(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 March 2015 (05.03.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/031731 A1

(51) International Patent Classification:
C12N 15/82 (2006.01)

(21) International Application Number:
PCT/US2014/053364

(22) International Filing Date:
29 August 2014 (29.08.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/872,134 30 August 2013 (30.08.2013) US

(71) Applicant: **DOW AGROSCIENCES LLC** [US/US]; 9330 Zionsville Road, Indianapolis, Indiana 46268 (US).

(72) Inventors: **KUMAR, Sandeep**; 201 Wyndotte Drive, Carmel, Indiana 46032 (US). **ASBERRY, Andrew**; 5346 North New Jersey Street, Indianapolis, Indiana 46220 (US).

(74) Agents: **ADDISON, Bradford G.** et al.; Barnes & Thornburg LLP, 11 South Meridian Street, Indianapolis, Indiana 46204 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

[Continued on next page]

(54) Title: CONSTRUCTS FOR EXPRESSING TRANSGENES USING REGULATORY ELEMENTS FROM SETARIA UBIQUITIN GENES

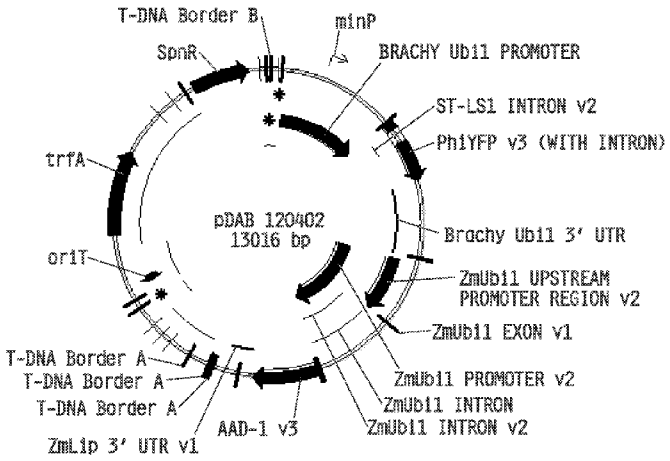

FIG. 34

(57) Abstract: Provided are constructs and methods for expressing a transgene in plant cells and/or plant tissues using the regulatory elements, including the promoters and/or 3'-UTRs, isolated from Setaria italica ubiquitin genes. In one embodiment an expression vector is provided wherein the regulatory elements of a ubiquitin gene are operably linked to a polylinker sequence. In accordance with one mbodiment a plant, plant tissue, or plant cell is provided comprising a promoter operably linked to a non-ubiquitin transgene.

WO 2015/031731 A1

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

CONSTRUCTS FOR EXPRESSING TRANSGENES USING REGULATORY ELEMENTS FROM SETARIA UBIQUITIN GENES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 61/872,134, filed August 30, 2013, which is hereby incorporated by reference in its entirety.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 68 KB ACII (Text) file named "Setaria_UBI_SEQ_LIST_ST25" created on August 15, 2014.

BACKGROUND

Plant transformation is an attractive technology for use in introducing agronomically desirable traits or characteristics into different crop plant species. Plant species are developed and/or modified to have particular desirable traits. Generally, desirable traits include, for example, improving nutritional value quality, increasing yield, conferring pest or disease resistance, increasing drought and stress tolerance, improving horticultural qualities (*e.g.*, 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.

Transgenic plants comprising multiple transgenes stacked at a single genomic locus are produced via plant transformation technologies. Plant transformation technologies result in the introduction of transgene into a plant cell, recovery of a fertile transgenic plant that contains the stably integrated copy of the transgene in the plant genome, and subsequent transgene expression via transcription and translation of the transgene(s) results in transgenic plants that possess desirable traits and phenotypes. Each transgene in a stack typically requires an independent promoter for gene expression, and thus multiple promoters are used in a transgene stack.

The need for co-expression of multiple transgenes for regulating the same trait frequently results in the repeated use of the same promoter to drive expression of the multiple transgenes. However, the repeated use of promoters comprising sequences that share a high level of sequence identity may lead to homology-based gene silencing (HBGS). HBGS has been observed to occur frequently in transgenic plants (Peremarti et al., 2010) when repetitive DNA sequences are used within a transgene. In addition, repeated use of similar DNA sequences in transgene constructs

has proven to be challenging in *Agrobacterium* due to recombination and instability of the plasmid.

Described herein are ubiquitin regulatory elements (e.g., promoters and 3'-UTR) that share low levels of sequence identity or homology with the Maize ubiquitin1 promoter. Further described are constructs and methods utilizing ubiquitin regulatory elements.

SUMMARY

Disclosed herein are constructs and methods for expressing a transgene in plant cells and/or plant tissues. In one embodiment regulatory elements of a ubiquitin gene are purified from *Panicum virgatum, Brachypodium distachyon, or Setaria italica* genomes and recombined with sequences not natively linked to said regulatory elements to create an expression vector for expressing transgenes in plant cells not native to the ubiquitin regulatory sequences. In one embodiment an expression vector is provided wherein the regulatory elements of a ubiquitin gene are operably linked to a polylinker sequence. Such an expression vector eases the insertion of a gene or gene cassette into the vector in an operably linked state with the ubiquitin gene regulatory sequences.

In an embodiment, a construct is provided comprising a *Panicum virgatum, Brachypodium distachyon,* or *Setaria italica* ubiquitin promoter. In an embodiment, a gene expression cassette is provided comprising a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter operably linked to a transgene. In an embodiment, a gene expression cassette includes a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin 5' -UTR operably linked to a transgene. In an embodiment, a gene expression cassette includes a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin 5' -UTR operably linked to a promoter. In an embodiment, a gene expression cassette includes a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin intron operably linked to a transgene. In an embodiment, a gene expression cassette includes a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin intron operably linked to a promoter. In an embodiment, a construct includes a gene expression cassette comprising *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin 3'-UTR. In an embodiment, a gene expression cassette includes *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin 3'-UTR operably linked to a transgene. In an embodiment, a gene expression cassette includes at least one, two, three, five, six, seven, eight, nine, ten, or more transgenes.

In an embodiment, a gene expression cassette includes independently a) a *Panicum virgatum, Brachypodium distachyon,* or *Setaria italica* ubiquitin promoter, b) a *Panicum*

virgatum, Brachypodium distachyon or *Setaria italica* ubiquitin intron, c) a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin 5'-UTR, and d) a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin 3'-UTR.

In accordance with one embodiment a nucleic acid vector is provided comprising a promoter operably linked to a non-ubiquitin transgene, wherein the promoter consists of SEQ ID NO: 17 or 41 or a sequence having 90% sequence identity with SEQ ID NO: 17 or 41. In a further embodiment the nucleic acid vector comprises a gene cassette, wherein the gene cassette comprises a promoter, a non-ubiquitin transgene and a 3' untranslated region, wherein the promoter consists of SEQ ID NO: 17 or 41 operably linked to a first end of a transgene, wherein the second end of the transgene is operably linked to a 3' untranslated sequence consisting of SEQ ID NO: 6.

Methods of growing plants expressing a transgene using the *Panicum virgatum, Brachypodium distachyon*, or *Setaria italica* promoters, 5' -UTRs, introns, and 3'-UTRs are disclosed herein. Methods of culturing plant tissues and cells expressing a transgene using the *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* promoters, 5' -UTRs, introns, and 3'-UTRs are also disclosed herein.

In accordance with one embodiment a plant, plant tissue, or plant cell is provided comprising a promoter operably linked to a non-ubiquitin transgene, wherein the promoter comprises SEQ ID NO: 3. In accordance with one embodiment a non-Setaria plant or plant cell is provided comprising SEQ ID NO: 3, or a sequence that has 90% sequence identity with SEQ ID NO: 3 operably linked to a transgene. In one embodiment the plant is a corn variety. In one embodiment a plant, plant tissue, or plant cell is provided comprising a promoter operably linked to a non-ubiquitin transgene, wherein the promoter consists of SEQ ID NO: 17, 40, 41 or 42. In one embodiment a non-Setaria plant or plant cell is provided comprising a gene cassette, wherein the gene cassette comprises a promoter operably linked to a transgene, further wherein the promoter consists SEQ ID NO: 17. In one embodiment a non-Setaria plant or plant cell is provided comprising a gene cassette, wherein the gene cassette comprises a promoter operably linked to a transgene, further wherein the promoter consists SEQ ID NO: 41. In a further embodiment the promoter is operably linked to a first end of a transgene, wherein the second end of the transgene is operably linked to a 3' untranslated sequence consisting of SEQ ID NO: 6.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the protein alignment of *Zea mays* ubiquitin (ZM Ubi1) protein sequence to *Brachypodium distachyon* and *Setaria italic* ubiquitin sequences used for promoter identification.

The Zm Ubi1 protein sequence is disclosed herein as SEQ ID NO:22. The *S. italica* Ubi2 protein sequence is disclosed herein as SEQ ID NO:23. The *B. distachyon* Ubi1 promoter sequence is disclosed herein as SEQ ID NO:24. The *B. distachyon* Ubi1C protein sequence is disclosed herein as SEQ ID NO:25. The consensus sequence is disclosed herein as SEQ ID NO:26.

FIG. 2 shows the alignment of *Zea mays* ubiquitin (ZM Ubi1) promoter polynucleotide sequence to *Brachypodium distachyon* and *Setaria italica* ubiquitin promoter polynucleotides identified herein. The *Zea may* Ubiquitin 1 (Zm-Ubi) promoter sequence is disclosed herein as SEQ ID NO:27. The *B. distachyon* Ubi1 promoter sequence is disclosed herein as SEQ ID NO:16. The *B. distachyon* Ubi1-C promoter sequence is disclosed herein as SEQ ID NO:15. The *S. italica* Ubi2 promoter sequence is disclosed herein as SEQ ID NO:17.

FIG. 3 is a plasmid map showing the synthesized *Setaria italica* Ubiquitin2 promoter genetic element.

