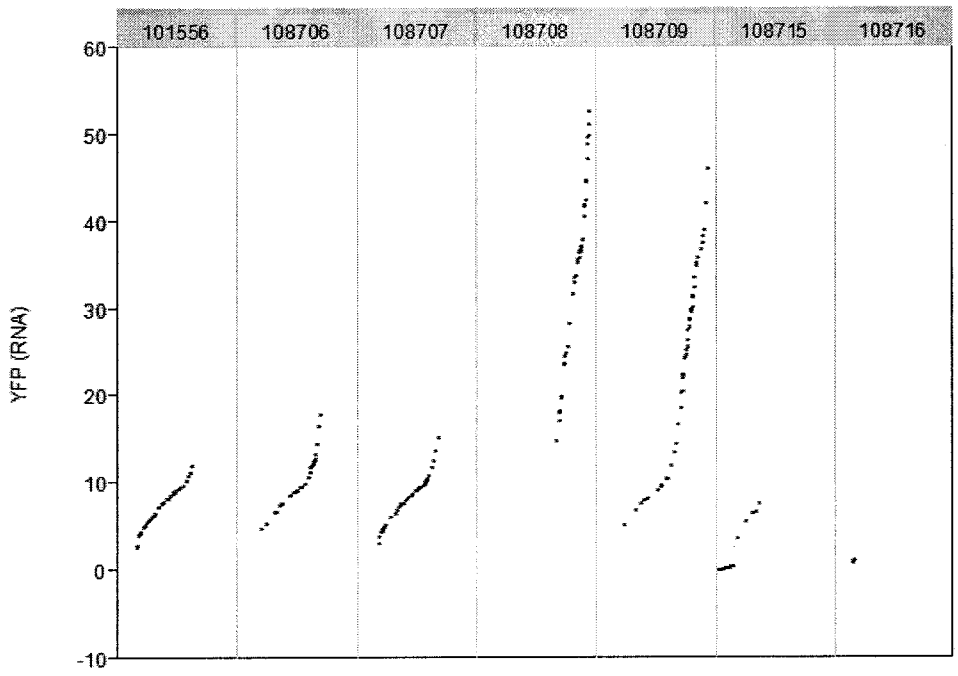


FIG. 31B

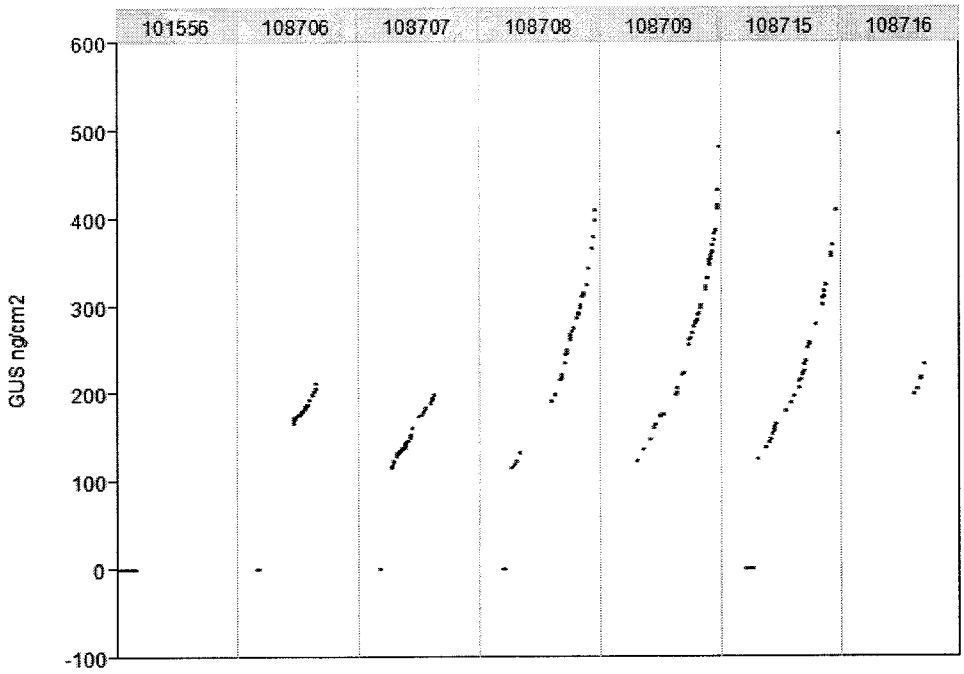


FIG. 32A

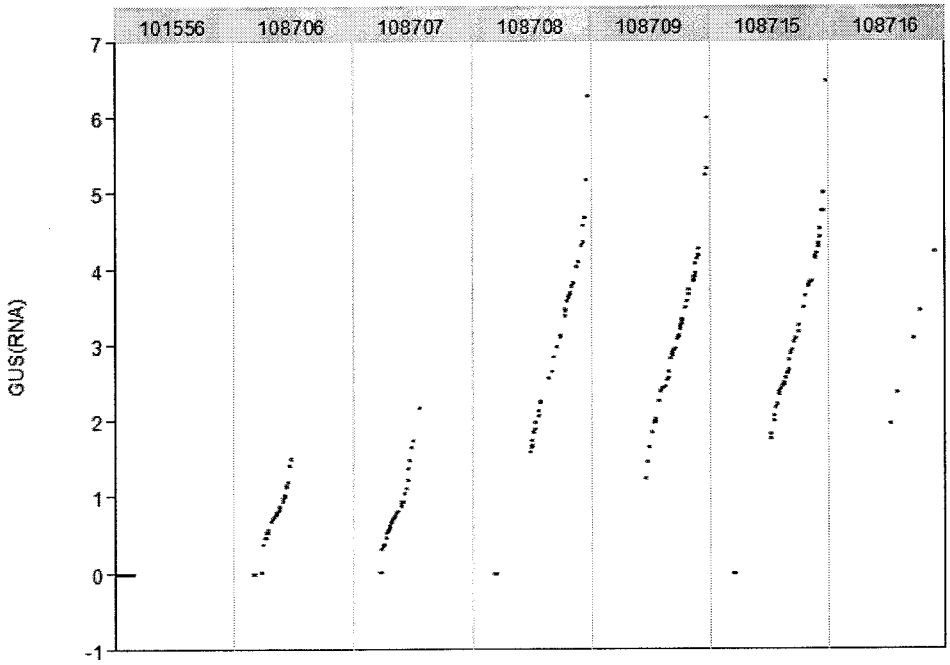


FIG. 32B

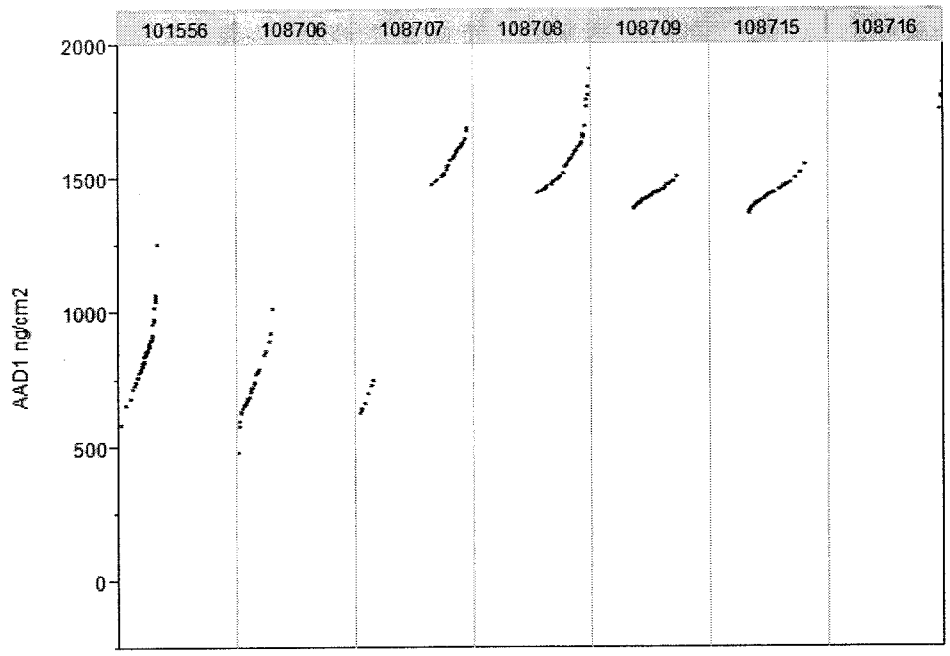


FIG. 33A

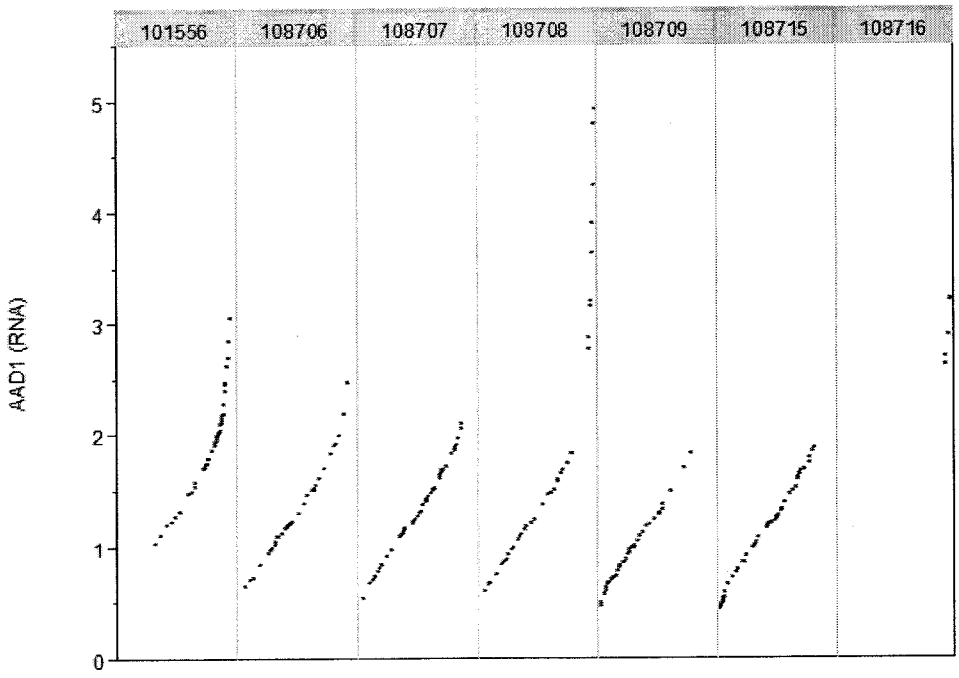
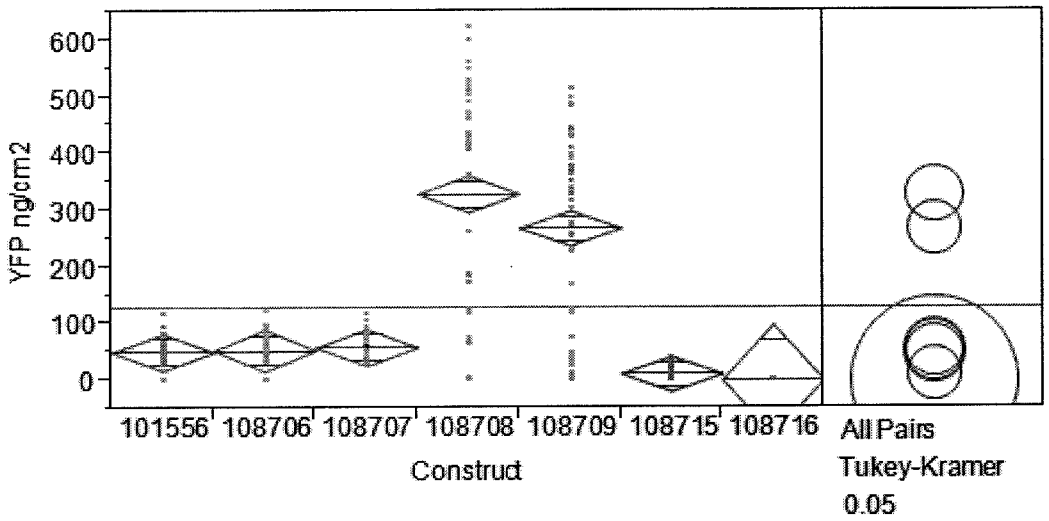


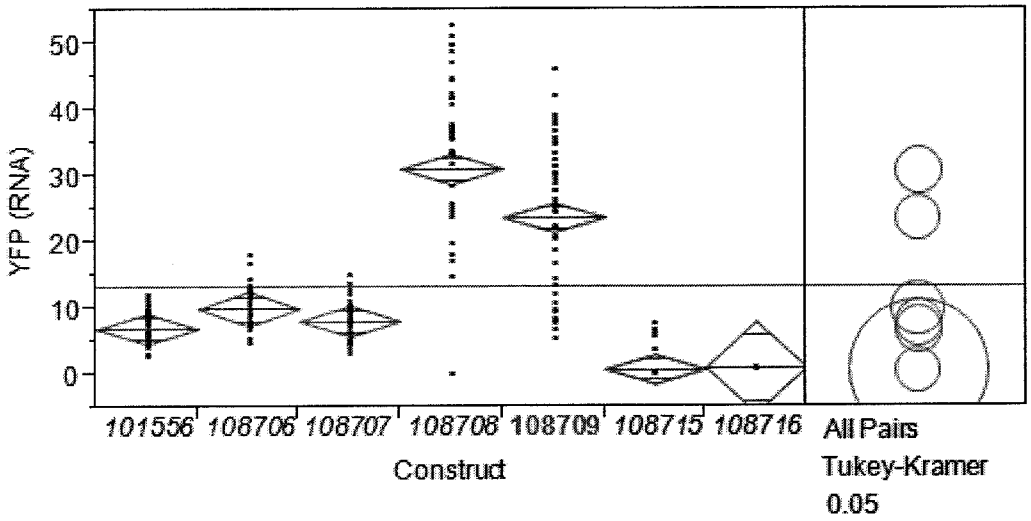
FIG. 33B



108708	A	328.3276
108709	A	267.6876
108707	B	57.63336
108706	B	52.6654
101556	B	49.75972
108715	B	10.63202
108716	B	0

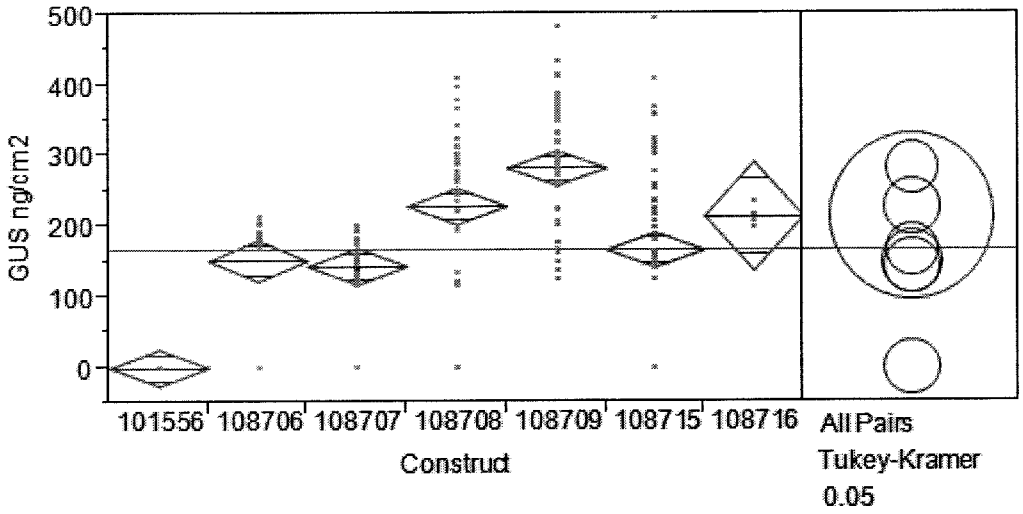
Levels not connected by same letter are significantly different.