FIG. 4 is plasmid map showing the synthesized *Brachypodium distachyon* Ubiquitin1 C promoter genetic element and flanking seamless cloning overhang location.

FIG. 5 is plasmid map showing the synthesized *Brachypodium distachyon* Ubiquitin1 promoter genetic element and flanking seamless cloning overhang location.

FIG. 6 is plasmid map showing the expression vector containing *Setaria italica* ubiquitin2 (SI-Ubi2) promoter fused to PhiYFP reporter gene.

FIG. 7 is plasmid map showing the expression vector containing *Brachypodium distachyon* Ubiquitin1 C promoter fused to PhiYFP reporter gene.

FIG. 8 is a plasmid map showing the expression vector containing *Brachypodium distachyon* Ubiquitin1 promoter fused to PhiYFP reporter gene.

FIG. 9 is a plasmid map showing the expression vector containing OS Act1 (Rice Actin1) promoter fused to PhiYFP reporter gene.

FIG. 10 is a plasmid map showing the expression vector containing ZM Ubi1 promoter fused to PhiYFP reporter gene.

FIG. 11 is a plasmid map showing the binary destination vector used to build binary expression vectors using Gateway technology.

FIG. 12 is a plasmid map showing the binary expression vector containing *Setaria italica* Ubiquitin2 (SI-Ubi2) promoter fused to yellow fluorescent protein (Phi YFP) marker gene coding region containing ST-LS1 intron followed by fragment comprising a StPinII 3'UTR from potato.

FIG. 13 is a plasmid map showing the binary expression vector containing *Brachypodium distachyon* Ubiquitin1 C promoter fused to yellow fluorescent protein (Phi YFP)

marker gene coding region containing ST-LS1 intron followed by fragment comprising a StPinII 3'UTR from potato.

FIG. 14 is a plasmid map showing the binary expression vector containing *Brachypodium distachyon* Ubiquitin1 promoter fused to yellow fluorescent protein (Phi YFP) marker gene coding region containing ST-LS1 intron followed by fragment comprising a StPinII 3'UTR from potato.

FIG. 15 is a plasmid map showing the binary expression vector containing OS Act1 promoter fused to yellow fluorescent protein (Phi YFP) marker gene coding region containing ST-LS1 intron followed by fragment comprising a StPinII 3'UTR from potato.

FIG. 16 is a plasmid map showing the binary expression vector containing ZM Ubi1 promoter fused to yellow fluorescent protein (Phi YFP) marker gene coding region containing ST-LS1 intron followed by fragment comprising a StPinII 3'UTR from potato.

FIG. 17 shows YFP expression in a T_0 leaf where YFP is driven by the cross species ubiquitin and Os Act 1 promoters as depicted in Figures 12, 13, 14, 15, and 16.

FIG. 18 shows AAD1 expression in a T_0 leaf where AAD1 is driven by the Zm Ubi 1 promoter as depicted in Figures 12, 13, 14, 15, and 16.

FIG. 19 shows transient YFP expression driven by the *Brachypodium distachyon* and *Setaria italica* novel promoters as compared to YFP expression driven by the ZM Ubi1 and OS Act1 promoters.

FIG. 20 shows YFP expression in calli tissues driven by the novel *Brachypodium distachyon* and *Setaria italica* promoters as compared to YFP expression driven by the ZM Ubi1 and OS Act1 promoters.

FIG. 21 shows YFP expression in root tissue driven by the novel *Brachypodium distachyon* and *Setaria italica* promoters as compared to YFP expression driven by the ZM Ubi1 and OS Act1 promoters.

FIG. 22 is a plasmid map showing the synthesized *Panicum virgatum* Ubiquitin1 promoter genetic element and flanking seamless cloning overhang location.

FIG. 23 is plasmid map showing the synthesized *Panicum virgatum* Ubiquitin1 3'UTR genetic element and flanking seamless cloning overhang location.

FIG. 24 is plasmid map showing the synthesized *Brachypodium distachyon* Ubiquitin1C 3'UTR genetic element and flanking seamless cloning overhang location.

FIG. 25 is plasmid map showing the synthesized *Brachypodium distachyon* Ubiquitin1 3'UTR genetic element and flanking seamless cloning overhang location.

FIG. 26 is plasmid map showing the synthesized *Setaria italica* ubiquitin2 (SI-Ubi2) 3'UTR genetic element and flanking seamless cloning overhang location.

FIG. 27 is plasmid map showing the expression vector containing *Panicum virgatum* Ubiquitin1 promoter and 3'UTR fused to PhiYFP reporter gene.

FIG. 28 is plasmid map showing the expression vector containing *Brachypodium distachyon* Ubiquitin1 C promoter and 3'UTR fused to PhiYFP reporter gene.

FIG. 29 is a plasmid map showing the expression vector containing *Setaria italica* ubiquitin2 promoter and 3'UTR fused to PhiYFP reporter gene.

FIG. 30 is plasmid map showing the expression vector containing *Brachypodium distachyon* Ubiquitin1 promoter and 3'UTR fused to PhiYFP reporter gene.

FIG. 31 is a plasmid map showing the binary expression vector containing *Brachypodium distachyon* Ubiquitin1 C promoter fused to yellow fluorescent protein (Phi YFP) marker gene coding region containing ST-LS1 intron followed by fragment comprising a *Brachypodium distachyon* Ubiquitin1 C 3'UTR.

FIG. 32 is a plasmid map showing the binary expression vector containing *Panicum virgatum* Ubiquitin1 promoter fused to yellow fluorescent protein (Phi YFP) marker gene coding region containing ST-LS1 intron followed by fragment comprising a *Panicum virgatum* Ubiquitin1 3'UTR.

FIG. 33 is a plasmid map showing the binary expression vector containing *Setaria italica* ubiquitin2 promoter fused to yellow fluorescent protein (Phi YFP) marker gene coding region containing ST-LS1 intron followed by fragment comprising a *Setaria italica* ubiquitin2 3'UTR.

FIG. 34 is a plasmid map showing the binary expression vector containing *Brachypodium distachyon* Ubiquitin1 promoter fused to yellow fluorescent protein (Phi YFP) marker gene coding region containing ST-LS1 intron followed by fragment comprising a *Brachypodium distachyon* Ubiquitin1 3'UTR.

FIG. 35 presents the *Brachypodium distachyon* Ubiquitin1 C coding sequence and putative promoter (upstream sequence of ATG). The upstream promoter sequence is underlined, the 5'-UTR sequence is presented in uppercase, the intron is boxed, the Ubi1 CDS is in italics, the 3'-UTR (underlined) and the transcription termination sequence is downstream of TAA (Translational Stop Codon).

FIG. 36 presents the *Brachypodium distachyon* Ubiquitin 1 coding sequence and putative promoter. The upstream promoter is underlined, the 5'UTR sequence is in uppercase, the intron

is boxed, the CDS is in italics, the 3'-UTR (underlined) and transcription termination sequence is downstream of TAA (Translational Stop Codon).

FIG. 37 presents the ***Setaria italica*** **Ubiquitin2** coding sequence and putative promoter. The upstream promoter is underlined, the 5'UTR sequence is in uppercase, the intron is boxed, the CDS is in italics, the 3'-UTR (underlined) and transcription termination sequence is downstream of TAA (Translational Stop Codon).

FIG. 38 presents the *Panicum virgatum* (Switchgrass)Ubiquitin 1 coding sequence and putative promoter. The upstream promoter is underlined, the 5'UTR sequence is in uppercase, the intron is boxed, the CDS is in italics, the 3'-UTR (underlined) and transcription termination sequence is downstream of TAA (Translational Stop Codon).

DETAILED DESCRIPTION

DEFINITIONS

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

The term "about" as used herein means greater or lesser than the value or range of values stated by 10 percent, but is not intended to designate any value or range of values to only this broader definition. Each value or range of values preceded by the term "about" is also intended to encompass the embodiment of the stated absolute value or range of values.

As used herein, the term" 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.

A "promoter" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. A promoter may contain specific sequences that are recognized by transcription factors. These factors may bind to a promoter DNA sequence, which results in the recruitment of RNA polymerase. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. The promoter may be operatively associated with other expression control sequences, including enhancer and repressor sequences.

For the purposes of the present disclosure, a "gene," includes a DNA region encoding a gene product (see infra), as well as all DNA regions which regulate the production of the gene product, whether or not such regulatory sequences are adjacent to coding and/or transcribed sequences. Accordingly, a gene includes, but is not necessarily limited to, promoter sequences, terminators, translational regulatory sequences such as ribosome binding sites and internal ribosome entry sites, enhancers, silencers, insulators, boundary elements, replication origins, matrix attachment sites and locus control regions.

As used herein the terms "native" or "natural" define a condition found in nature. A "native DNA sequence" is a DNA sequence present in nature that was produced by natural means or traditional breeding techniques but not generated by genetic engineering (e.g., using molecular biology/transformation techniques).

As used herein a "transgene" is defined to be a nucleic acid sequence that encodes a gene product, including for example, but not limited to, an mRNA. In one embodiment the transgene is an exogenous nucleic acid, where the transgene sequence has been introduced into a host cell by genetic engineering (or the progeny thereof) where the transgene is not normally found. In one example, a transgene encodes an industrially or pharmaceutically useful compound, or a gene encoding a desirable agricultural trait (e.g., an herbicide-resistance gene). In yet another example, a transgene is an antisense nucleic acid sequence, wherein expression of the antisense nucleic acid sequence inhibits expression of a target nucleic acid sequence. In one embodiment the transgene is an endogenous nucleic acid, wherein additional genomic copies of the endogenous nucleic acid are desired, or a nucleic acid that is in the antisense orientation with respect to the sequence of a target nucleic acid in a host organism.

As used herein the term "non-ubiquitin transgene" is any transgene that has less than 80% sequence identity with the *Zea may* Ubiquitin 1 coding sequence (SEQ ID NO:27).

"Gene expression" as defined herein is the conversion of the information, contained in a gene, into a gene product.

A "gene product" as defined herein is any product produced by the gene. For example the gene product can be the direct transcriptional product of a gene (e.g., mRNA, tRNA, rRNA, antisense RNA, interfering RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of a mRNA. Gene products also include RNAs which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins modified by, for example, methylation, acetylation, phosphorylation, ubiquitination, ADP-ribosylation, myristilation, and glycosylation. 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 term "intron" is defined as 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 corresponding sequence in RNA molecules transcribed therefrom. A construct described herein can also contain sequences that enhance translation and/or mRNA stability such as introns. An example of one such intron is the first intron of gene II of the histone H3 variant of *Arabidopsis thaliana* or any other commonly known intron sequence. Introns can be used in combination with a promoter sequence to enhance translation and/or mRNA stability.

As used herein, the terms "5' untranslated region" or "5'-UTR" is defined as the untranslated segment in the 5' terminus of pre-mRNAs or mature mRNAs. For example, on mature mRNAs, a 5'-UTR typically harbors on its 5' end a 7-methylguanosine cap and is involved in many processes such as splicing, polyadenylation, mRNA export towards the cytoplasm, identification of the 5' end of the mRNA by the translational machinery, and protection of the mRNAs against degradation.

As used herein, the terms "transcription terminator" is defined as the transcribed segment in the 3' terminus of pre-mRNAs or mature mRNAs. For example, longer stretches of DNA beyond "polyadenylation signal" site is transcribed as a pre-mRNA. This DNA sequence usually contains one or more transcription termination signals for the proper processing of the pre-mRNA into mature mRNA.

As used herein, the term "3' untranslated region" or "3'-UTR" is defined as the untranslated segment in a 3' terminus of the pre-mRNAs or mature mRNAs. For example, on mature mRNAs this region harbors the poly-(A) tail and is known to have many roles in mRNA stability, translation initiation, and mRNA export.

As used herein, the term "polyadenylation signal" designates a nucleic acid sequence present in mRNA transcripts that allows for transcripts, when in the presence of a poly-(A) polymerase, to be polyadenylated on the polyadenylation site, for example, located 10 to 30

bases downstream of the poly-(A) signal. Many polyadenylation signals are known in the art and are useful for the present invention. An exemplary sequence includes AAUAAA and variants thereof, as described in Loke J., et al., (2005) Plant Physiology 138(3); 1457-1468.

The term "isolated" as used herein means having been removed from its natural environment, or removed from other compounds present when the compound is first formed. The term "isolated" embraces materials isolated from natural sources as well as materials (e.g., nucleic acids and proteins) recovered after preparation by recombinant expression in a host cell, or chemically-synthesized compounds such as nucleic acid molecules, proteins, and peptides.

The term "purified," as used herein relates to the isolation of a molecule or compound in a form that is substantially free of contaminants normally associated with the molecule or compound in a native or natural environment, or substantially enriched in concentration relative to other compounds present when the compound is first formed, and means having been increased in purity as a result of being separated from other components of the original composition. The term "purified nucleic acid" is used herein to describe a nucleic acid sequence which has been separated, produced apart from, or purified away from other biological compounds including, but not limited to polypeptides, lipids and carbohydrates, while effecting a chemical or functional change in the component (*e.g.*, a nucleic acid may be purified from a chromosome by removing protein contaminants and breaking chemical bonds connecting the nucleic acid to the remaining DNA in the chromosome).

As used herein, the terms "homology-based gene silencing" or "HBGS" are generic terms that include 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. 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.

As used herein, the terms "nucleic acid molecule", "nucleic acid", or "polynucleotide" (all three terms are synonymous with one another) refer 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 thereof. "A nucleotide" may refer to a ribonucleotide, deoxyribonucleotide, or a

modified form of either type of nucleotide. A nucleic acid molecule is usually at least 10 bases in length, unless otherwise specified. The terms may refer to a molecule of RNA or DNA of indeterminate length. The terms include 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 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 term "base position," refers to the location of a given base or nucleotide residue within a designated nucleic acid. A designated nucleic acid may be defined by alignment with a reference nucleic acid.

As used herein, the term "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 bonding of a pyrimidine to a 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 terms "specifically hybridizable" and "specifically complementary" refers to a sufficient degree of complementarity such that stable and specific binding occurs between an oligonucleotide and the DNA or RNA target. Oligonucleotides need not be 100% complementary to its target sequence to specifically hybridize. 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 an 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 Na^+ and/or Mg^{2+} concentration) of a 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 term "stringent conditions" encompasses 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 5-6 x SSC buffer at 65-70°C for 16-20 hours; wash twice in 2 x 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 an embodiment, specifically hybridizable nucleic acid molecules can remain bound under very high stringency hybridization conditions. In an embodiment, specifically hybridizable nucleic acid molecules can remain bound under high stringency hybridization conditions. In an embodiment, specifically hybridizable nucleic acid molecules can remain bound under moderate stringency hybridization conditions.

As used herein, the term "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, an 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 terms "Polymerase chain reaction" or "PCR" define a procedure or technique in which minute amounts of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Pat. No. 4,683,195 issued July 28, 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 51:263 (1987); Erlich, ed., PCR Technology, (Stockton Press, NY, 1989).

As used herein, the term "primer" refers to an oligonucleotide capable of acting as a point of initiation of synthesis along a complementary strand when conditions are suitable for

synthesis of a primer extension product. The synthesizing conditions include the presence of four different deoxyribonucleotide triphosphates and at least one polymerization-inducing agent such as reverse transcriptase or DNA polymerase. These are present in a suitable buffer, which may include constituents which are co-factors or which affect conditions such as pH and the like at various suitable temperatures. A primer is preferably a single strand sequence, such that amplification efficiency is optimized, but double stranded sequences can be utilized.

As used herein, the term "probe" refers to an oligonucleotide that hybridizes to a target sequence. In the TaqMan® or TaqMan®-style assay procedure, the probe hybridizes to a portion of the target situated between the annealing site of the two primers. A probe includes about eight nucleotides, about ten nucleotides, about fifteen nucleotides, about twenty nucleotides, about thirty nucleotides, about forty nucleotides, or about fifty nucleotides. In some embodiments, a probe includes from about eight nucleotides to about fifteen nucleotides. A probe can further include a detectable label, e.g., a fluorophore (Texas-Red®, Fluorescein isothiocyanate, etc.,). The detectable label can be covalently attached directly to the probe oligonucleotide, e.g., located at the probe's 5' end or at the probe's 3' end. A probe including a fluorophore may also further include a quencher, e.g., Black Hole Quencher™, Iowa Black™, etc.

As used herein, the terms "sequence identity" or "identity" can be used interchangeably and refer to nucleic acid residues in two sequences that are the same when aligned for maximum correspondence over a specified comparison window.

As used herein, the term "percentage of sequence identity" refers to a value determined by comparing two optimally aligned sequences (*e.g.*, nucleic acid sequences or amino acid sequences) over a comparison window, wherein the portion of a sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to a reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. A percentage is calculated by determining the number of positions at which an identical nucleic acid 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. 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.

The National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST™; Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-10) 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 term "operably linked" refers to two components that have been placed into a functional relationship with one another. The term, "operably linked," when used in reference to a regulatory sequence and a coding sequence, means that the regulatory sequence affects the expression of the linked coding sequence. "Regulatory sequences," "regulatory elements", or "control elements," refer to nucleic acid sequences that influence the timing and level/amount of transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters; translation leader sequences; 5' and 3' untranslated regions, introns; enhancers; stem-loop structures; repressor binding sequences; termination sequences; polyadenylation recognition sequences; etc. Particular regulatory sequences may be located upstream and/or downstream of a coding sequence operably linked thereto. Also, particular regulatory sequences operably linked to a coding sequence may be located on the associated complementary strand of a double-stranded nucleic acid molecule. Linking can be accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. However, elements need not be contiguous to be operably linked.

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; lipofection; microinjection (Mueller *et al.* (1978) Cell 15:579-85); *Agrobacterium*-mediated transfer; direct DNA uptake; whiskers-mediated transformation; and microprojectile bombardment.

As used herein, the term "transduce" refers to a process where a virus transfers nucleic acid into a cell.

The terms "polylinker" or "multiple cloning site" as used herein defines a cluster of three or more Type -2 restriction enzyme sites located within 10 nucleotides of one another on a nucleic acid sequence. Constructs comprising a polylinker are utilized for the insertion and/or excision of nucleic acid sequences such as the coding region of a gene.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence. Type -2 restriction enzymes recognize and cleave DNA at the same site, and include but are not limited to XbaI, BamHI, HindIII, EcoRI, XhoI, SalI, KpnI, AvaI, PstI and SmaI.

The term "vector" is used interchangeably with the terms "construct", "cloning vector" and "expression vector" and means the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence. A "non-viral vector" is intended to mean any vector that does not comprise a virus or retrovirus. In some embodiments a "vector " is a sequence of DNA comprising at least one origin of DNA replication and at least one selectable marker gene. Examples include, but are not limited to, a plasmid, cosmid, bacteriophage, bacterial artificial chromosome (BAC), 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.

The term "plasmid" defines a circular strand of nucleic acid capable of autosomal replication in either a prokaryotic or a eukaryotic host cell. The term includes nucleic acid which may be either DNA or RNA and may be single- or double-stranded. The plasmid of the definition may also include the sequences which correspond to a bacterial origin of replication.

The term "selectable marker gene" as used herein defines a gene or other expression cassette which encodes a protein which facilitates identification of cells into which the selectable marker gene is inserted. For example a “selectable marker gene” encompasses reporter genes as well as genes used in plant transformation to, for example, protect plant cells from a selective agent or provide resistance/tolerance to a selective agent. In one embodiment 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 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 or pat (resistance against glufosinate ammonium or phosphinothricin), 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. Examples of "reporter genes" that can be used as a selectable marker gene include the visual observation of expressed reporter gene proteins such as proteins encoding β-glucuronidase (GUS), luciferase, green fluorescent protein (GFP), yellow fluorescent protein (YFP), DsRed, β-galactosidase, chloramphenicol acetyltransferase (CAT), alkaline phosphatase, and the like. The phrase "marker-positive" refers to plants that have been transformed to include a selectable marker gene.