FIG. 34A



108708	A			31.02019
108709		B		23.68044
108706			C	9.966029
108707			C	8.0728
101556			C	6.954422
108716			C	D 1.01158
108715				D 0.767854

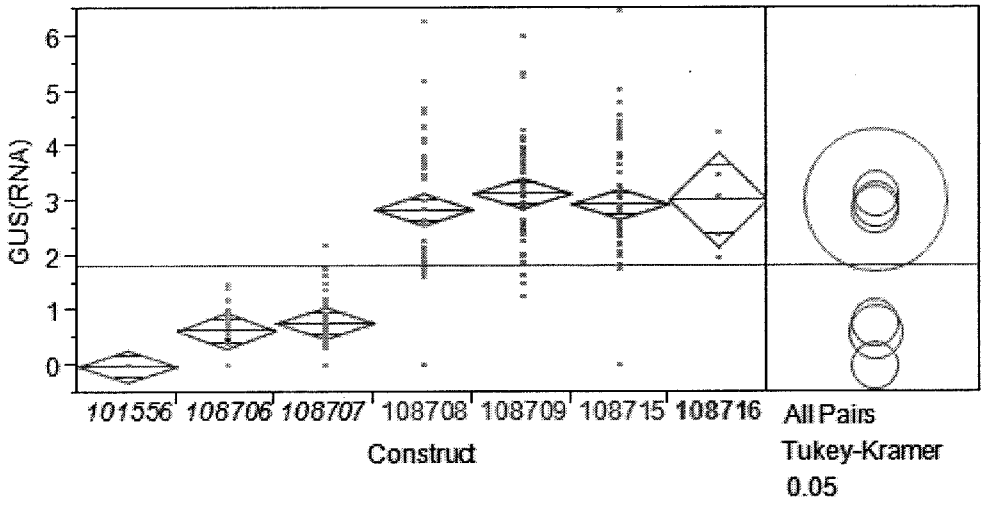
FIG. 34B



Level		Mean
108709	A	282.22909
108708	B	228.17205
108716	A B C	213.97739
108715	C	166.52102
108706	C	151.27776
108707	C	143.22297
101556	D	0.00000

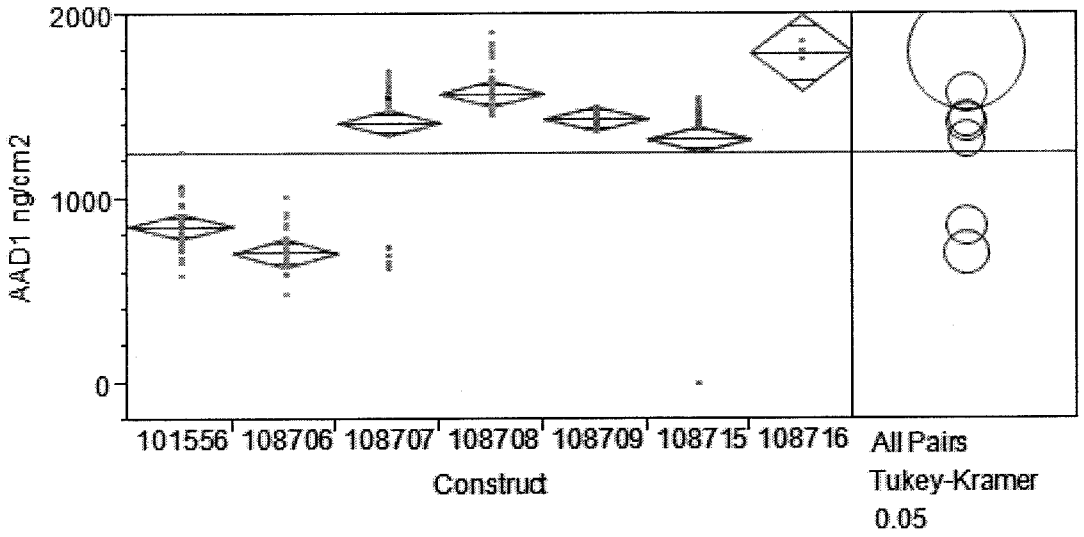
Levels not connected by same letter are significantly different.

FIG. 35A



108709	A		3.14136
108716	A		3.0266
108715	A		2.949466
108708	A		2.851752
108707		B	0.784242
108706		B	C 0.647211
101556			C 0

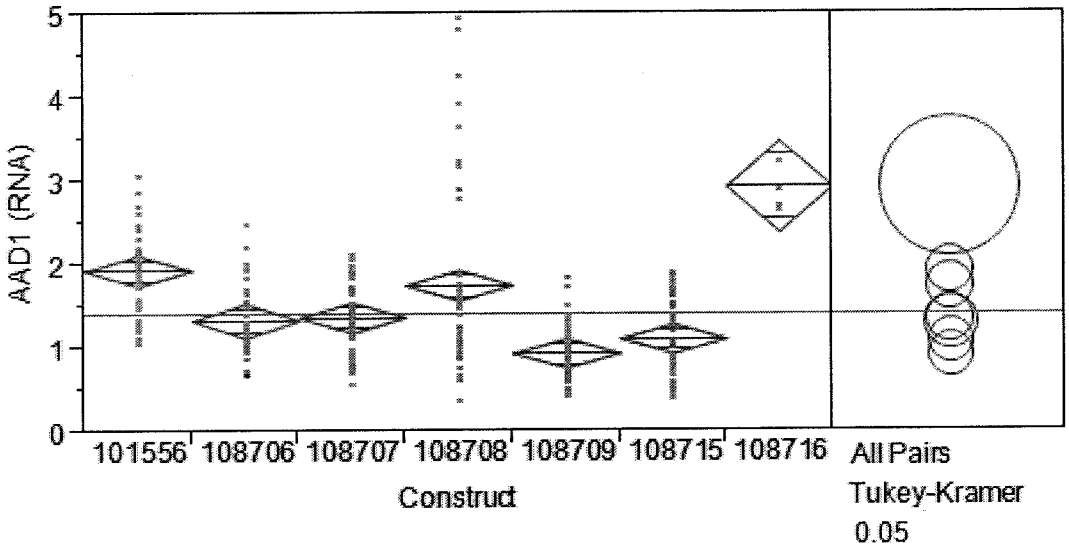
FIG. 35B



Level			Mean
108716	A		1795.4332
108708	A	B	1574.8654
108709		B	C 1437.2658
108707			C 1417.0101
108715			C 1325.8135
101556			D 856.5800
108706			D 710.8802

Levels not connected by same letter are significantly different.

FIG. 36A



108716	A				2.9338
101556		B			1.936933
108708		B	C		1.752869
108707			C	D	1.368713
108706				D E	1.333686
108715				D E	1.11043
108709				E	0.937108

FIG. 36B

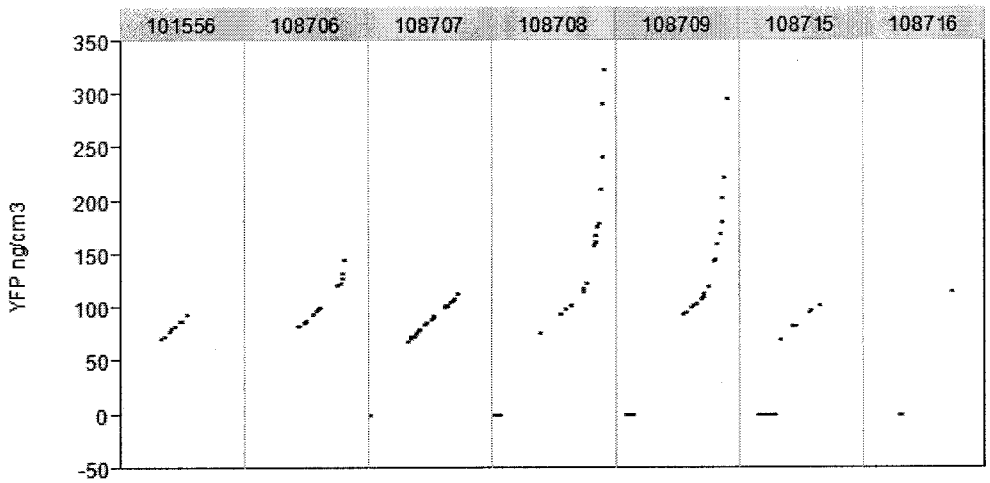


FIG. 37A

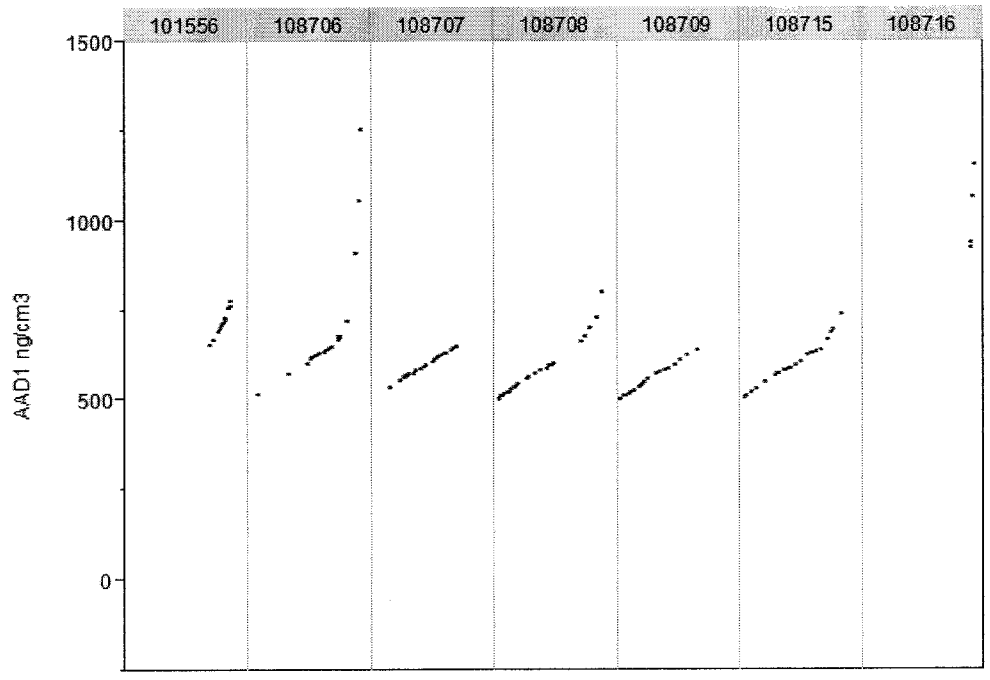


FIG. 37B

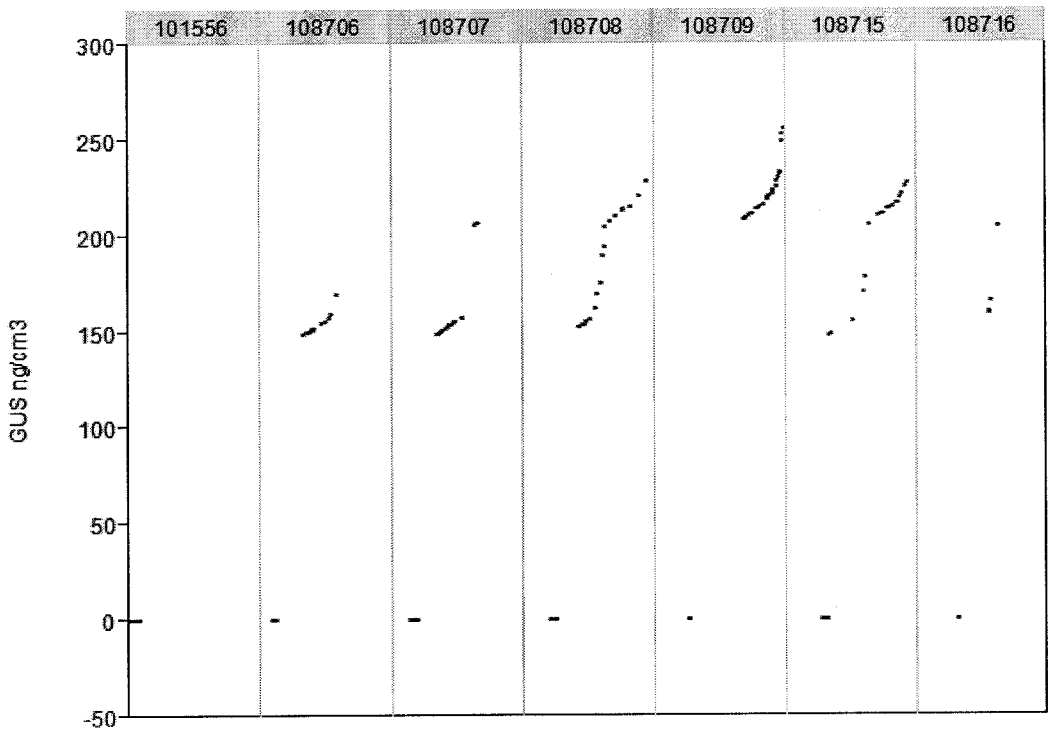
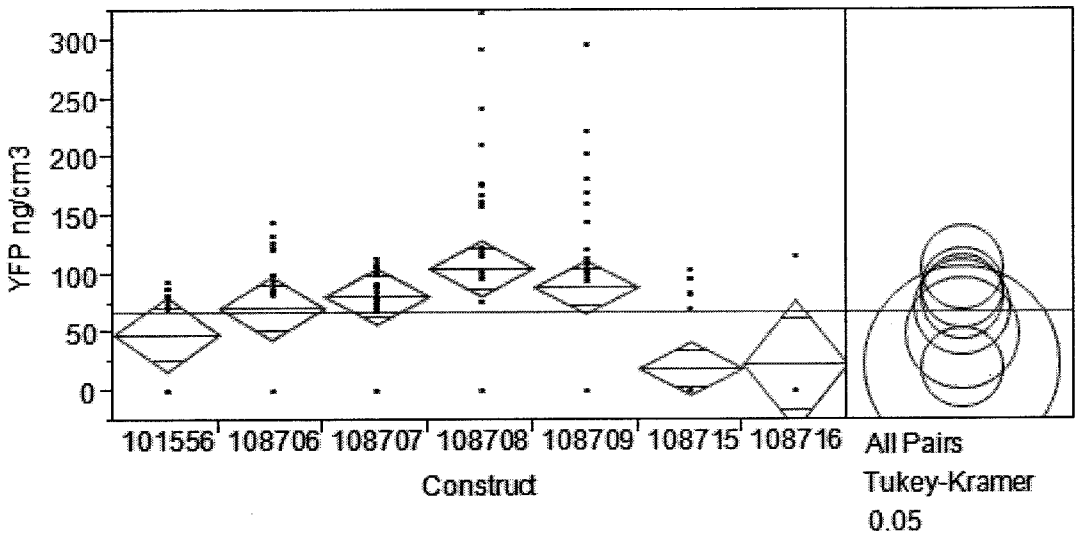

101556
108706
108707
108708
108709
108715
108716
300
250
200
150
100
50
0
-50
GUS ng/cm3

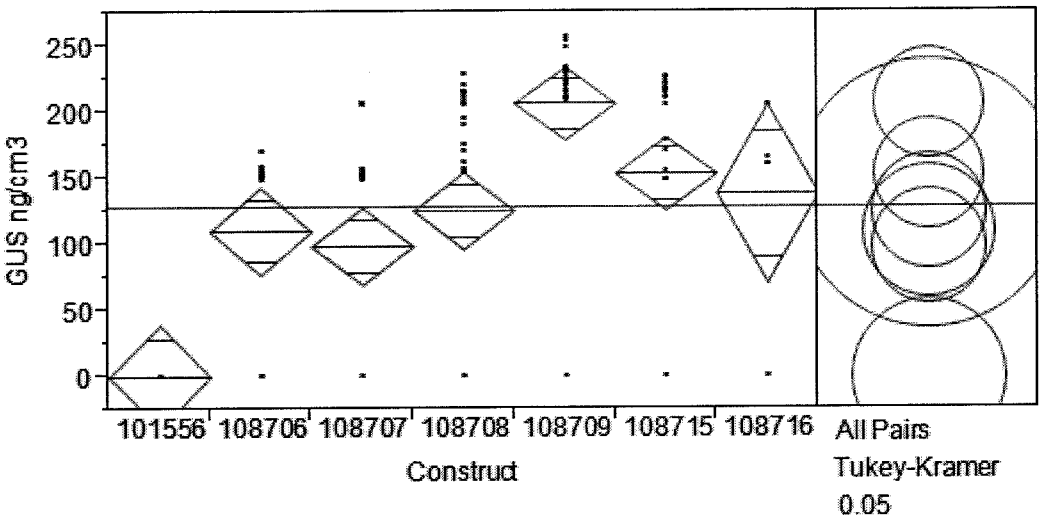
FIG. 37C



Level		Mean
108708	A	105.53451
108709	A	89.43307
108707	A	81.80872
108706	A B	71.76678
101556	A B	49.58332
108716	A B	23.01201
108715	B	19.94290

Levels not connected by same letter are significantly different.