As used herein, the term "detectable marker" refers to a label capable of detection, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator, or enzyme. Examples of detectable markers include, but are not limited to, the following: fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In an embodiment, a detectable marker can be attached by spacer arms of various lengths to reduce potential steric hindrance.

As used herein, the term "detecting" is used in the broadest sense to include both qualitative and quantitative measurements of a specific molecule, for example, measurements of a specific polypeptide.

As used herein, the terms "cassette", "expression cassette" and "gene expression cassette" refer to a segment of DNA that can be inserted into a nucleic acid or polynucleotide at specific restriction sites or by homologous recombination. As used herein the segment of DNA comprises a polynucleotide that encodes a polypeptide of interest, and the cassette and restriction sites are designed to ensure insertion of the cassette in the proper reading frame for transcription and translation. In an embodiment, an expression cassette can include a polynucleotide that encodes a polypeptide of interest and having elements in addition to the polynucleotide that facilitate transformation of a particular host cell. In an embodiment, a gene expression cassette may also include elements that allow for enhanced expression of a polynucleotide encoding a polypeptide of interest in a host cell. These elements may include,

but are not limited to: a promoter, a minimal promoter, an enhancer, a response element, a terminator sequence, a polyadenylation sequence, and the like.

As used herein a "linker" or "spacer" is a bond, molecule or group of molecules that binds two separate entities to one another. Linkers and spacers may provide for optimal spacing of the two entities or may further supply a labile linkage that allows the two entities to be separated from each other. Labile linkages include photocleavable groups, acid-labile moieties, base-labile moieties and enzyme-cleavable groups.

As used herein, the term “control” refers to a sample used in an analytical procedure for comparison purposes. A control can be “positive” or “negative”. For example, where the purpose of an analytical procedure is to detect a differentially expressed transcript or polypeptide in cells or tissue, it is generally preferable to include a positive control, such as a sample from a known plant exhibiting the desired expression, and a negative control, such as a sample from a known plant lacking the desired expression.

As used herein, the term “plant” includes a whole plant and any descendant, cell, tissue, or part of a plant. A class of plant 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 dicot and monocot plants. The term “plant parts” include any part(s) of a plant, including, for example and without limitation: seed (including mature seed and immature seed); a plant cutting; a plant cell; a plant cell culture; a plant organ (e.g., pollen, embryos, flowers, fruits, shoots, leaves, roots, stems, and explants). A plant tissue or plant organ may be a seed, protoplast, callus, or any other group of plant cells that is organized into a structural or functional unit. A plant cell or tissue culture may be capable of regenerating a plant having the physiological and morphological characteristics of the plant from which the cell or tissue was obtained, and of regenerating a plant having substantially the same genotype as the plant. In contrast, some plant cells are not capable of being regenerated to produce plants. Regenerable cells in a plant cell or tissue culture may be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks, or stalks.

Plant parts include harvestable parts and parts useful for propagation of progeny plants. Plant parts useful for propagation include, for example and without limitation: seed; fruit; a cutting; a seedling; a tuber; and a rootstock. A harvestable part of a plant may be any useful part of a plant, including, for example and without limitation: flower; pollen; seedling; tuber; leaf; stem; fruit; seed; and root.

A plant cell is the structural and physiological unit of the plant, comprising a protoplast and a cell wall. A plant cell may be in the form of an isolated single cell, or an aggregate of cells (e.g., a friable callus and a cultured cell), and may be part of a higher organized unit (e.g., a plant tissue, plant organ, and plant). Thus, a plant cell may be a protoplast, a gamete producing cell, or a cell or collection of cells that can regenerate into a whole plant. As such, a seed, which comprises multiple plant cells and is capable of regenerating into a whole plant, is considered a "plant cell" in embodiments herein.

The term "protoplast," as used herein, refers to a plant cell that had its cell wall completely or partially removed, with the lipid bilayer membrane thereof naked, and thus includes protoplasts, which have their cell wall entirely removed, and spheroplasts, which have their cell wall only partially removed, but is not limited thereto. Typically, a protoplast is an isolated plant cell without cell walls which has the potency for regeneration into cell culture or a whole plant.

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).

EMBODIMENTS

As disclosed herein novel recombinant constructs are provided for expressing a non-ubiquitin transgene using the regulatory sequences of a ubiquitin gene from *Panicum virgatum, Brachypodium distachyon,* or *Setaria italica.* These constructs can be used to transform cells, including plant cells, to produce complete organisms that express the transgene gene product in their cells.

Regulatory Elements

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 transgenic crops to drive the expression of multiple genes.

Development of transgenic products is becoming increasingly complex, which requires stacking multiple transgenes into a single locus. Traditionally, each transgene usually requires a promoter for expression wherein multiple promoters are required to express different transgenes within one gene stack. This frequently leads to repetitive use of the same promoter within one transgene stack to obtain similar levels of expression patterns of different transgenes for expression of a single polygenic trait. Multi-gene constructs driven by the same promoter are known to cause gene silencing resulting in less efficacious transgenic products 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 performance of a transgenic plant produced to express transgenes. Repetitive sequences within a transgene may lead to gene intra locus homologous recombination resulting in polynucleotide rearrangements.

It is desirable to use diversified promoters for the expression of different transgenes in a gene stack. In an embodiment, diversified constitutive ubiquitin obtained from different plant species can drive transcription of multiple transcription units, including RNAi, artificial miRNA, or hairpin-loop RNA sequences.

Provided are methods and constructs using a constitutive ubiquitin (Ubi1) promoter to express non-ubiquitin transgenes in plant. In an embodiment, a promoter can be the *Brachypodium distachyon* ubiquitin1 C (Ubi1C) promoter.

CTGCTCGTTCAGCCCACAGTAACACGCCGTGCGACATGCAGATGCCC
TCCACCACGCCGACCAACCCCAAGTCCGCCGCGCTCGTCCACGGCGC
CATCCGCATCCGCGCGTCAACGTCATCCGGAGGAGGCGAGCGCGATG
TCGACGGCCACGGCGGCGGCGGACACGACGGCGACGCCCCGACTCC
GCGCGCGCGTCAAGGCTGCAGTGGCGTCGTGGTGGCCGTCCGCCTGC
ACGAGATCCCCGCGTGGACGAGCGCCGCCTCCACCCAGCCCCTATAT
CGAGAAATCAACGGTGGGCTCGAGCTCCTCAGCAACCTCCCCACCCC
CCCTTCCGACCACGCTCCCTTCCCCCGTGCCCCTCTTCTCCGTAAACC
CGAGCCGCCGAGAACAACACCAACGAAAGGGCGAAGAGAATCGCCA
TAGAGAGGAGATGGGCGGAGGCGGATAGTTTCAGCCATTCACGGAG
AAATGGGGAGGAGAGAACACGACATCATACGGACGCGACCCTCTAG
CTGGCTGGCTGTCCTAAAGAATCGAACGGAATCGCTGCGCCAGGAGA
AAACGAACGGTCCTGAAGCATGTGCGCCCGGTTCTTCCAAAACACTT
ATCTTTAAGATTGAAGTAGTATATATGACTGAAATTTTTACAAGGTTT
TTCCCCATAAAACAGGTGAGCTTATCTCATCCTTTTGTTTAGGATGTA
CGTATTATATATGACTGAATATTTTTTATTTTCATTGAATGAAGATTTT
CGACCCCCCCAAAAATAAAAAACGGAGGGAGTACCTTTGTGCCGTGTA
TATGGACTAGAGCCATCGGGACGTTTCCGGAGACTGCGTGGTGGGGG
CGATGGACGCACAACGACCGCATTTTCGGTTGCCGACTCGCCGTTCG
CATCTGGTAGGCACGACTCGTCGGGTTCGGCTCTTGCGTGAGCCGTG
ACGTAACAGACCCGTTCTCTTCCCCCGTCTGGCCATCCATAAATCCCC

CCTCCATCGGCTTCCCTTTCCTCAATCCAGCACCCTGATT (SEQ ID NO:1)

In an embodiment, a promoter can be the *Brachypodium distachyon* ubiquitin 1 (Ubi1) promoter.

GGCGTCAGGACTGGCGAAGTCTGGACTCTGCAGGGCCGAACTGCTGA
AGACGAAGCAGAGGAAGAGAAAGGGAAGTGTTCGACTTGTAATTTG
TAGGGGTTTTTTTTAGAGGAACTTGTAATTTGTAGGTGGGCTGGCCTC
GTTGGAAAAACGATGCTGGCTGGTTGGGCTGGGCCGATGTACGCTTG
CAAACAACTTGTGGCGGCCCGTTCTGGACGAGCAGGAGTTTCTTTTTT
GTTCTCACTTTTCTGGTCTTCTTTAGTTACGGAGTACCTTTTGTTTTTT
AAAGGAGTTACCTTTTTTTTAGGAATTCTTTAGTTACCTTTCGCTTGCT
CTCAAAAAATATTTAACTTTCGCTTTTTTTCATTTTAATTTTTGCAACT
ATTTACGAGTTTCATGAATGCTTATTTTCCAGCATATCATTATTTGCA
AGTATTTTTATGCCGTATGTATTGGACGAGAGCCATCGGGACTGTTCC
AGAGACTGCGTGGTGGGGACGGCTCCCAACCGCCTTTTCTATCTCTGT
TCGCATCCGGTGGCCGACTTGGCTCGCGCGTGAGCCGTGACGTAACA
GACTTGGTCTCTTCCCCATCTGGCCATCTATAAATTCCCCCATCGATC
GACCCTCCCTTTCC (SEQ ID NO:2)

In an embodiment, a promoter can be the *Setaria italica* ubiquitin 2 (Ubi2) promoter.