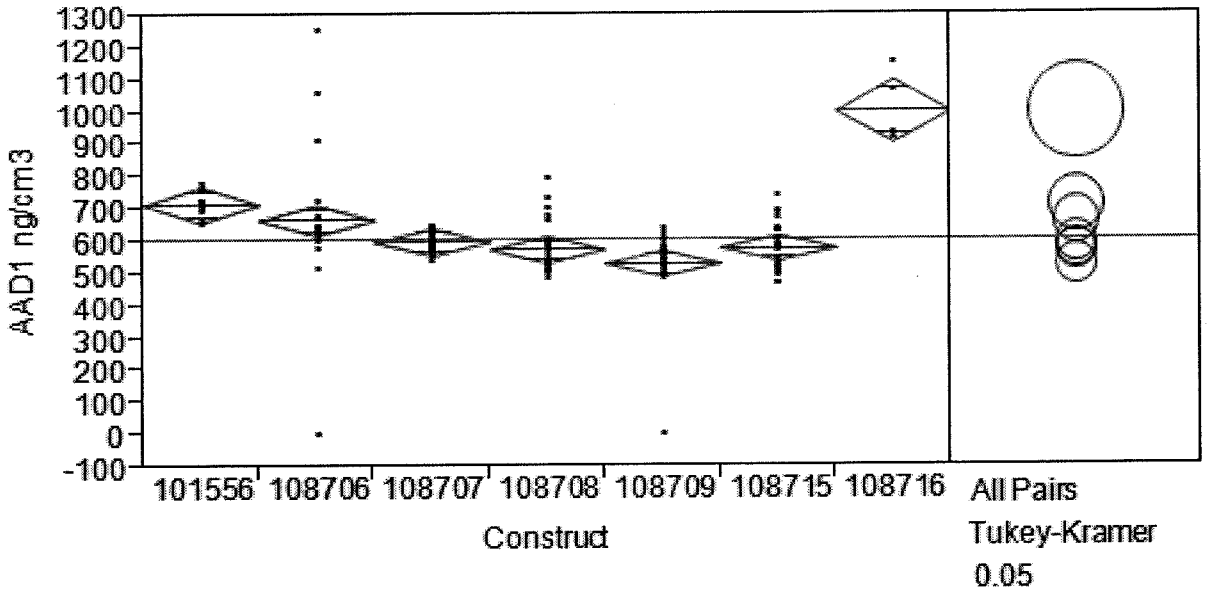
FIG. 38A



Level		Mean
108709	A	206.13021
108715	A B	153.06273
108716	A B	138.02009
108708	B	125.00936
108706	B	109.63065
108707	B	98.24549
101556	C	0.00000

Levels not connected by same letter are significantly different.

FIG. 38B



Level		Mean
108716	A	1002.8448
101556	B	715.1248
108706	B C	666.1088
108707	C D	597.8005
108715	C D	578.7276
108708	C D	574.1088
108709	D	530.3379

Levels not connected by same letter are significantly different.

FIG. 38C

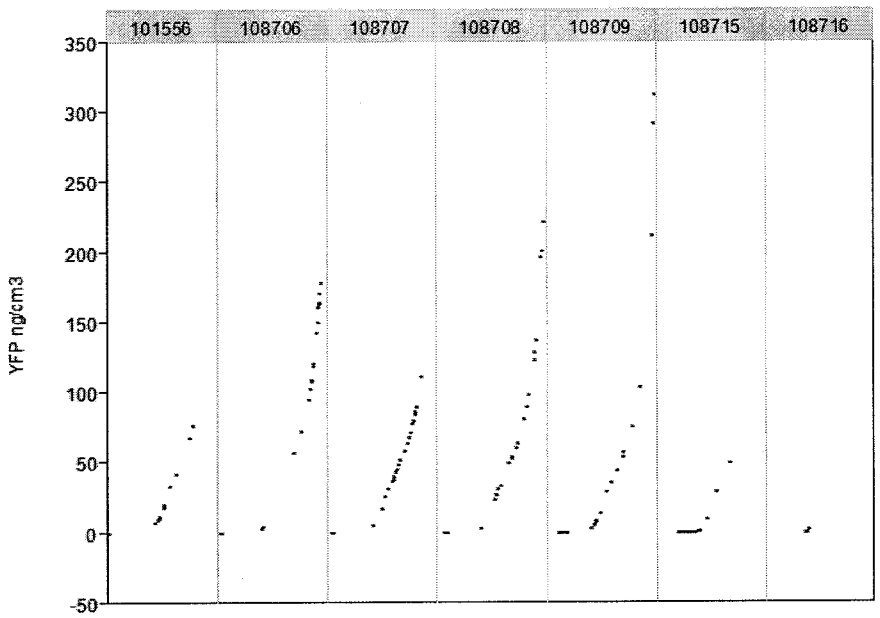


FIG. 39A

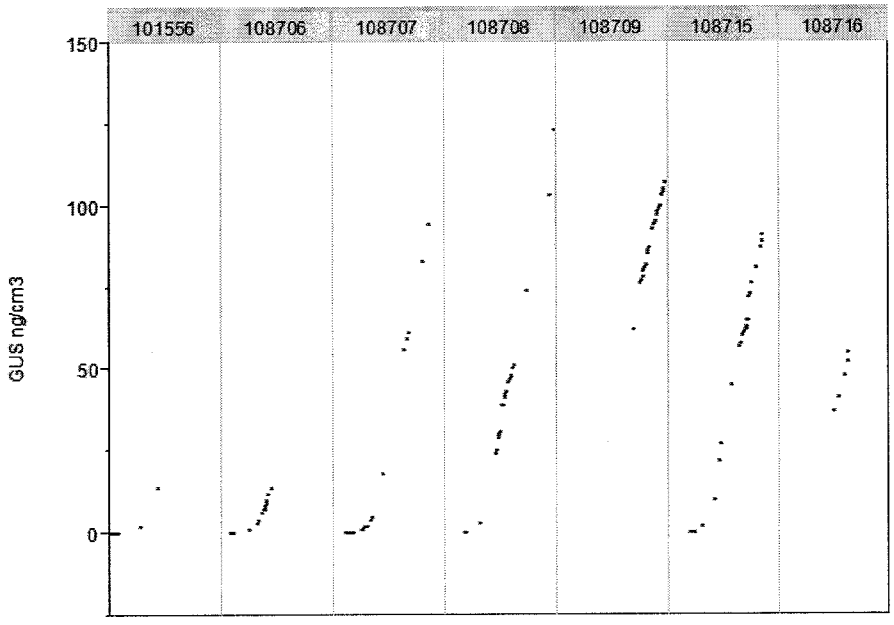


FIG. 39B

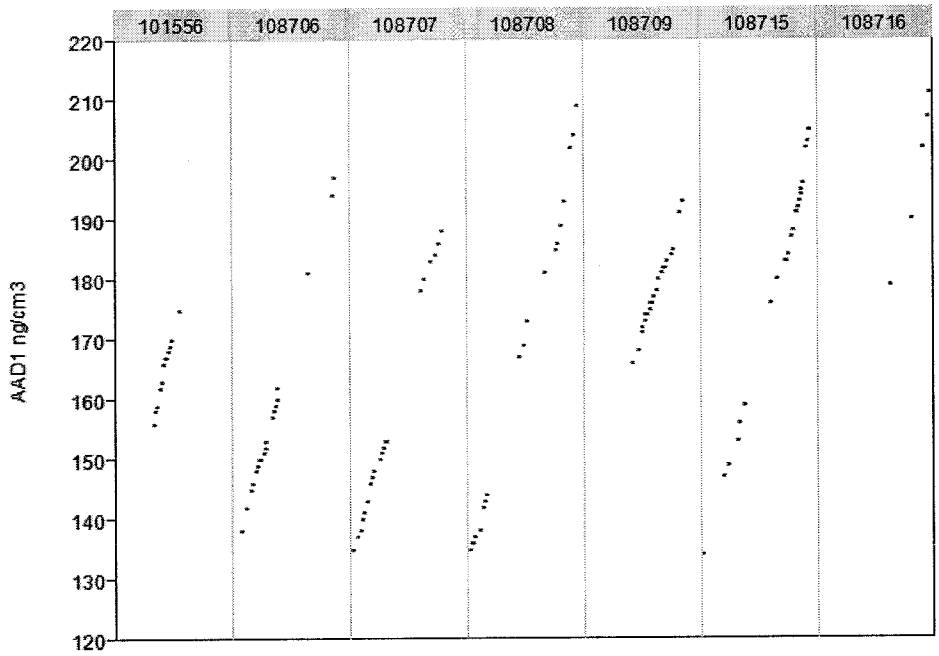
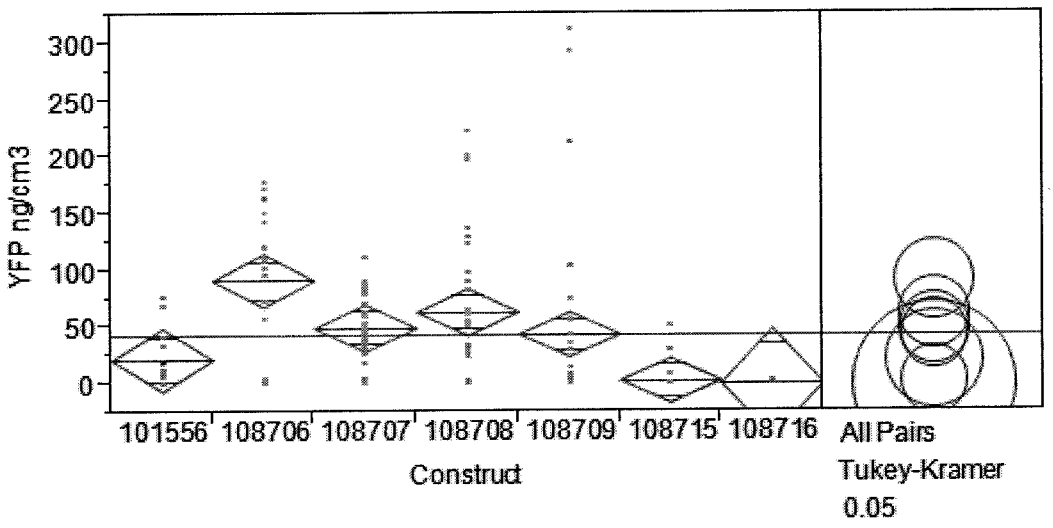
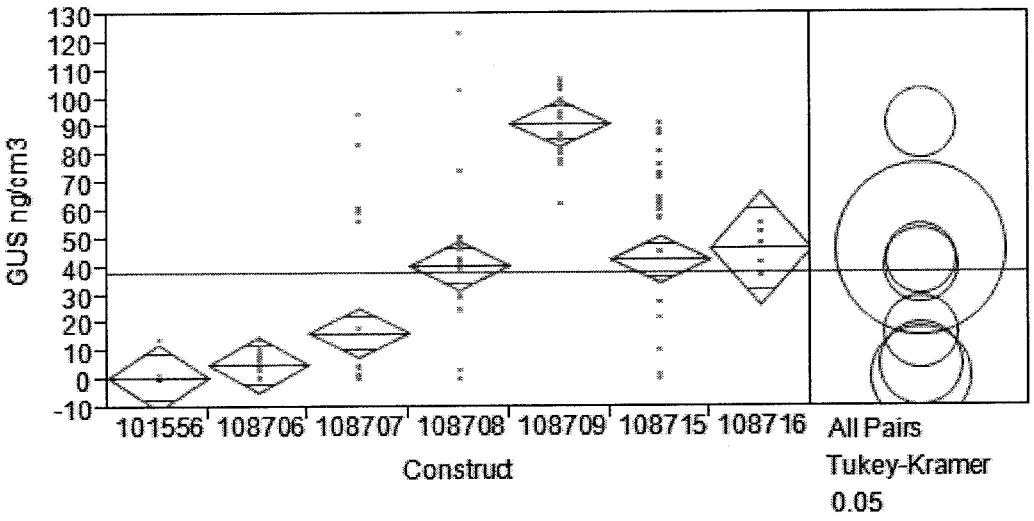

101556
108706
108707
108708
108709
108715
108716
AAD1 ng/cm3
220
210
200
190
180
170
160
150
140
130
120

FIG. 39C



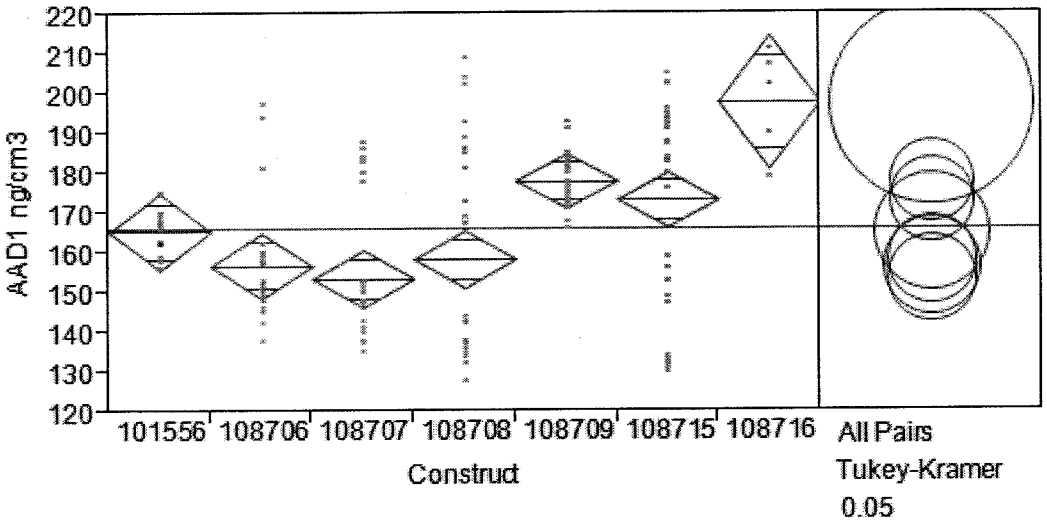
Level		Mean
108706	A	91.380952
108708	A B	63.222222
108707	A B	49.185185
108709	B C	43.600000
101556	B C	21.666667
108715	C	3.000000
108716	B C	0.400000

FIG. 40A



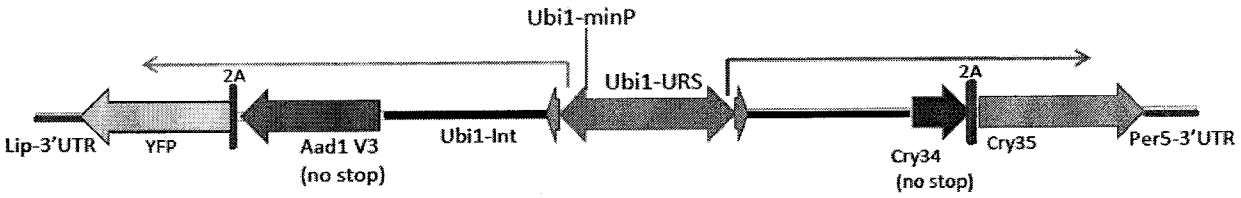
Level		Mean
108709	A	91.200000
108716	B C	46.600000
108715	B	42.766667
108708	B	40.629630
108707	C D	16.814815
108706	D	5.523810
101556	D	1.066667