TGCGTCTGGACGCACAAGTCATAGCATTATCGGCTAAAATTTCTTAAT
TTCTAAATTAGTCATATCGGCTAAGAAAGTGGGGAGCACTATCATTT
CGTAGAACAAGAACAAGGTATCATATATATATATATATATAATATTT
AAACTTTGTTAAGTGGAATCAAAGTGCTAGTATTAATGGAGTTTCAT
GTGCATTAAATTTTATGTCACATCAGCAATTTTGTTGACTTGGCAAGG
TCATTTAGGGTGTGTTTGGAAGACAGGGGCTATTAGGAGTATTAAAC
ATAGTCTAATTACAAAACTAATTGCACAACCGCTAAGCTGAATCGCG
AGATGGATCTATTAAGCTTAATTAGTCCATGATTTGACAATGTGGTGC
TACAATAACCATTTGCTAATGATGGATTACTTAGGTTTAATAGATTCG
TCTCGTGATTTAGCCTATGGGTTCTGCTATTAATTTTGTAATTAGCTCA
TATTTAGTTCTTATAATTAGTATCCGAACATCCAATGTGACATGCTAA
AGTTTAACCCTGGTATCCAAATGAAGTCTTATGAGAGTTTCATCACTC
CGGTGGTATATGTACTTAGGCTCCGTTTTCTTCCACCGACTTATTTTTA
GCACCCGTCACATTGAATGTTTAGATACTAATTAGAAGTATTAAACG
TAGACTATTTACAAAATCCATTACATAAGACGAATCTAAACGGCGAG
ACGAATCTATTAAACCTAATTAGTCCATGATTTGACAATGTGTTGCTA
CAGTAAACATTTGCTAATGATGGATTAATTAGGCTTAATAGATTCGTC
TCGCCGTTTAGCCTCCACTTATGTAATGGGTTTTCTAAACAATCTACG
TTTAATACTCCTAATTAGTATCTAAATATTCAATGTGACACGTGCTAA
AAATAAGTCAGTGGAAGGAAGAGAACGTCCCCTTAGTTTTCCATCTT
ATTAATTGTACGATGAAACTGTGCAGCCAGATGATTGACAATCGCAA
TACTTCAACTAGTGGGCCATGCACATCAGCGACGTGTAACGTCGTGA
GTTGCTGTTCCCGTAG (SEQ ID NO:3)

In an embodiment, a promoter can be the *Panicum virgatum (Switchgrass)* ubiquitin 1 promoter.

TTGAATTTTAATTTCAAATTTTGCAGGGTAGTAGTGGACATCACAATA
CATATTTAGAAAAAGTTTTATAATTTTCCTCCGTTAGTTTTCATATAAT
TTTGAACTCCAACGATTAATCTATTATTAAATATCCCGATCTATCAAA
ATAATGATAAAAATTTATGATTAATTTTTCTAACATGTGTTATGGTGT

GTACTATCGTCTTATAAAATTTCAACTTAAAACTCCACCTATACATGG
AGAAATGAAAAAGACGAATTACAGTAGGGAGTAATTTGAACCAAAT
GGAATAGTTTGAGGGTAAAATGAACTAAACAATAGTTTAGGAGGTTA
TTCAGATTTTAGTTATAGTTGAGAGGAGTAATTTAGACTTTTTCCTAT
CTTGAATTGTTGACGGCTCTCCTATCGGATATCGGATGGAGTCTTTCA
GCCCAACATAACTTCATTCGGGCCCAAACGTTCGTCCATCCAGCCTA
GGGAGAACATTTTGCCCATGATATCTGTTTTTCTTTTTTTCTATTTTCA
CTGGTATTATAGGAGGGAAATATACAACGTGTTCACCTTTGGTTTCAT
TCTTGTTCCATCTGAATTTATCTAAAACTGTGTTTGAACTTCGTAAGA
ATTTTGTTCGATCTGTCCGGTACATCGTGTTGATAGGTGGCCTCCGAG
ATTCTTCTTTTTAACCGGCAAAGTAAAATAATCTCAGCTCCAGCCTAA
CGTCAATTATCAGAGAGAGAAAAAAATATTTTTTTATGATTGATCGG
AAACCAACCGCCTTACGTGTCGATCCTGGTTCCTGGCCGGCACGGCG
GAGGAAAGCGACCGACCTCGCAACGCCGGCGCACGGCGCCGCCGTG
TTGGACTTGGTCTCCCGCGACTCCGTGGGCCTCGGCTTATCGCCGCCG
CTCCATCTCAACCGTCCGCTTGGACACGTGGAAGTTGATCCGTCGCGC
ACCAGCCTCGGAGGTAACCTAACTGCCCGTACTATAAATCCGGGATC
CGGCCTCTCCAATCCCCATCGCCA (SEQ ID NO:35)

In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter. In an embodiment, the ubiquitin promoter is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter. In an embodiment, a nucleic acid construct is provided comprising a promoter, wherein the promoter is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35. In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter that is operably linked to a polylinker. In an embodiment, a gene expression cassette is provided comprising a ubiquitin promoter that is operably linked to a non-ubiquitin transgene. In one embodiment the promoter consists of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35. In an illustrative embodiment, a gene expression cassette comprises a ubiquitin promoter that is operably linked to the 5' end of a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof.

In addition to a promoter, a 3'-untranslated gene region (*i.e.,* 3'UTR) or terminator is needed for transcription termination and polyadenylation of the mRNA. Proper transcription termination and polyadenylation of mRNA is important for stable expression of transgene. The transcription termination becomes more critical for multigene stacks to avoid transcription read-through into next transgene. Similarly, non-polyadenylated aberrant RNA (aRNA) is a substrate for plant RNA-dependent RNA polymerases (RdRPs) to convert aRNA into double stranded RNA (dsRNA) leading to small RNA production and transgene silencing. Strong

transcription terminators therefore are very useful both for single gene and multiple gene stacks. While a promoter is necessary to drive transcription, a 3'-UTR gene region can terminate transcription and initiate polyadenylation of a resulting mRNA transcript for translation and protein synthesis. A 3'-UTR gene region aids stable expression of a transgene.

In accordance with one embodiment a nucleic acid construct is provided comprising a ubiquitin transcription terminator. In an embodiment, the ubiquitin transcription terminator is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin transcription terminator. In an embodiment, a nucleic acid construct is provided comprising a transcription terminator, wherein the transcription terminator is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36. In an embodiment, a nucleic acid construct is provided comprising a ubiquitin transcription terminator that is operably linked to a polylinker. In an embodiment, a gene expression cassette is provided comprising a ubiquitin transcription terminator that is operably linked to the 3' end of a non-ubiquitin transgene. In one embodiment the transcription terminator consists of SEQ ID NO: 4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36. In an illustrative embodiment, a gene expression cassette comprises a ubiquitin transcription terminator that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof. In one embodiment a nucleic acid vector is provided comprising a transcription terminator operably linked to either a polylinker sequence, a non-ubiquitin transgene or a combination of both, wherein the transcription terminator comprises SEQ ID NO: 6 or a sequence that has 90% sequence identity with SEQ ID NO: 6. In one embodiment the transcription terminator is less than 1kb in length, and in a further embodiment the transcription terminator consists of the 3'UTR sequence of SEQ ID NO: 6.

In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter as described herein and a 3'-UTR. In an embodiment, the nucleic acid construct comprises a ubiquitin 3'-UTR. In an embodiment, the ubiquitin 3'-UTR is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin 3'-UTR. In an embodiment, a 3'-UTR can be the *Brachypodium distachyon* ubiquitin1 C (Ubi1C) 3'-UTR.

GTTTGTCAAAAACTGGCCTACAGTCTGCTGCCCCTGTTGGTCTGCCCC
TTGGAAGTAGTCGTGTCTATGGTTATGTGAGAAGTCGTTGTGTTCTTT
CTAATCCCGTACTGTTTGTGTGAACATCTGCTGCTGTCGTATTGCATC
GTGAAGAATCCTGTTATGAATAAGTGAACATGAACCTTGTTCTGTGA

TTACGGCTTCGTGGTTATGCGAACGTTCTTACAAACGCAATTGCACCT
GATGTAAAATCGTTTTTGCTAGCTGTATGGAACAAGTGCTCATGATGT
TCATGCAAGATGCAATTCCAGCTTTTGTTGGTTTGTCATCTTTGTACT
GTGCTTACCGCACATAAAGATTGCATCTTGCTTATTGCTTTGTTGCTTT
GGTGCTCGTCCGCTTCTCCTTGCACCTTATCAAACCTTTGTTTAGATTC
TCTTCTTATAGCACTTGGTAACTCTCAGCTTTACAACGCCAGTACTGT
TTCTGAAATTTCATGACTGATAAAGCTGATAGATGGAGTACTAATAT
ATGACATCTTTCCATAAATGTTCGGGTGCAGAGATATGGAGGCCCCA
GGATCCTATTTACAGGATGAACCTACCTGGGCCGCTGTACGCATGAC
ATCCGCGAGCAAGTCTGAGGTTCTCAATGTACACATGAAATTGATTTT
TGCTGCGTTTGGCTTGGCTGATCGTTGCATTTGTTCTGATTCATCAGA
GTTAAATAACGGATATATCAGCAAATATCCGCAGCATCCACACCGAC
CACACGTCCGGTTAACAGAGTCCCCCTGCCTTGCTTTAATTATTACGG
AGTACTCCGCTATTAATCCTTAGATATGTTTCGAAGGAACTCAAACCT
TCCTCCATCTGCAAATCTCAGTGCTTCAAAACTGGAATTAGATAATTG
AAACCTTCATTCGGTTGCAATTCACAACTGCAAATTGAACAGCACTG
TCAATTTCAATTTCGGGTTCACGATTCCACCGATAGGTTGACATGATC
CATGATCCACCCATTGTACAAC (SEQ ID NO:4)

In an embodiment, a 3'-UTR can be the *Brachypodium distachyon* ubiquitin 1 (Ubi1) 3'-UTR.