FIG. 40B

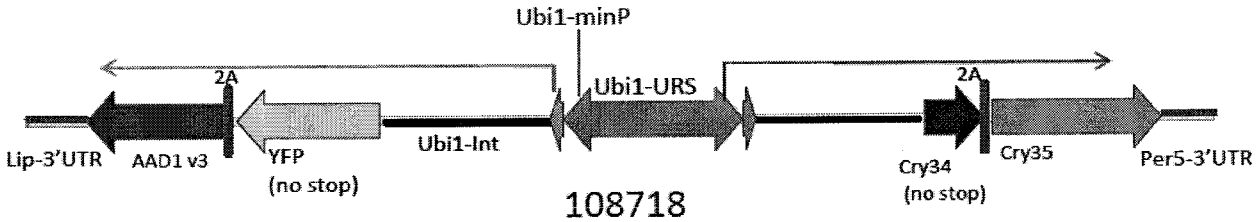


Level		Mean
108716	A	197.80000
108709	A B	178.00000
108715	A B C	173.30000
101556	B C D	165.40000
108708	C D	158.33333
108706	D	156.71429
108707	D	153.44444

FIG. 40C



108717



108718

FIG. 41

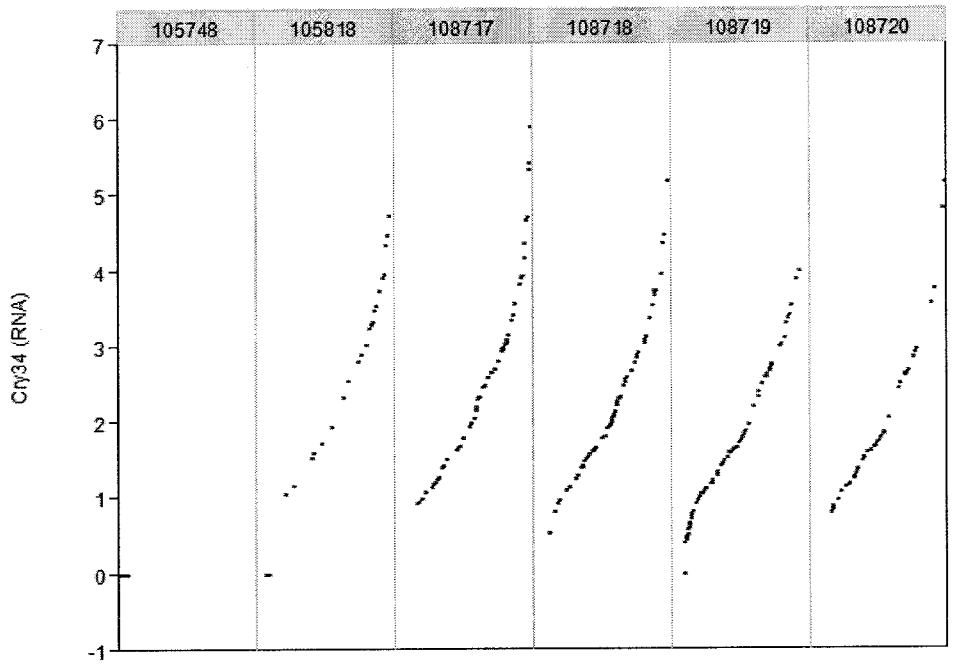


FIG. 42A

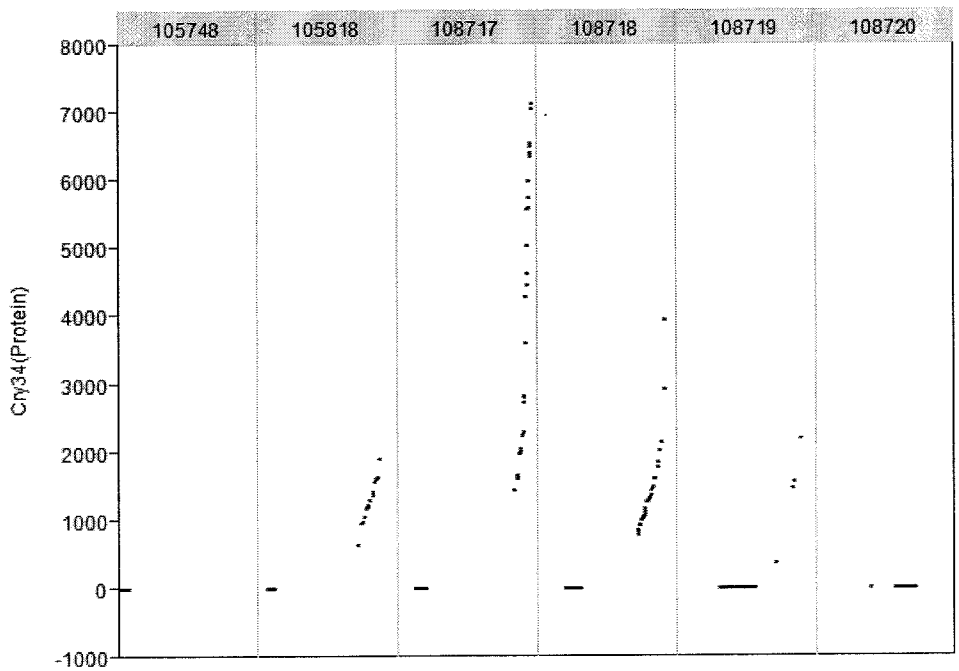


FIG. 42B

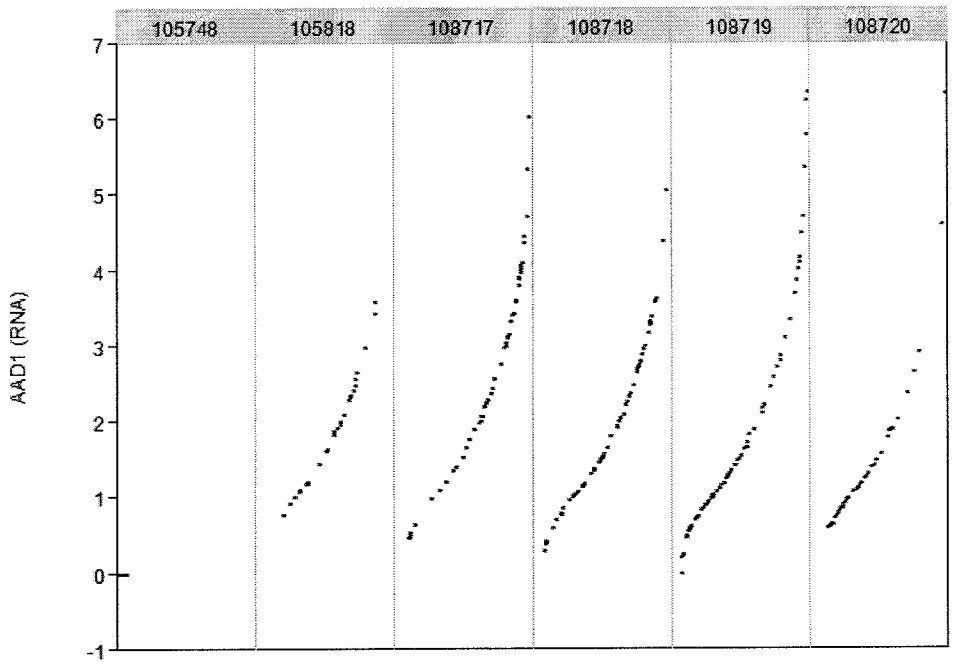


FIG. 43A

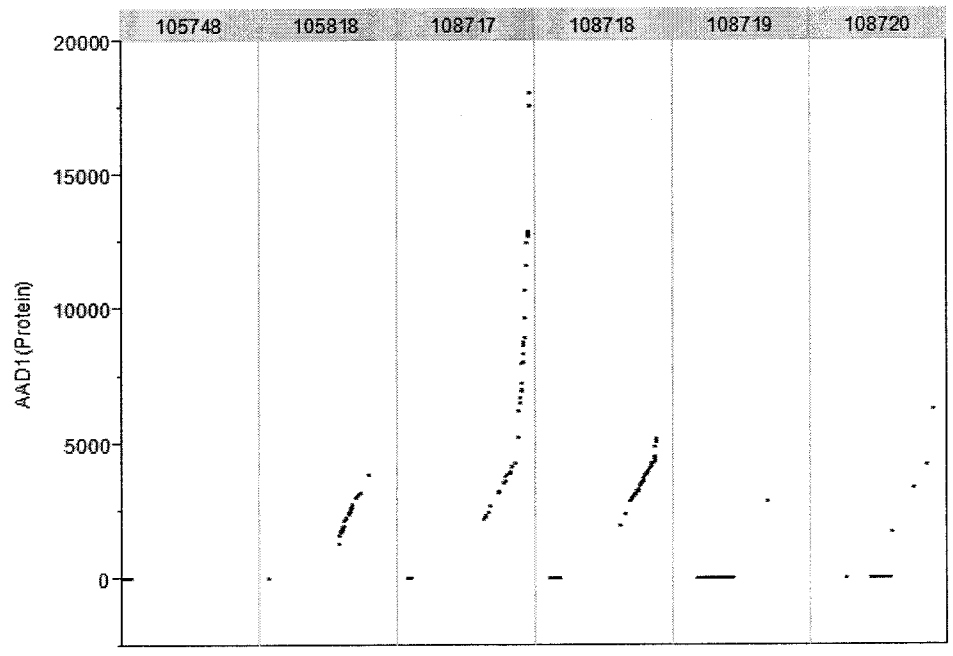


FIG. 43B

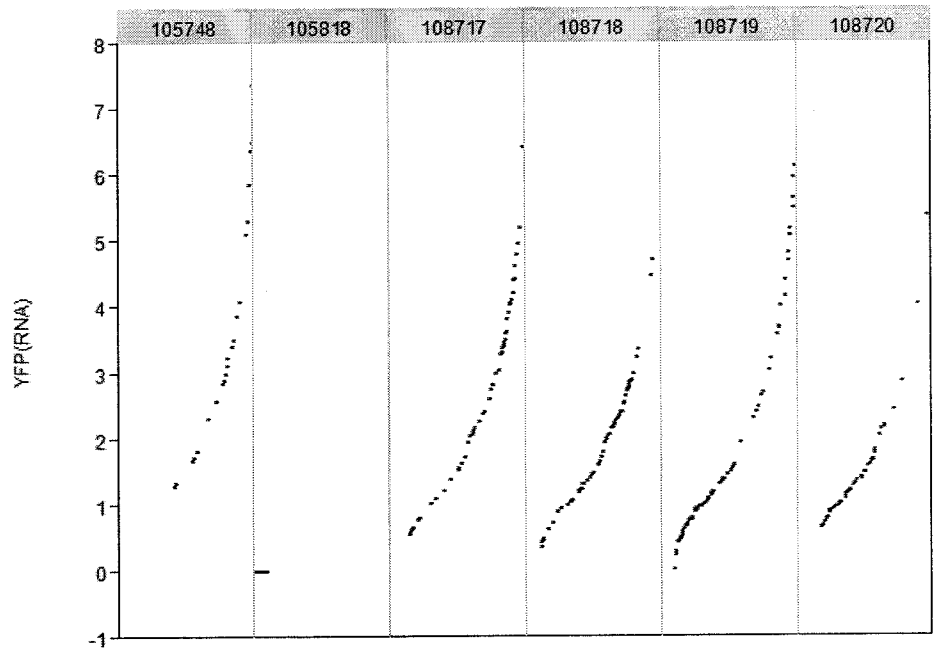


FIG. 44A

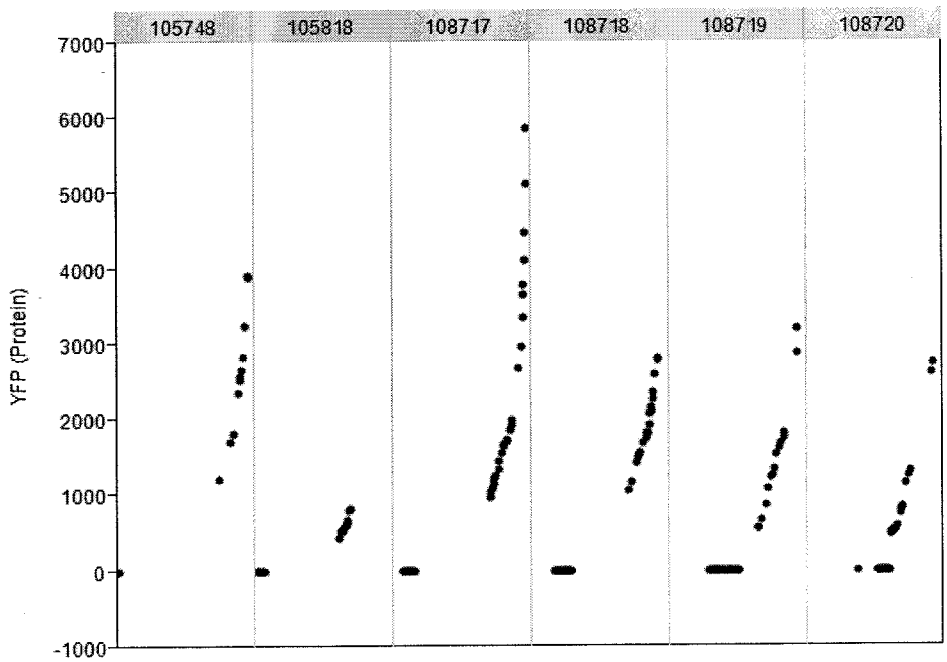


FIG. 44B

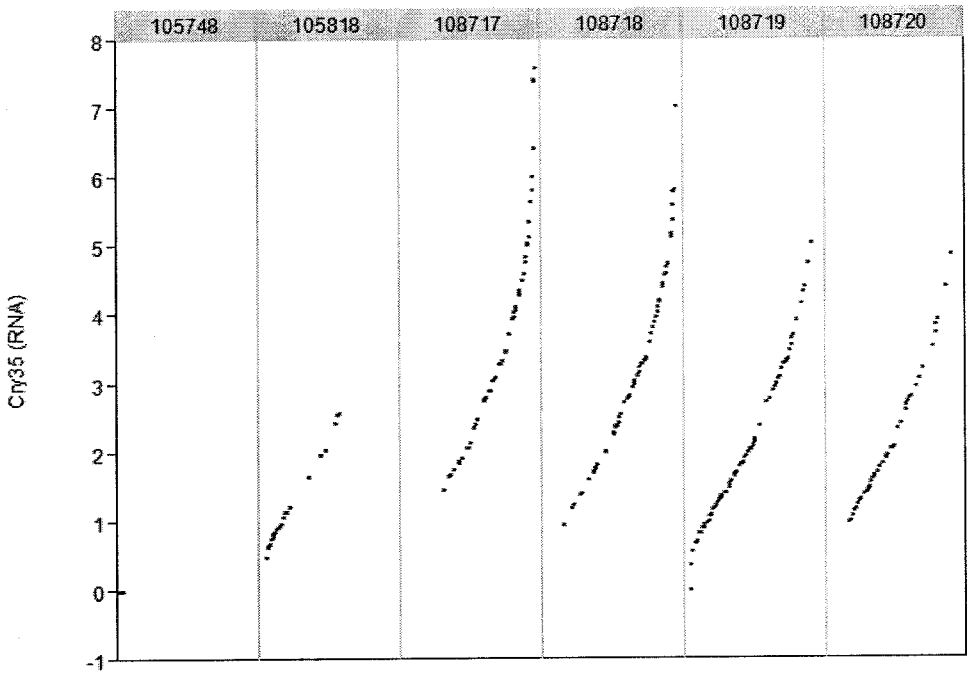


FIG. 45A

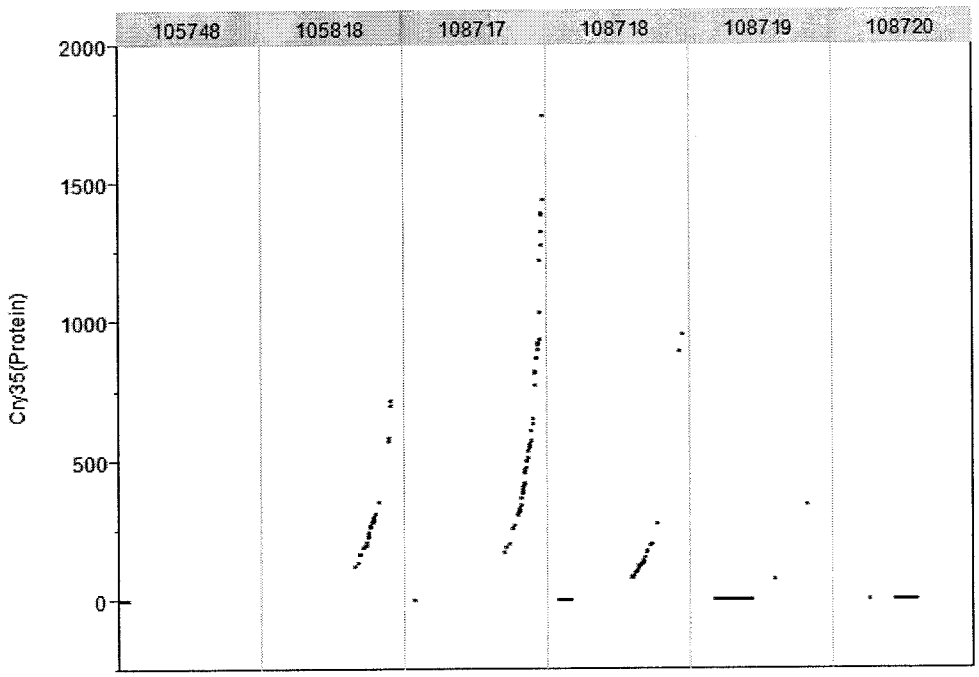


FIG. 45B

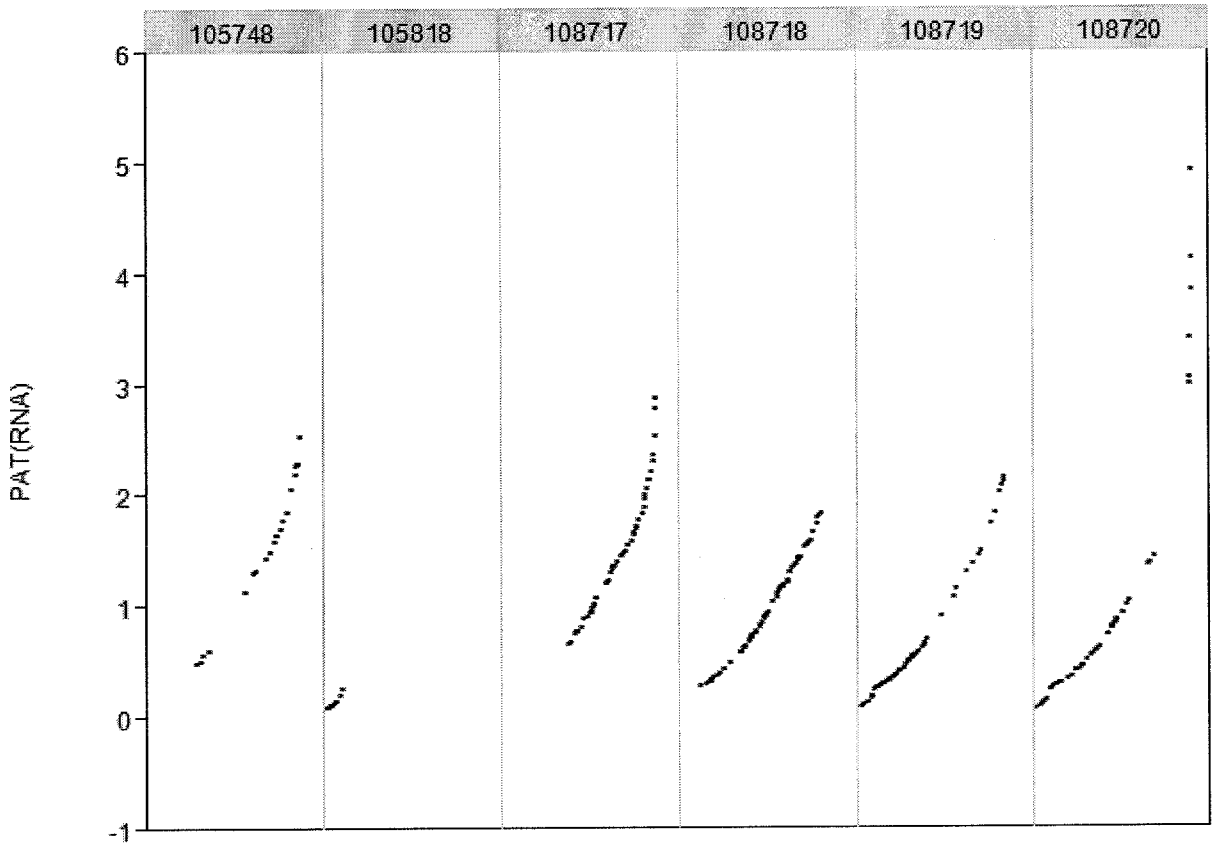
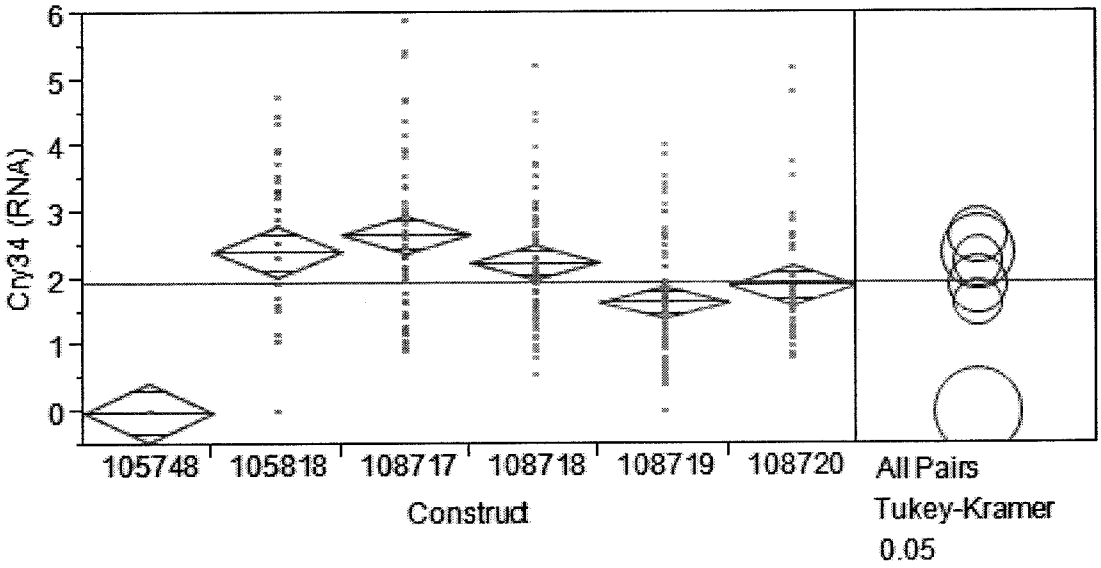
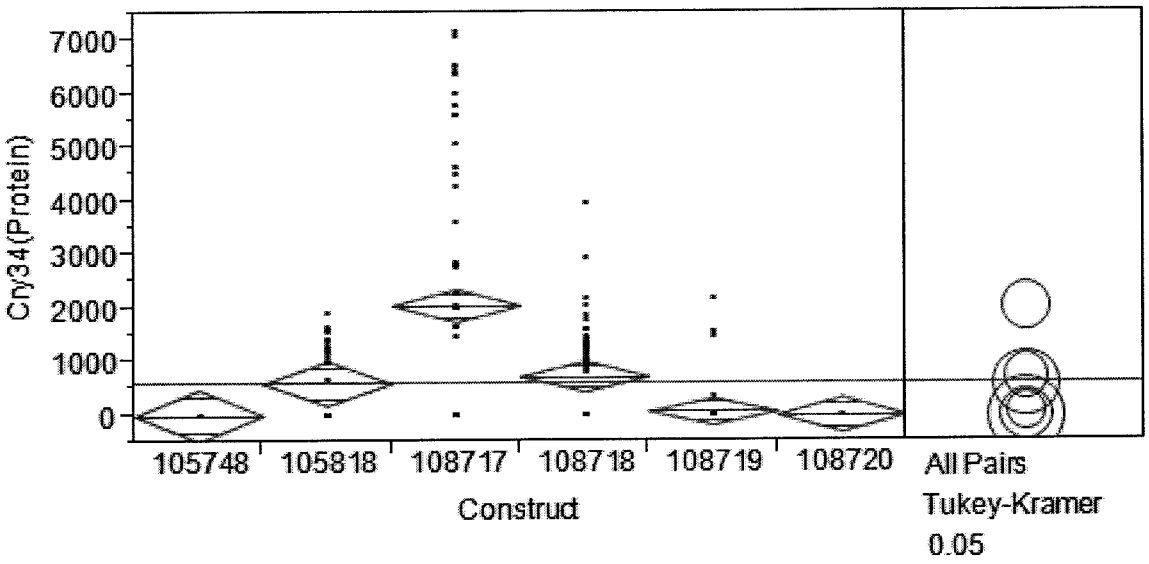

105748
105818
108717
108718
108719
108720
PAT(RNA)
6
5
4
3
2
1
0
-1

FIG. 46



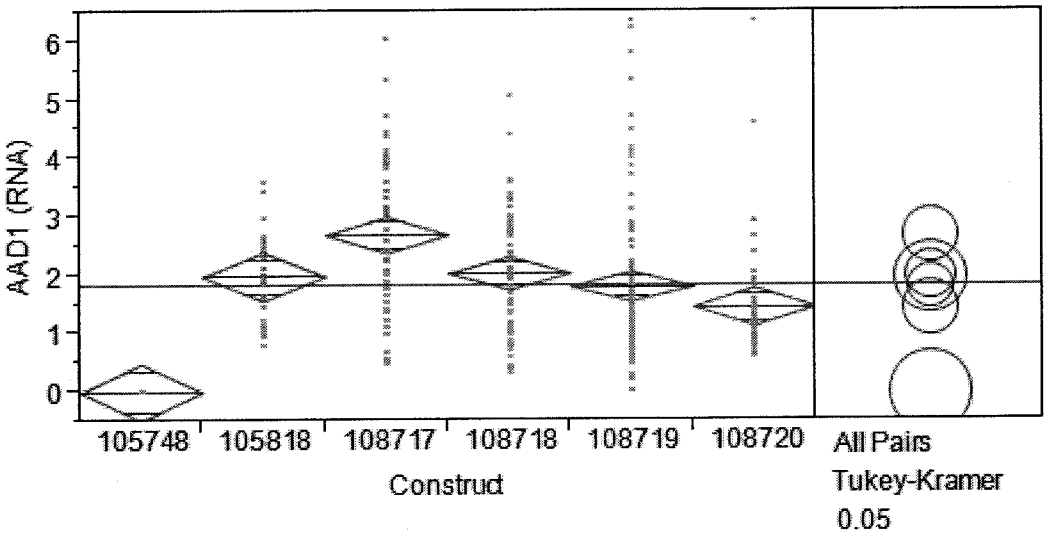
Level		Mean
108717	A	2.6770370
105818	A B	2.4213333
108718	A B	2.2554545
108720	B C	1.9048980
108719	C	1.6506329
105748	D	0.0000000

FIG. 47A



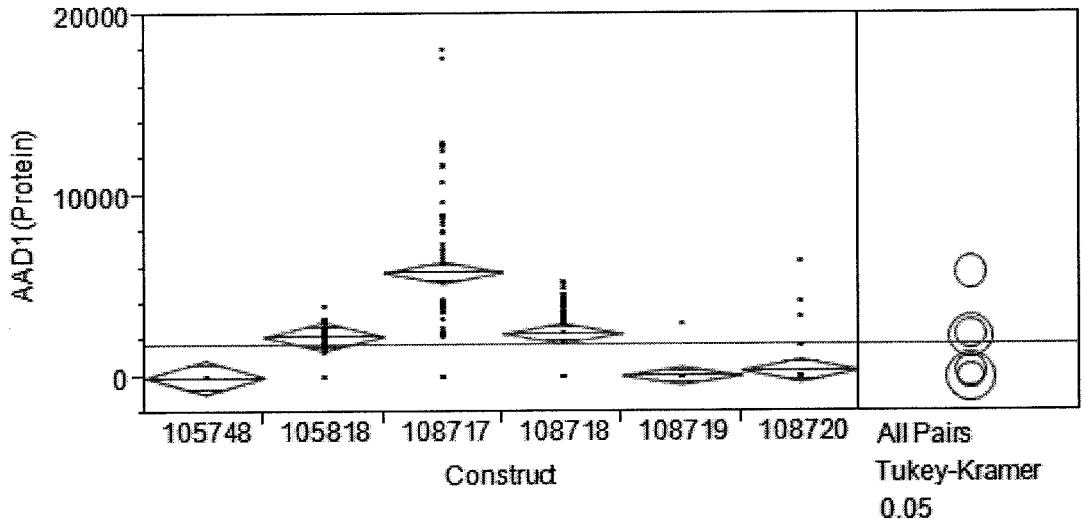
Level		Mean
108717	A	2044.7248
108718	B	719.1750
105818	B C	596.9441
108719	C	66.9930
105748	B C	0.0000
108720	C	0.0000

FIG. 47B



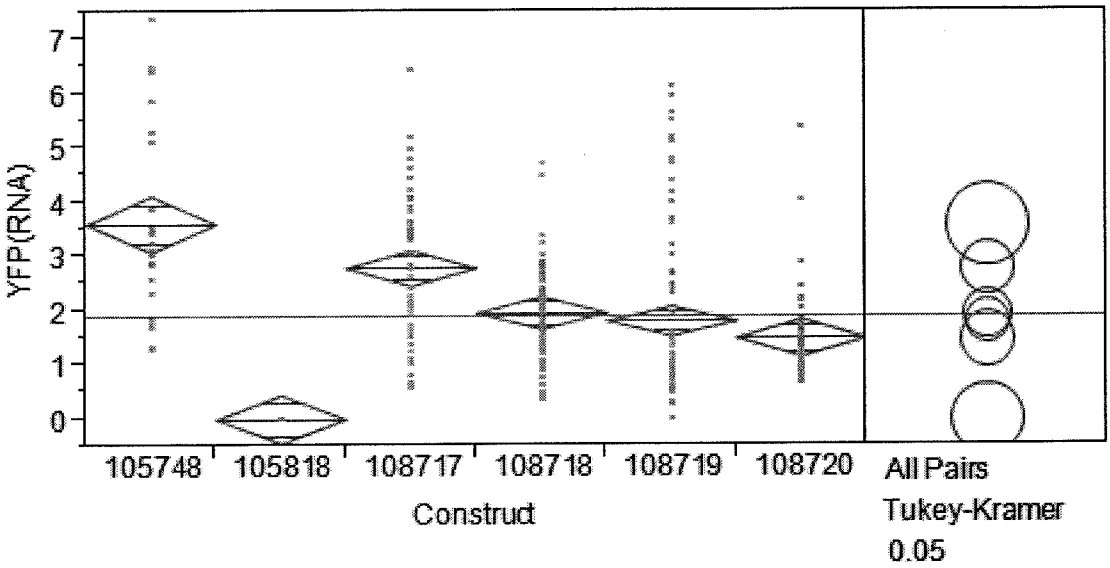
Level		Mean
108717	A	2.6842593
108718	B	2.0268182
105818	A B	1.9756667
108719	B	1.8015190
108720	B	1.4540816
105748	C	0.0000000