GCTTCTGCCGAACTGGTTCACAGTCTGCTGCCCTTGGTGGTCTGCCCC
TTAGTGGTCATGCCTTTTGTTATGTGTCTTGCGTCCCAATCCTGTATCG
TTTGTGTGAACATCTCTGCTGCTGTATAGCAGCTTGAATCCTGTTATG
AATTTGTGAACCTGAACCTTGTTCCGTGAATCATGTTATGAATAAGTG
AACCTGAACCTTGTTCCGTGATTATTGTTACAATCTGTTGTGCCGTAT
GGTTGGTCGTGTGTGATTTATGTTGAACTGGAGAACCAAGTTCGTTCC
AGGACATATTGCAACCTAAGCTAAACCATGTAGAACTACTTGTTCTG
GGAGACATAAAACGTCATTTTTATGCATTCGTAACATTTAAGCATACT
ACAATAATTGTATTGTCCTTTTCCTACTCATCCTTGAAACCATATGCC
TCTTCTCAGCGCCTCTACATGCAGTGTGCTCAGAACAAACAGGCCCT
GCCAGCTGCTTTTCAATTTTCCAATTAATAACCACAATAGTCGGACTA
TGGCATCTGTGGGTGACTATGCAAGATGTTGCTGTCAGGTCTCTGAA
ACTTTTCCCATGTATCTGTTGAAATTACCCAGTAAATTCATGCCTCTA
TTTAATCTGGCATGGTTGATTTTCAAACAGAATGTGTTTTTTTTTGTTC
TGGAAGCTATATTGGTAAATAAATACAAAGCTGGAGTGTGATTATAT
TTCCAACAGATATTCAAGAAAATCTCAGTTGATTTATTTACTACTGTA
GTATATATATATATCTTACAGTTGACTTCTCATATTTCAAACGACATG
TGAGCACATTGTTCAGTTTCTTAGGATGTGTTGTGTGCTCAAAGGTGT
AATTTTGCATTCTGCCCTCCGAGTAAACACTACACGTATTTTTTTGAG
TGGCAGTGCATTTGATTACAAGGCAACAACAACAAAAACCTATGGCA
AGATATCCTTCTTAGAGGCTGCCAGGATCATTTTGACTGAACTATGTA
AGGCTGAAGAAAAGG (SEQ ID NO:5)

In an embodiment, a 3'-UTR can be the *Setaria italica* ubiquitin 2 (Ubi2) 3'-UTR.

GCCCATCGGTCATGGATGCTTCTACTGTACCTGGGTCGTCTGGTCTCT
GCCTGTGTCACCTTTGAAGTACCTGTGTCGGGATTGTGTTTGGTCATG
AACTGCAGTTTGTCTTTGATGTTCTTTTGTCTGGTCTTATGAACTGGTT
GTATCTGTATGTTTACTGTAAACTGTTGTTGCGGTGCAGCAGTATGGC
ATCCGAATGAATAAATGATGTTTGGACTTAAATCTGTACTCTGTTTGT

TTTCGGTTATGCCAGTTCTATATTGCCTGAGATCAGAATGTTTAGCTT
TTGAGTTCTGTTTGGCTTGTGGTCGACTCCTGTTTCTTACTTGAGGCGT
AACTCTGTTCTGGCAAACTCAAATGTCTAACTGAATGTTTTAGGACTT
AATTGTTGGACAGATTAACGTGTTTGGTTTGTTTCTAGATTGTGATTC
GGAAGGCTTGTTAGTTGTGGAATCAAGGAGAGCAGCTAGGTCTGTGC
AGAACGTTATTTTGGATTTAAGCCTTCTCAGATTATGCCATTACTCTA
AACCTAATGATATCATATTTCACTCGGGGATGTTGGAGTAGTCTTTTC
TTTCTCCTGCAGACAAAATGATTTTGCTTTCGTGTGTGTACATGATTTT
GTGCAACTGTTGCAACAACTGAAGTAGACAAGTTTTGACCTCACCAG
AAGAATGAAAAAGATTTTGGAATTTGTTACATCGACAAACCATTGTA
ACTTGGCCCATCAGAATGCACAGAAGAGCGGCTACAAATTGACATGC
GTTGCAAACTTTGCAATAGTTGATGCACATGTTTGCCATTGCCTGCCA
GTCTTAGGAAAAGTGTGTGGTTCGAGAAATCTAAGCATATGTGCTCT
GCTCACATTGCGTGGAACCCACACAGCTTTGTCACACTCTTGTCCACT
CCAGAAGTCATTCCTGGCGCTGTTTACCCCTGGTAAAAGGTAACCGA
AAACTTCTCAAGGCTGTACCCAAAACTGGAAGGAAATTTGGAGGAAA
TCTTTGCTTTTGATCGGCTCACTCTTTC(SEQ ID NO:6)

In an embodiment, a 3' -UTR can be the *Panicum virgatum (Switchgrass)* ubiquitin 1 3' -UTR.

GCCTAGTGCTCCTGAGTTGCCTTTTGTCGTTATGGTCAACCTCTGGTTT
AAGTCGTGTGAACTCTCTGCATTGCGTTGCTAGTGTCTGGTTGTGGTT
GTAATAAGAACATGAAGAACATGTTGCTGTGGATCACATGACTTTTT
TTTTTGAACCGGAAGATCACATGACTTTCATGGCTTTAAGTTCCTGAA
CTCTGAAATCTGGACCCCTTTTTAAGCTCTGAACTCATCATTCTTGCA
TTTACATCTGGTGTTGATCTTATTGATGTGATGCAGTCCTGCTGAAAT
AGTCAATGTAGATTCATGACTGACTGATTGCGTTTATGGTGTGTATGT
TGTTAACAAGCTGAAGGTCGTGTGGTGTCTTTCCAGTTAGACGAAGT
GTGCTTTATTGTAGCGTGTAGTGCTGCTGGATGATTGATGAACTGAAA
CATTCTGCATTTAGCAACTAGCGAGCCAAAGGTGATGACTGAGTTTC
TGTAGACCTGTTTTTTTATGCCCATGGTCGTTCTTCAATTGCACTTGAT
TTTCACATTAGCTGGATCATAATCTGAGCAGACTACTCAAAAGTACA
AAGTTCATCTTCGCTATGACGCTTTGCCACTAGGATTTTCTTTGTATG
ATTTGTTTACAAATCCTGTAATCTAGTCAAAAGAAAAGCCAAAATTTT
TCTTTGTATGATTTGTTTACAAATCCTCTAATCTAGTCAAAGAAAAGC
CAAATTTATCCCTCCTGGTCCCCTACATCACGTAGCTATGTGGCCCGC
AAGCAGATGAAAGCAGCCCCGTCAGCCGACGCCGACGCCGACGCCA
ACACATCCTGCTCCTCCCTCGCCGGCGCCGGCGCCGGCGAGGCCGCA
CCGCCGCTGCCCCGTGGCCGCAGGCACACGGTGCCGCACTGCCGCCG
CCCCGTGGCCGCAGGCACACGGTGCCGCACTGCCGCCGCCTCCCCTT
CCGGCATTGCCGGACGGCTGGGCTACTGTCCCCGCCGCCTTCCCAAT(
SEQ ID NO:36)

In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter as described herein and a 3'-UTR, wherein the 3'-UTR is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36. In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter as described herein and the 3'-UTR wherein the ubiquitin promoter and 3'-UTR are both operably linked to opposite ends of a polylinker. In an

embodiment, a gene expression cassette is provided comprising a ubiquitin promoter as described herein and a 3'-UTR, wherein the ubiquitin promoter and 3'-UTR are both operably linked to opposite ends of a non-ubiquitin transgene. In one embodiment the a 3'-UTR, consists of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36. In one embodiment, a gene expression cassette is provided comprising a ubiquitin promoter as described herein and a 3'-UTR, wherein the ubiquitin promoter comprises SEQ ID NO: 3 and the 3'-UTR comprises SEQ ID NO: 6 wherein the promoter and 3'-UTR are operably linked to opposite ends of a non-ubiquitin transgene. In one embodiment the a 3'-UTR, consists of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36. In one embodiment the promoter consists of SEQ ID NO: 3, 17 or 40 and the 3'-UTR, consists of SEQ ID NO:6. In an illustrative embodiment, a gene expression cassette comprises a ubiquitin 3'-UTR that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof. In a further embodiment the transgene is operably linked to a ubiquitin promoter and a 3'-UTR from the same ubiquitin gene isolated from *Panicum virgatum, Brachypodium distachyon,* or *Setaria italica.*

In one embodiment a vector is provided comprising a first transgene and/or polylinker and a second transgene and/or polylinker wherein the first transgene and/or polylinker is operably linked to a promoter comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:35 and operably linked to a 3'-UTR, comprising a sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36 and the second transgene and/or polylinker is operably linked to a promoter comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:35 and operably linked to a 3'-UTR, comprising a sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36, further wherein the promoter of the first transgene and/or polylinker and second transgene and/or polylinker are derived from Ubi genes from different plant species. In a further embodiment the vector is provided with a third transgene and/or polylinker wherein the third transgene and/or polylinker polylinker is operably linked to a promoter comprising a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:35 and operably linked to a 3'-UTR, comprising a sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36, further wherein the promoter of the third transgene and/or polylinker is derived from Ubi genes from a different plant species from the promoter of the first and second transgene and/or polylinker.

Transgene expression may also be regulated by an intron region located downstream of the promoter sequence. Both a promoter and an intron can regulate transgene expression. While a promoter is necessary to drive transcription, the presence of an intron can increase expression levels resulting in mRNA transcript for translation and protein synthesis. An intron gene region aids stable expression of a transgene.

In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter as described herein and an intron. In one embodiment the intron is operably linked to the 3' end of the promoter. In an embodiment, a nucleic acid construct is provided comprising a ubiquitin intron operably linked to the 3' end of a ubiquitin promoter isolated from *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* or a derivative of such promoter sequence. In an embodiment, the ubiquitin intron is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin intron, or a derivative of such intron sequence.