FIG. 48A



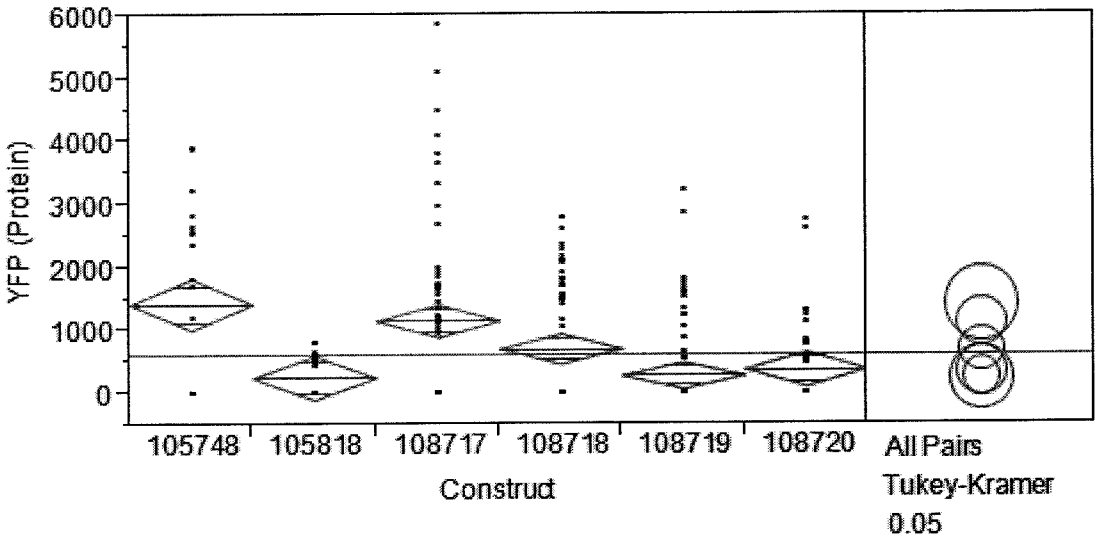
Level		Mean
108717	A	5763.8761
108718	B	2379.1465
105818	B	2237.5350
108720	C	315.9063
108719	C	34.4708
105748	C	0.0000

FIG. 48B



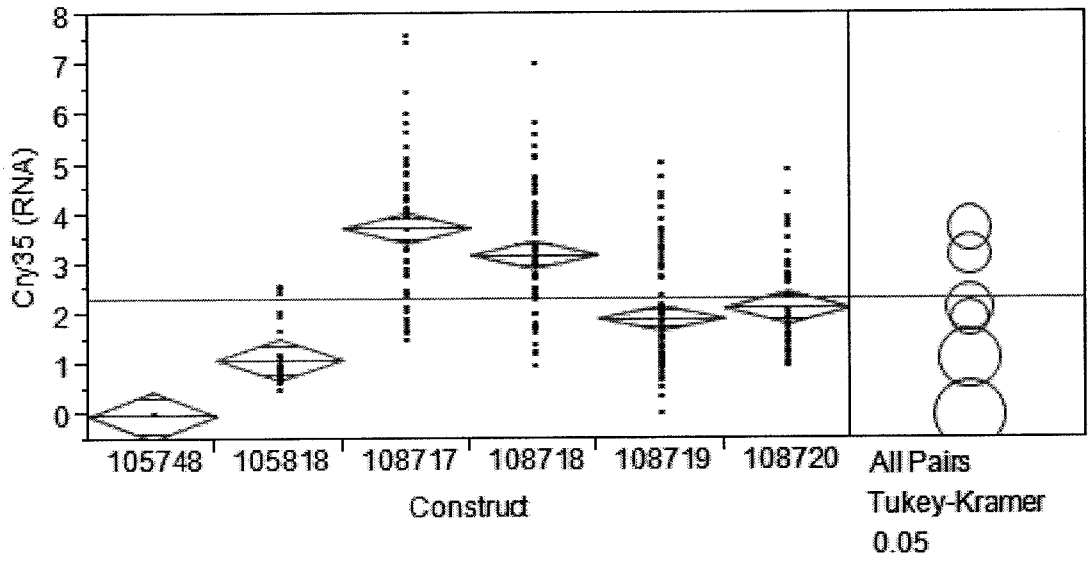
Level		Mean
105748	A	3.5986364
108717	A	2.7827778
108718	B	1.9533333
108719	B	1.8054430
108720	B	1.4918367
105818	C	0.0000000

FIG. 49A



Level		Mean
105748	A	1420.6883
108717	A B	1154.0353
108718	B C	706.0426
108720	C	381.0429
108719	C	283.5958
105818	C	251.6821

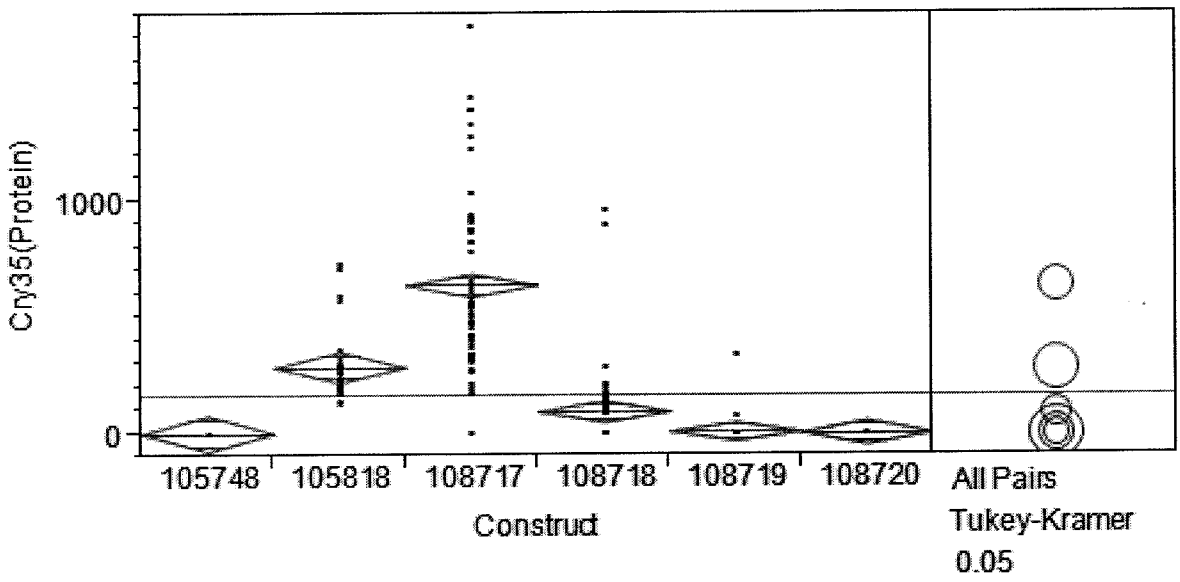
FIG. 49B



Level		Mean
108717	A	3.7442593
108718	A	3.2030303
108720	B	2.1365306
108719	B	1.9309302
105818	C	1.1190000
105748	D	0.0000000

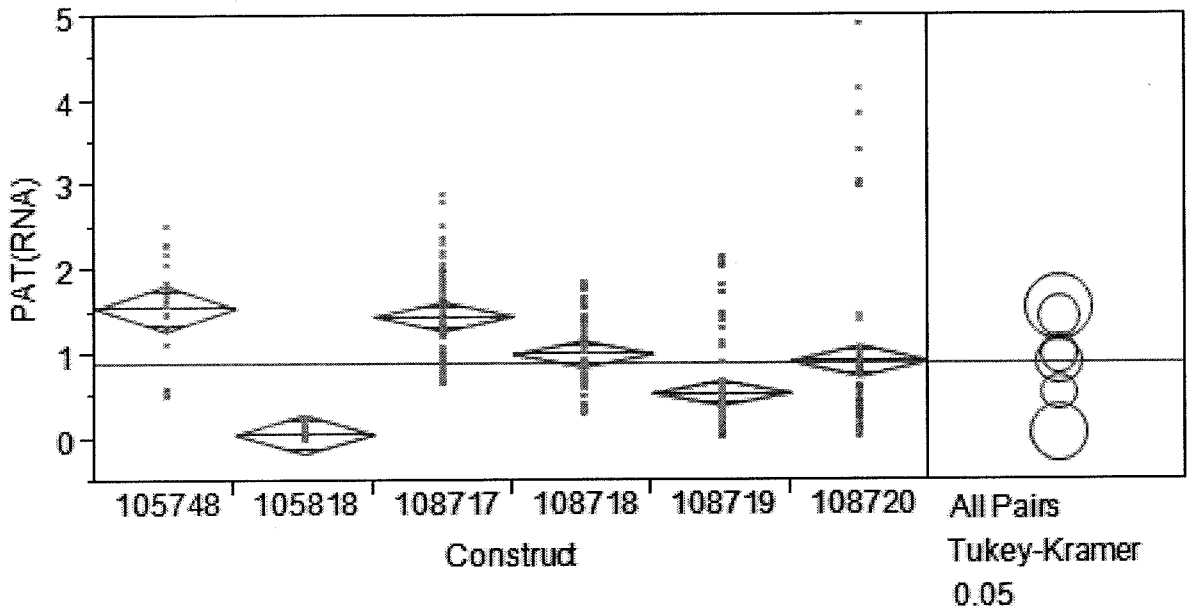
Levels not connected by same letter are significantly different.

FIG. 50A



Level		Mean
108717	A	635.82520
105818	B	283.54176
108718	C	90.97016
108719	C	4.91214
105748	C	0.00000
108720	C	0.00000

FIG. 50B



Level			Mean
105748	A		1.5627273
108717	A		1.4614815
108718		B	1.0131818
108720		B	0.9251020
108719		C	0.5496203
105818		D	0.0706667