In an embodiment, an intron can be the *Brachypodium distachyon* ubiquitin1 C intron.

GTATGCAGCCTCGCTTCCTCCTCGCTACCGTTTCAATTCTGGAGTAGGTCGTAGAGGA
TACCATGTTGATTTGACAGAGGGAGTAGATTAGATACTTGTAGATCGAAGTGCGGAT
GTTCCATGGTAGATGATACCATGTTGATTTCGATTAGATCGGATTAAATCTTTGTAGA
TCGAAGTGCGCATGTTCCATGAATTGCCTGTTACCAGTAGATTCAAGTTTTTCTGTGT
TATAGAGGTGGGATCTACTCGTTGAGATGATTAGCTCCTAGAGGACACCATGCCGTT
TTGGAAAATAGATCAGAACCGTGTAGATCGATGTGAGCATGTGTTCCTGTAGATCCA
AGTTCTTTCGCATGTTACTAGTTGTGATCTATTGTTTGTGTAATACGCTCTCGATCTAT
CCGTGTAGATTTCACTCGATTACTGTTACTGTGGCTTGATCGTTCATAGTTGTTCGTTA
GGTTTGATCGAACAGTGTCTGAACCTAATTGGATATGTATTCTTGATCTATCAACGTG
TAGGTTTCAGTCATGTATTTATGTACTCCCTCCGTCCCAAATTAACTGACGTGGATTT
TGTATAAGAATCTATACAAATCCATGTCAGTTAATTCGGGATGGAGTACCATATTCA
ATAATTTGTTTATTGCTGTCCACTTATGTACCATATGTTTGTTGTTCCTCATGTGGATT
CTACTAATTATCATTGATTGGTGATCTTCTATTTTGCTAGTTTCCTAGCTCAATCTGGT
TATTCATGTAGATGTGTTGTTGAAATCGGAGACCATGCTTGTTATTAGATAGTTTATT
GCTTATCAGTTTCATGTTCTGGTTGATGCAACACATATTCATGTTCGCTATCTGGTTG
CTGCTTGATATTCTCTGATTTACATTCATTATAAGAATATATTCTGCTCTGGTTGTTGC
TTCTCATGACTTTACCTACTCGGTAGGTGACTTACCTTTTGGTTTACAATTGTCAACTA
TGCAG (SEQ ID NO:7)

In an embodiment, an intron can be the *Brachypodium distachyon* ubiquitin 1 (Ubi1) intron

GTATGTAGCCTCTCGATTCCTCCTCAGCCCTGCCCTCGATTTGGTGTACGCGTTGAGA
TGATGATCTCGTAGATGTCTAGATGACACCATGTCGATTTGAAATAGATCAGATCCG
TGTAGATCGATGAGCTCCTGTGTACCTGTGGATTCAAGTTATTTTCGCATGCTATTGT
TGTGATCTACTAGATCTAGTGTGTGTATTCTATGCTATCGATTTCTCCGTGTAGATTTC
ACTCGATTACTGTTACTGTGGCTTGATCGGCCATAGATGTTGGTTAAGGTTTGATCGG
TTAGTGTTTGAACCTGCGTGGATATCTAGCATCCATCTATTATCGTGTAGGTTTCGAA
CAAACAAGCACTATTATTGTACTGATGGTTCGTCTATGGTTGGTTTTGACCGTTTTAG
TGTTGAACGAGCCTTCTGTATTTGTTTATTGCTGTCCAGTGATGTACCATGTTCGTTG
AGTGTCGGATTATACTAATTATTGTTGATTGATAATCTTGTAGTTTGCTTTTCCTAATT
TATTTATCGTAGTCCTGATTTGCCTCAGCTGTGCCTCACCCGTGCGATGGTCAATCAA
CTTGTTAGCCCAATCTGCTTAATCATGTACATTTGTTGTTAGAATCAGAGATCAAGCC

AATTAGCTATCTTATTGCTTATCTGTTCCATGTTCTGATCGATGTAACAGTCTACACTT
TTGCTCTGTGCTACTTGATTAAAACATTCTGACTTAAATTCATGATTGGAAGTTTCAG
ATCTGATTGTTGCCTTACTTGACTAATATCTATTCATGTGACACCTCTCTGTCTTGGTA
ACTTACCGCTGTTTGTTTGTAATTTCTGACTATGCAG (SEQ ID NO:8)

In an embodiment, an intron can be the *Setaria italica* ubiquitin 2 (Ubi2) intron 1

GTCACGGGTTCCTTCCCCACCTCTCCTCTTCCCCACCGCCATAAATAG (SEQ ID NO:9)

In an embodiment, an intron can be the *Setaria italica* ubiquitin 2 (Ubi2) intron 2

GTACGGCGATCGTCTTCCTCCTCTAGATCGGCGTGATCTGCAAGTAGTTGATTTGGTA
GATGGTTAGGATCTGTGCACTGAAGAAATCATGTTAGATCCGCGATGTTTCTGTTCGT
AGATGGCTGGGAGGTGGAATTTTTGTGTAGATCTGATATGTTCTCCTGTTTATCTTGT
CACGCTCCTGCGATTTGTGGGGATTTTAGGTCGTTGATCTGGGAATCGTGGGGTTGCT
TCTAGGCTGTTCGTAGATGAGGTCGTTCTCACGGTTTACTGGATCATTGCCTAGTAGA
TCAGCTCGGGCTTTCGTCTTTGTATATGGTGCCCATACTTGCATCTATGATCTGGTTCC
GTGGTGTTACCTAGGTTTCTGCGCCTGATTCGTCCGATCGATTTTGTTAGCATGTGGT
AAACGTTTGGTCATGGTCTGATTTAGATTAGAGTCGAATAGGATGATCTCGATCTAG
CTCTTGGGATTAATATGCATGTGTCACCAATCTGTTCCGTGGTTAAGATGATGAATCT
ATGCTTAGTTAATGGGTGTAGATATATATGCTGCTGTTCCTCAATGATGCCGTAGCTT
TTACCTGAGCAGCATGGATCCTCCTGTTACTTAGGTAGATGCACATGCTTATAGATCA
AGATATGTACTGCTACTGTTGGAATTCTTTAGTATACCTGATGATCATCCATGCTCTT
GTTACTTGTTTTGGTATACTTGGATGATGGCATGCTGCTGCTTTTTGTTGATTTGAGCC
CATCCATATCTGCATATGTCACATGATTAAGATGATTACGCTGTTTCTGTATGATGCC
ATAGCTTTTATGTGAGCAACATGCATCCTCCTGGTTATATGCATTAATAGATGGAAG
ATATCTATTGCTACAATTTGATGATTATTTTGTACATACGATGATCAAGCATGCTCTT
CATACTTTGTTGATATACTTGGATAATGAAATGCTGCTGCACGTTCATTCTATAGCAC
TAATGATGTGATGAACACGCACGACCTGTTTGTGGCATCTGTTTGAATGTGTTGTTGC
TGTTCACTAGAGACTGTTTTATTAACCTACTGCTAGATACTTACCCTTCTGTCTGTTTA
TTCTTTTGCAG (SEQ ID NO:10)

In an embodiment, an intron can be the *Panicum virgatum (Switchgrass)* ubiquitin intron.

GTACTCCTACCTAATCCTCCTTAACTGATCTCTCCTCTATCACGTTGGTAATCTTCGA
ATGATCTGCTGCCTGGCTCGCTGTTCCCCCTCGTTATGCACTGTTTCCATCACGAGT
TTTTTTTTTCATCATCTAATCTATGCGGTTGCGGAAGAATTGTGGCTAGTGGAGTAG
TTTTCTGTGCTTGATCGGTAGATTCGATGTGTGGGTGTATGGATGTTTTCTGAAAAG
TTGCTGGATTAGTTTACGCTTTCAGGCCGCAGGTCTGTTCGAAATTGATTATGAAGT
CTATATGCTTTGGATCTATCGATTTCCAGTTTTATTCAGATGTAGGCCAAAAAATTG
TCGGCATTTGTGTGGAATTAGTTGGCCTTTAGGTCTGCACATTCATGGTGACGGCAC
AGTTGCTGCTGGCTGTTGCGTGGGACGAGTTATTATAGTTGTTTTTGTTTTTCCCTG
ATTGATTCACATTTTCAATGATAACTAGCCTTTGTCACCTAACCAAGTCCAGGTTGA
TCCTATCTGTGTTCTTCAGCTACCAGTTTGCATAGATGATGGTGTATTCGATTGCTTT
AGTAGGCCTTCTGATTTCACATCTAATTCTGTCATGAATATAGATAACTTTACATGC
TTTTGATATACTTTATATTTGAACTGTTCACTGTCCAGCCTATTTTGGATAATTGAGT
GCATTGGCTTTTGATGCCTGAATTATTCACATGTTCCTGGATAATTGACCTGTGTCA
CCTAGTTGACTGTTTTTTGAGGTGCCACCCGTCTGTTCAGCTGATTTGTGTATTCGA
TTGCTCTAGTTAATCTTTTGATTATGCAGCTAGTGCTTTGTCATATGTAGCTTTATAG
GCTTCTGATGTCCTTGGATATAGTTCAGTCTACTTGTCAAGTTGCTTTACAAGTAGT
AGCTCTGATTCTATTTGGCTTCCTGAGTCAGAGCTTTGCAAATTGCTTGTTGTTACA

TTACATCATATTACTTGAATTGCAGTTATTTAATGGTTGGATTGTTGCTGTTTACTTC TACATTTTTTGCTGTTTTATATTATACTAAAATGTTTGTGTTGCTGCTTTTCAG (SEQ ID NO:37)

In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter as described herein and an intron, wherein the intron is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:37. In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter as described herein, an intron sequence and a polylinker wherein the promoter and intron are operably linked to a polylinker. In an embodiment, a gene expression cassette is provided comprising a ubiquitin promoter as described herein, an intron sequence and a non-ubiquitin transgene wherein the promoter and intron are operably linked to the 5' end of the transgene. Optionally the construct further comprises a 3'-UTR that is operably linked to the 3' end of the non-ubiquitin transgene or polylinker. In one embodiment the promoter and 3'-UTR sequences are selected from those described herein and the intron sequence consists of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:37. In an embodiment, a gene expression cassette comprises a ubiquitin intron that is operably linked to a promoter, wherein the promoter is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter, or a promoter that originates from a plant (e.g., *Zea mays* ubiquitin 1 promoter), a virus (e.g., Cassava vein mosaic virus promoter) or a bacteria (e.g., *Agrobacterium tumefaciens* delta mas). In an illustrative embodiment, a gene expression cassette comprises a ubiquitin intron that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof.