FIG. 51

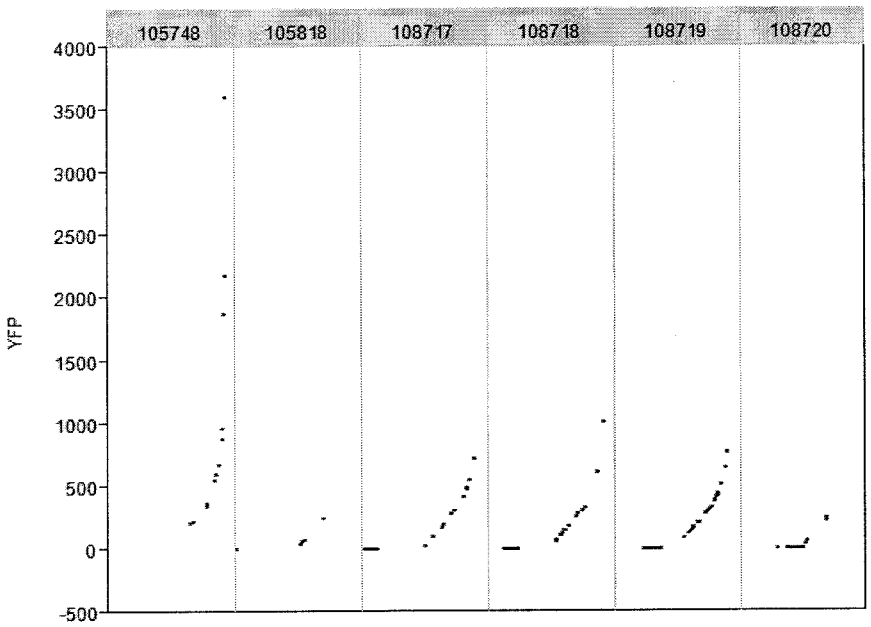


FIG. 52A

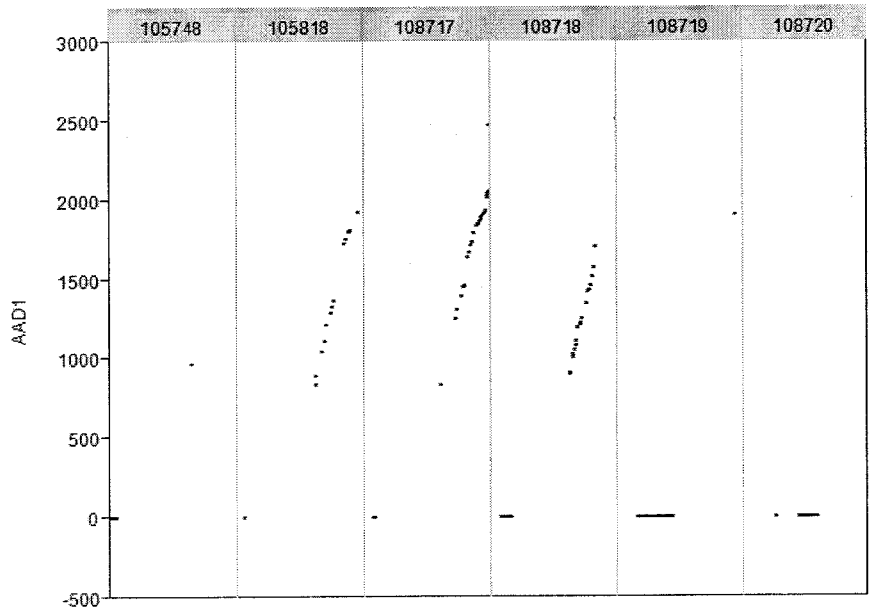


FIG. 52B

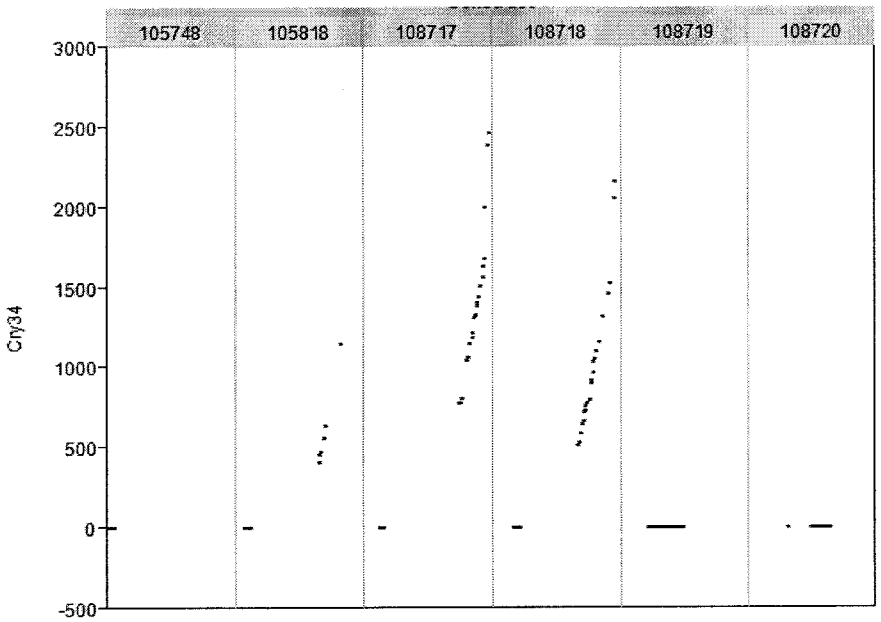


FIG. 52C

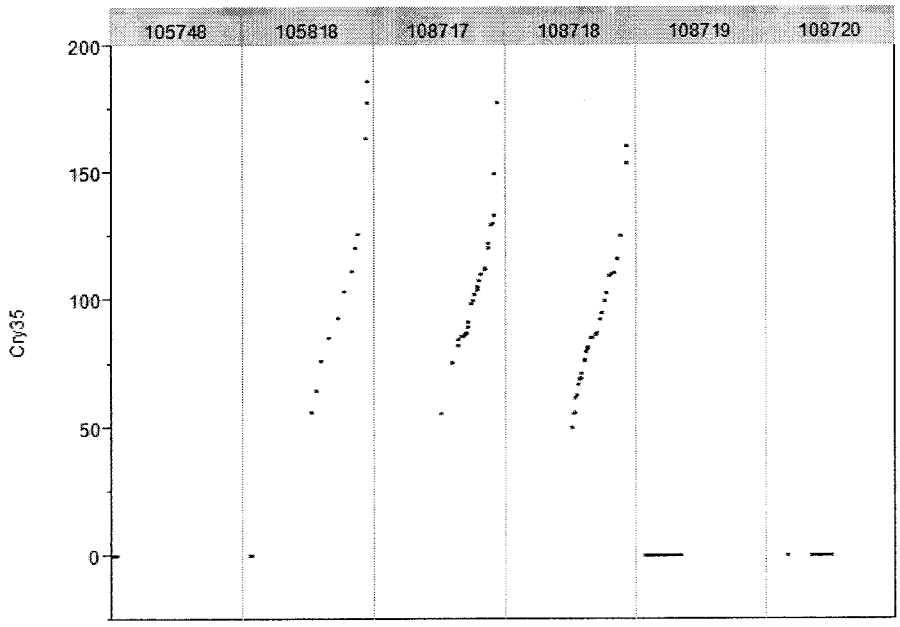
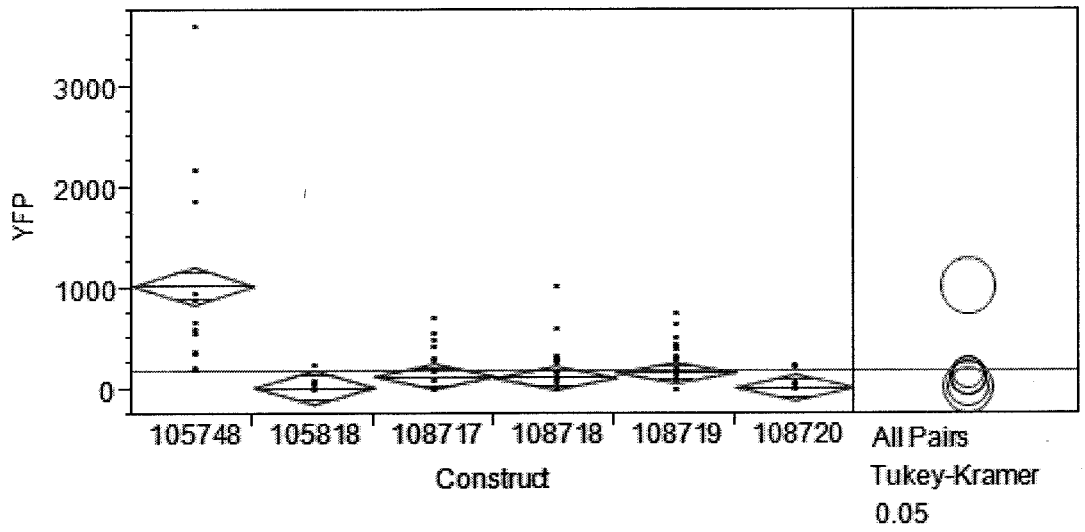
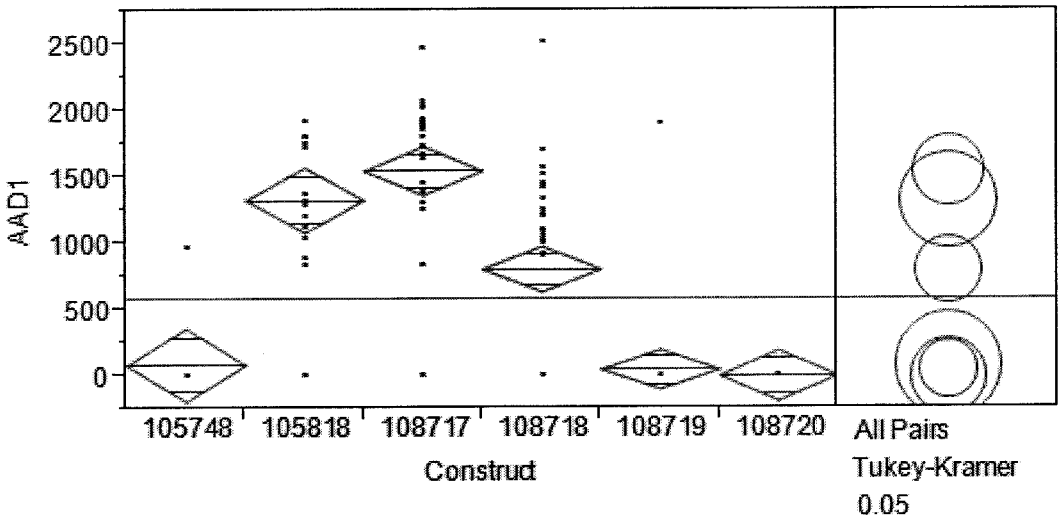


FIG. 52D



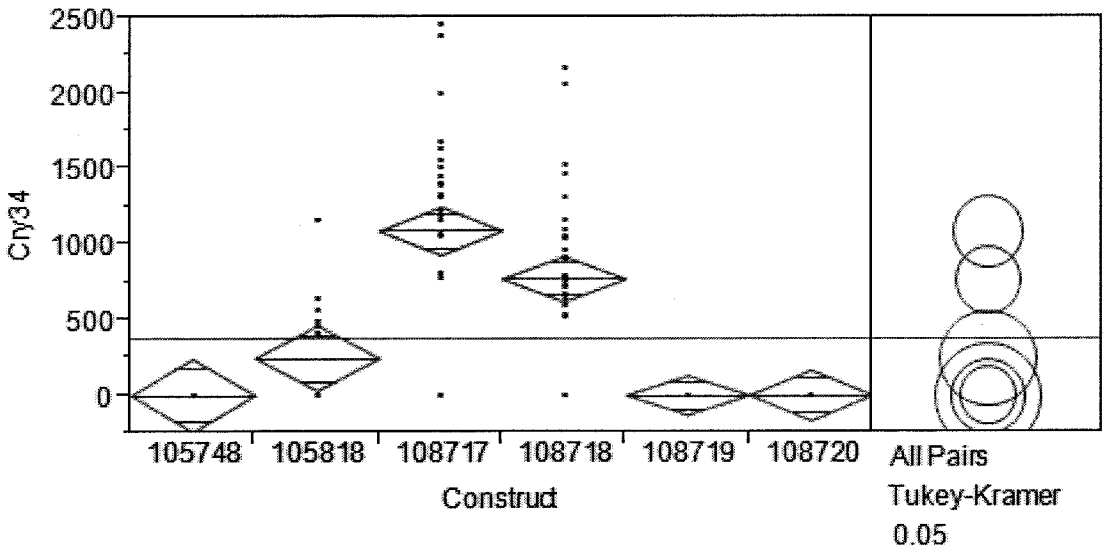
Level		Mean
105748	A	1033.4667
108719	B	169.8439
108717	B	136.1815
108718	B	119.0613
105818	B	27.5133
108720	B	22.4840

FIG. 53A



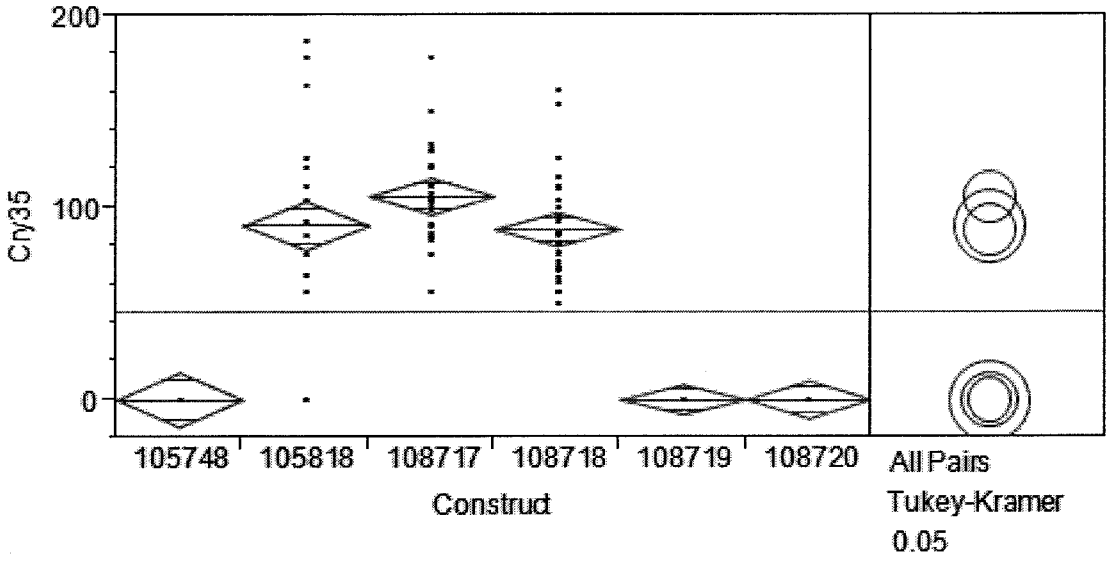
Level		Mean
108717	A	1544.6963
105818	A	1323.8000
108718	B	802.5000
105748	C	80.8917
108719	C	46.4927
108720	C	0.0000

FIG. 53B



Level		Mean
108717	A	1089.1778
108718	A	769.8065
105818	B	246.0533
105748	B	0.0000
108719	B	0.0000
108720	B	0.0000

FIG. 53C



Level		Mean
108717	A	106.08519
105818	A	90.74667
108718	A	88.80323
105748	B	0.00000
108719	B	0.00000
108720	B	0.00000

FIG. 53D

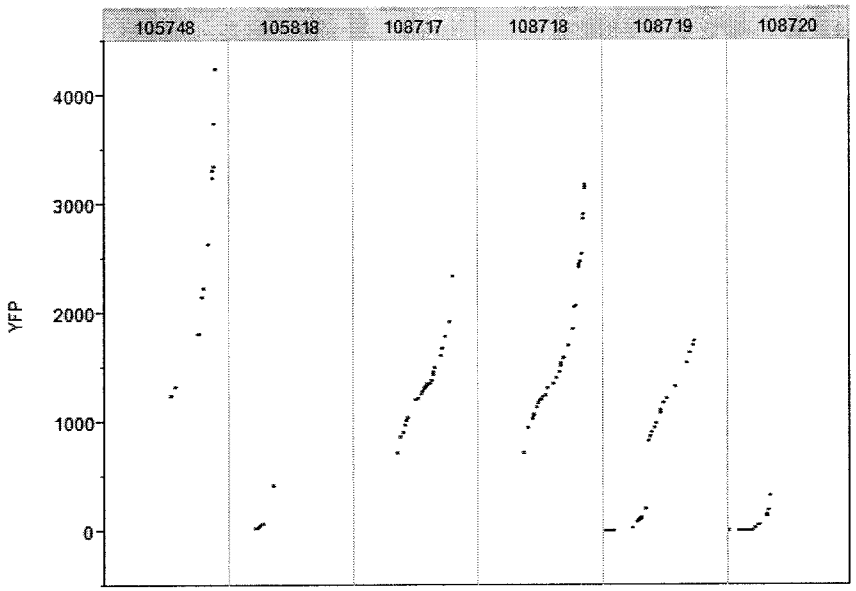


FIG. 54A

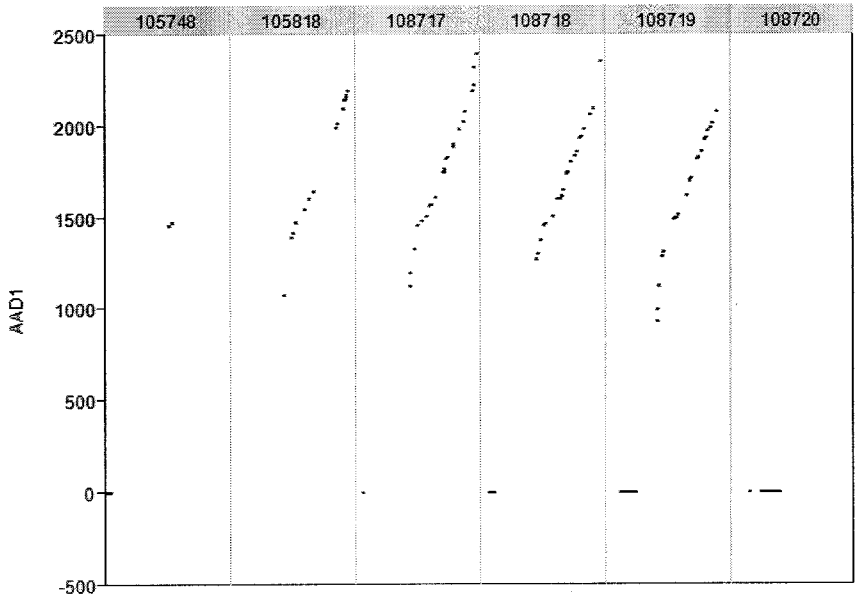


FIG. 54B

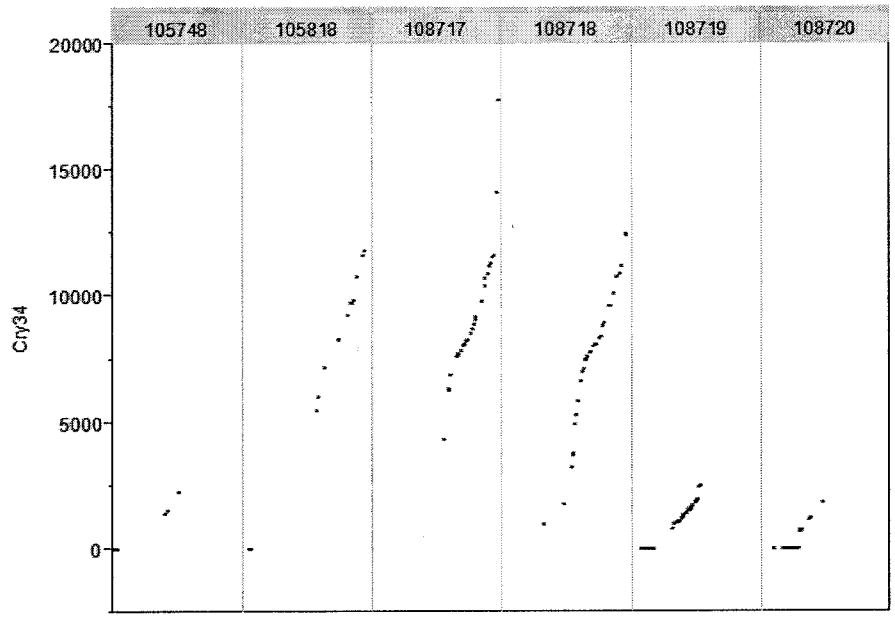


FIG. 54C

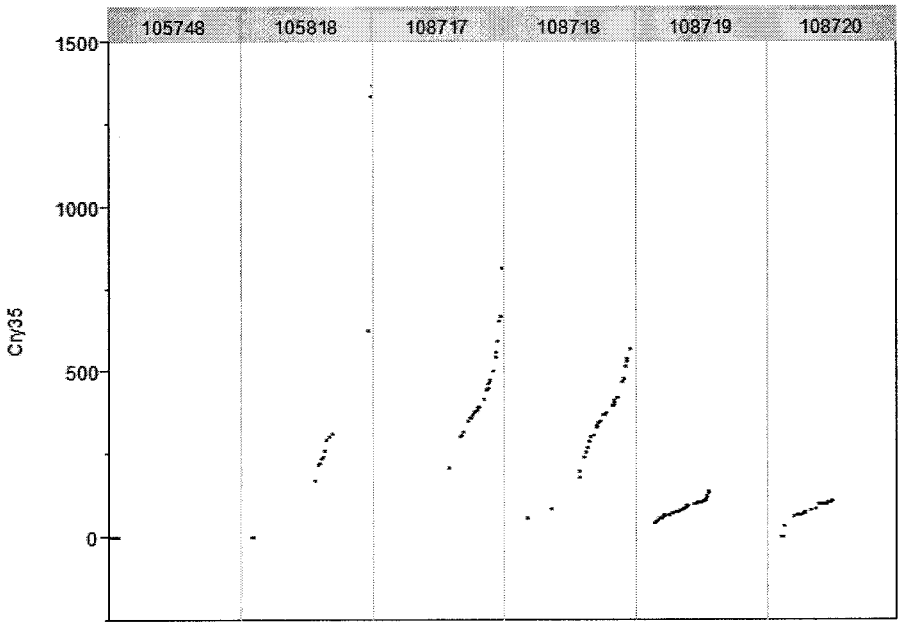
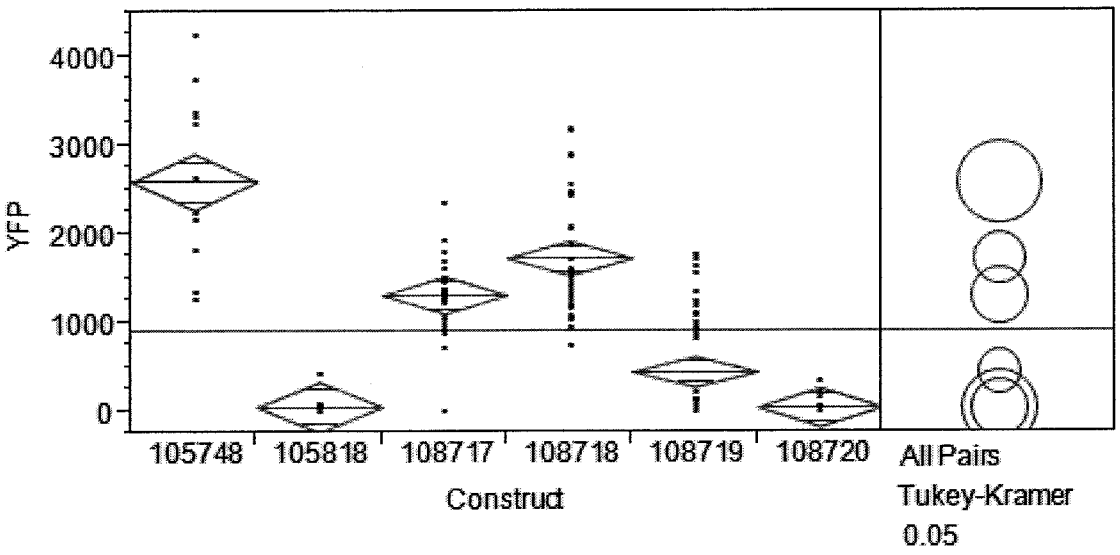


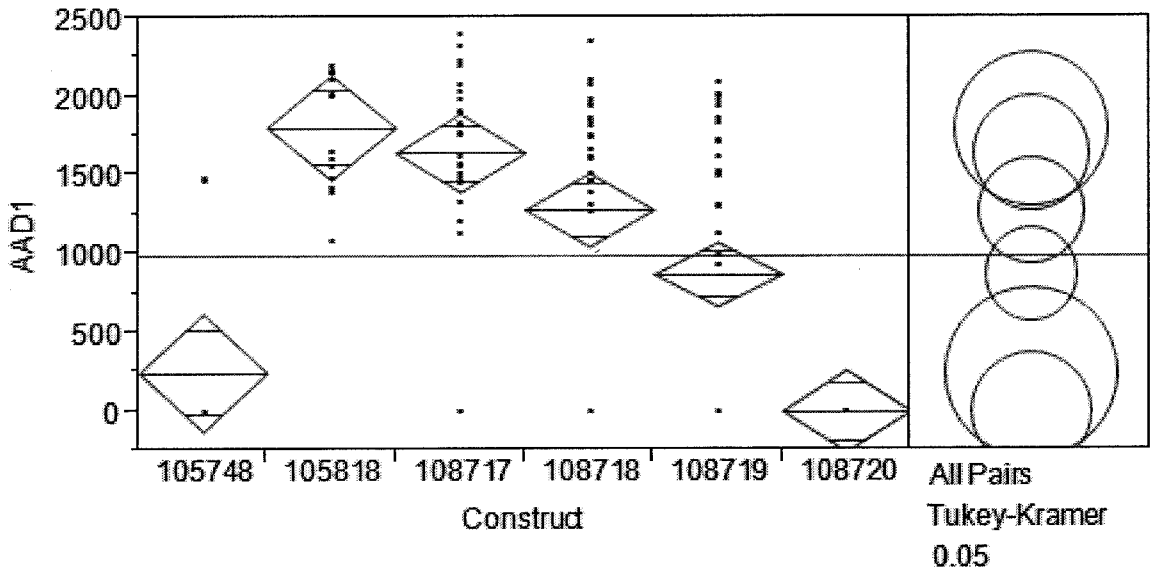
FIG. 54D



Level		Mean
105748	A	2589.6333
108718	B	1721.9581
108717	B	1305.2741
108719	C	438.8829
105818	C	43.6267
108720	C	37.4400

Levels not connected by same letter are significantly different.

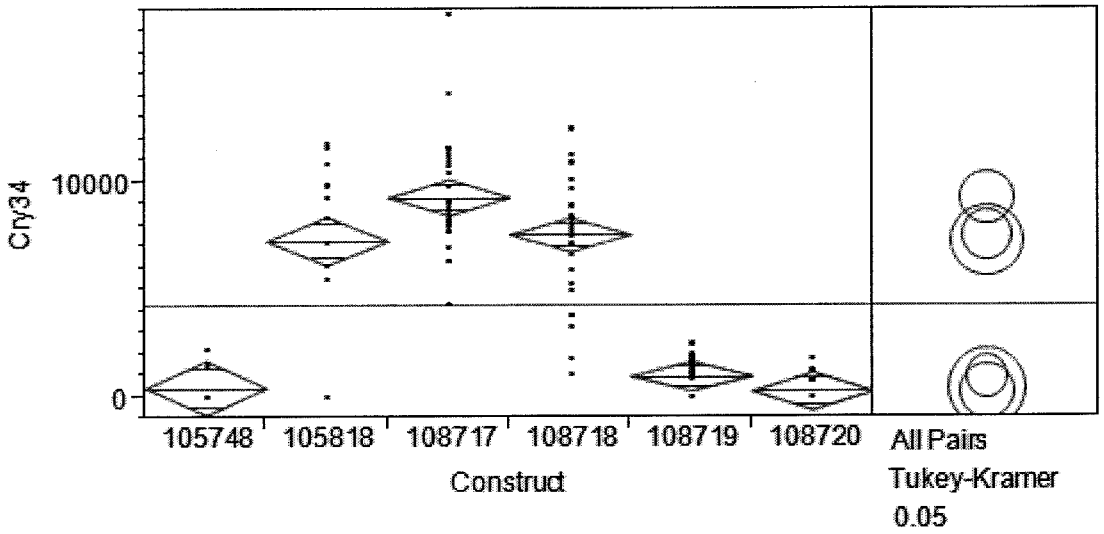
FIG. 55A



Level		Mean
105818	A	1803.9867
108717	A	1642.4370
108718	A B	1279.1677
108719	B	869.0707
105748	C	244.4083
108720	C	0.0000

Levels not connected by same letter are significantly different.

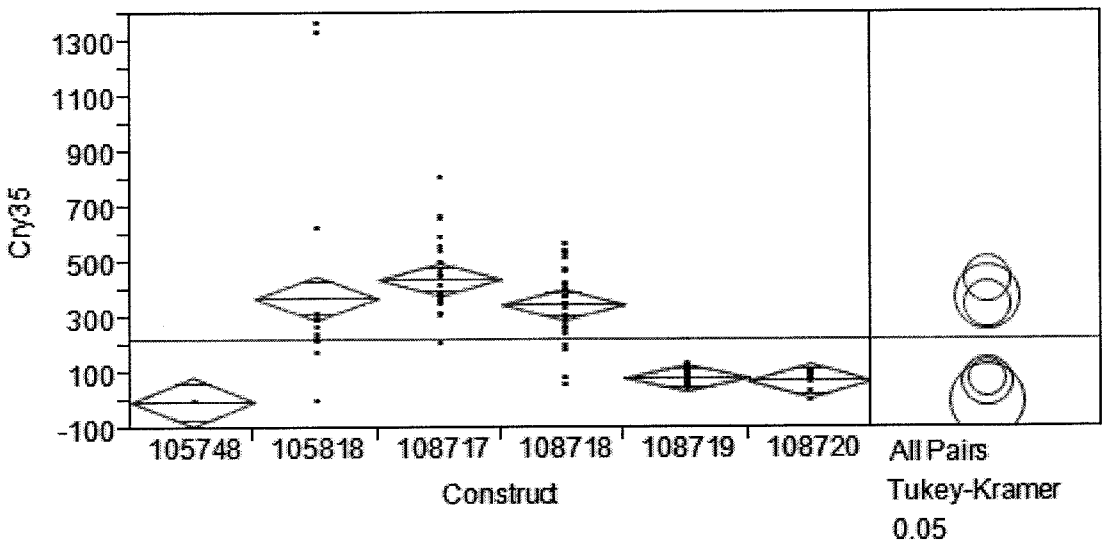
FIG. 55B



Level		Mean
108717	A	9285.7370
108718	B	7544.7484
105818	A B	7258.1533
108719	C	950.5341
105748	C	422.4500
108720	C	247.9240

Levels not connected by same letter are significantly different.

FIG. 55C



Level		Mean
108717	A	441.10741
105818	A	373.34667
108718	A	348.44839
108719	B	83.04634
108720	B	71.80800
105748	B	0.00000

Levels not connected by same letter are significantly different.

FIG. 55D

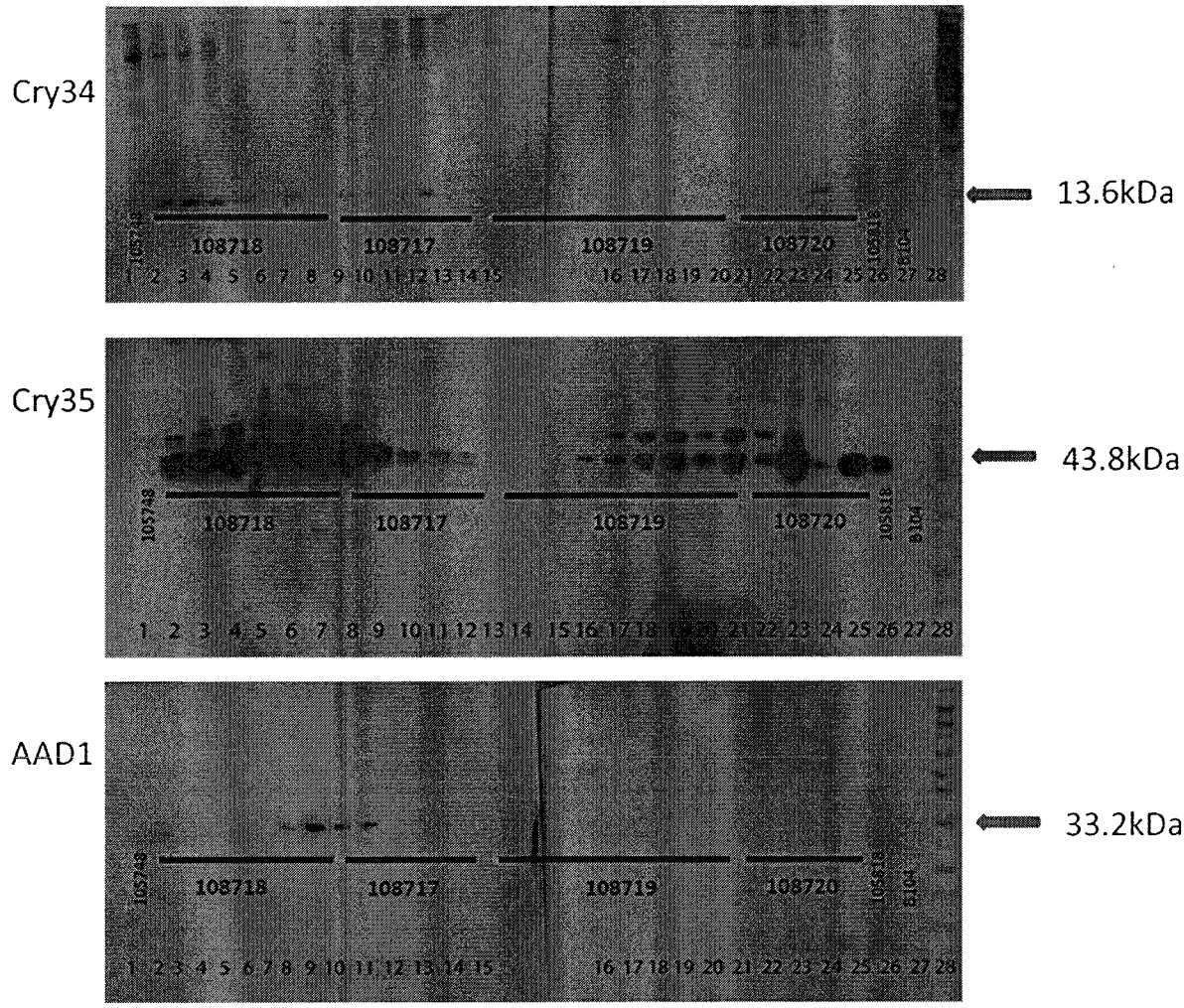
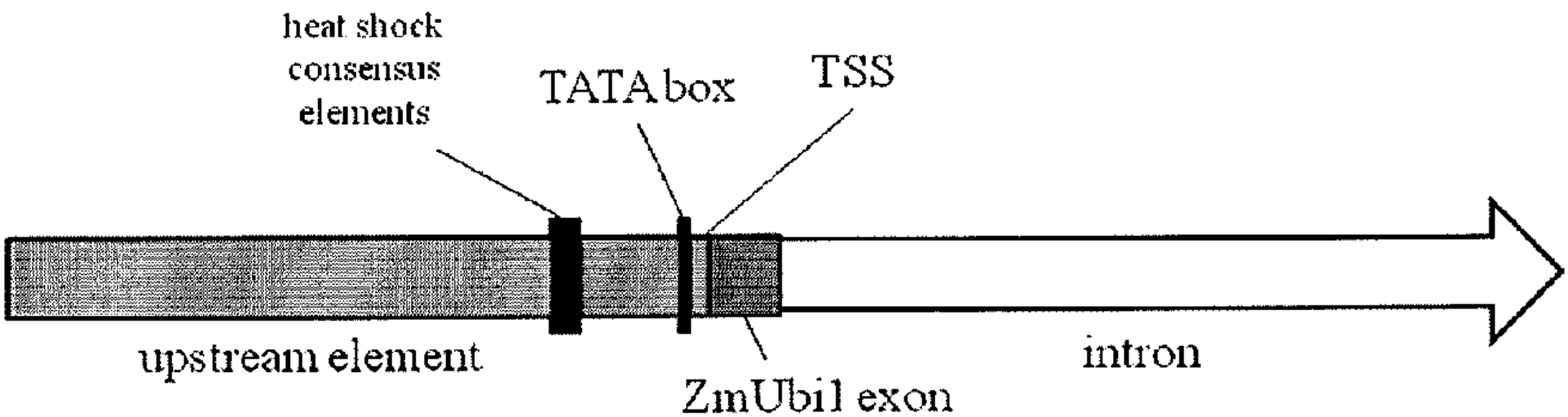

Cry34
13.6kDa
Cry35
43.8kDa
AAD1
33.2kDa
108718
108717
108719
108720

FIG. 56



maize Ubi1 promoter