Transgene expression may also be regulated by a 5'-UTR region located downstream of the promoter sequence. Both a promoter and a 5'-UTR can regulate transgene expression. While a promoter is necessary to drive transcription, the presence of a 5'-UTR can increase expression levels resulting in mRNA transcript for translation and protein synthesis. A 5'-UTR gene region aids stable expression of a transgene.

In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter as described herein and a 5'-UTR. In one embodiment the 5'-UTR is operably linked to the 3' end of the promoter. In an embodiment, a nucleic acid construct is provided comprising a ubiquitin a 5'-UTR operably linked to the 3' end of a ubiquitin promoter isolated from *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* or a derivative of such promoter sequence. In a

further embodiment the 3' end of the 5'-UTR is operably linked to the 5' end of a ubiquitin intron from *Panicum virgatum, Brachypodium distachyon* or *Setaria italica*, as described herein.

In an embodiment, a 5'-UTR can be the *Brachypodium distachyon* ubiquitin1 C (Ubi1C) 5'-UTR.

CCGATCGAAAAGTCCCCGCAAGAGCAAGCGACCGATCTCGTGAATCTCCGTCAAG (SEQ ID NO:11)

In an embodiment, a 5'-UTR can be the *Brachypodium distachyon* ubiquitin 1 (Ubi1) 5'-UTR.

CCAATCCAGCACCCCCGATCCCGATCGAAAATTCTCCGCAACAGCAAGCGATCGATC TAGCGAATCCCCGTCAAG (SEQ ID NO:12)

In an embodiment, a 5'-UTR can be the *Setaria italica* ubiquitin 2 (Ubi2) 5'-UTR1

AGAAATATCAACTGGTGGGCCACGCACATCAGCGTCGTGTAACGTGGACGGAGGAG CCCCGTGACGGCGTCGACATCGAACGGCCACCAACCACGGAACCACCCGTCCCCACC TCTCGGAAGCTCCGCTCCACGGCGTCGACATCTAACGGCTACCAGCAGGCGTACGGG TTGGAGTGGACTCCTTGCCTCTTTGCGCTGGCGGCTTCCGGAAATTGCGTGGCGGAG ACGAGGCGGGCTCGTCTCACACGGCACGGAAGAC (SEQ ID NO:13)

In an embodiment, a 5'-UTR can be the *Setaria italica* ubiquitin 2 (Ubi2) 5'-UTR2

CCGACCCCCTCGCCTTTCTCCCCAATCTCATCTCGTCTCGTGTTGTTCGGAGCACACC ACCCGCCCCAAATCGTTCTTCCCGCAAGCCTCGGCGATCCTTCACCCGCTTCAAG (SEQ ID NO:14)

In an embodiment, a 5' -UTR can be the *Panicum virgatum (Switchgrass)* ubiquitin 5' -UTR.

CAAGTTCGCGATCTCTCGATTTCACAAATCGCCGAGAAGACCCGAGCAGAGAAGTT CCCTCCGATCGCCTTGCCAAG (SEQ ID NO:38).

In an embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter as disclosed herein and a 5'-UTR, wherein the 5'-UTR is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:38. In an embodiment, a nucleic acid construct is provided comprising ubiquitin promoter, wherein the promoter is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35, and a 5'-UTR operably linked to a polylinker. In an embodiment, a gene expression cassette is provided comprising a ubiquitin promoter, wherein the promoter is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35, and a 5'-UTR sequences operably linked to a non-ubiquitin transgene.

Optionally, the construct can further comprise a ubiquitin intron as disclosed herein operably linked to the 3' end of the 5'-UTR and the 5' end of the non-ubiquitin transgene, and optionally further comprising a 3'-UTR that is operably linked to the 3' end of the non-ubiquitin transgene. In one embodiment the promoter, intron and 3'-UTR sequences are selected from those described herein and the 5'-UTR sequence consists of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:38. In one embodiment the 3'-UTR consists of SEQ ID NO:13 or SEQ ID NO: 14.

In an embodiment, a gene expression cassette comprises a ubiquitin 5'-UTR that is operably linked to a promoter, wherein the promoter is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter, or a promoter that originates from a plant (e.g., *Zea mays* ubiquitin 1 promoter), a virus (e.g., Cassava vein mosaic virus promoter) or a bacteria (e.g., *Agrobacterium tumefaciens* delta mas). In an illustrative embodiment, a gene expression cassette comprises a ubiquitin 5'-UTR that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof.

In one embodiment a nucleic acid construct is provided comprising a promoter and a polylinker and optionally one or more of the following elements:

a) a 5' untranslated region;

b) an intron; and

c) a 3' untranslated region,

wherein

the promoter consists of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35 or a sequence having 98% sequence identity with SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35;

the 5' untranslated region consists of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:38 or a sequence having 98% sequence identity with SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:38

the intron consists of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:37 or a sequence having 98% sequence identity with SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:37

the 3' untranslated region consists of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36 or a sequence having 98% sequence identity with SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36; further wherein said promoter is operably linked to said polylinker

and each optional element, when present, is also operably linked to both the promoter and the polylinker.

In one embodiment a nucleic acid construct is provided comprising a promoter and a non-ubiquitin transgene and optionally one or more of the following elements:

a) a 5' untranslated region;

b) an intron; and

c) a 3' untranslated region,

wherein

the promoter consists of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35 or a sequence having 98% sequence identity with SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35;

the 5' untranslated region consists of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:38 or a sequence having 98% sequence identity with SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:38

the intron consists of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:37 or a sequence having 98% sequence identity with SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:37

the 3' untranslated region consists of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36 or a sequence having 98% sequence identity with SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36; further wherein said promoter is operably linked to said transgene and each optional element, when present, is also operably linked to both the promoter and the transgene. In a further embodiment a transgenic cell is provided comprising the nucleic acid construct disclosed immediately above. In one embodiment the transgenic cell is a plant cell, and in a further embodiment a plant is provided wherein the plant comprises said transgenic cells.

In accordance with one embodiment transgene expression is regulated by a promoter operably linked to an intron and 5'-UTR region, wherein the intron and 5'-UTR region are located downstream of the promoter sequence. A promoter operably linked to an intron and 5'-UTR region can be used to drive transgene expression. While a promoter is necessary to drive transcription, the presence of the intron and 5'-UTR can increase expression levels resulting in mRNA transcript for translation and protein synthesis.

In an embodiment, a gene expression cassette comprises a promoter operably linked to a 5'-UTR and intron region. In an embodiment, a gene expression cassette comprises a ubiquitin promoter operably linked to a ubiquitin 5'-UTR and ubiquitin intron. In an embodiment, the ubiquitin promoter operably linked to a 5'-UTR and intron region is a *Panicum virgatum,*

Brachypodium distachyon or *Setaria italica* ubiquitin promoter operably linked to an intron and 5'-UTR.

In an embodiment, a promoter operably linked to a 5'-UTR and intron can be the *Brachypodium distachyon* ubiquitin1 C (Ubi1C) promoter operably linked to an intron and 5'-UTR. In one embodiment the promoter comprises or consists of the sequence of SEQ ID NO: 15:

CTGCTCGTTCAGCCCACAGTAACACGCCGTGCGACATGCAGATGCCCTCCACCACG
CCGACCAACCCCAAGTCCGCCGCGCTCGTCCACGGCGCCATCCGCATCCGCGCGTC
AACGTCATCCGGAGGAGGCGAGCGCGATGTCGACGGCCACGGCGGCGGCGGACAC
GACGGCGACGCCCCGACTCCGCGCGCGCGTCAAGGCTGCAGTGGCGTCGTGGTGG
CCGTCCGCCTGCACGAGATCCCCGCGTGGACGAGCGCCGCCTCCACCCAGCCCCTA
TATCGAGAAATCAACGGTGGGCTCGAGCTCCTCAGCAACCTCCCCACCCCCCCTTC
CGACCACGCTCCCTTCCCCCGTGCCCCTCTTCTCCGTAAACCCGAGCCGCCGAGAA
CAACACCAACGAAAGGGCGAAGAGAATCGCCATAGAGAGGAGATGGGCGGAGGC
GGATAGTTTCAGCCATTCACGGAGAAATGGGGAGGAGAGAACACGACATCATACG
GACGCGACCCTCTAGCTGGCTGGCTGTCCTAAAGAATCGAACGGAATCGCTGCGCC
AGGAGAAAACGAACGGTCCTGAAGCATGTGCGCCCGGTTCTTCCAAAACACTTATC
TTTAAGATTGAAGTAGTATATATGACTGAAATTTTTACAAGGTTTTTCCCCATAAAA
CAGGTGAGCTTATCTCATCCTTTTGTTTAGGATGTACGTATTATATATGACTGAATA
TTTTTTATTTTCATTGAATGAAGATTTTCGACCCCCCAAAAATAAAAAACGGAGGG
AGTACCTTTGTGCCGTGTATATGGACTAGAGCCATCGGGACGTTTCCGGAGACTGC
GTGGTGGGGGCGATGGACGCACAACGACCGCATTTTCGGTTGCCGACTCGCCGTTC
GCATCTGGTAGGCACGACTCGTCGGGTTCGGCTCTTGCGTGAGCCGTGACGTAACA
GACCCGTTCTCTTCCCCCGTCTGGCCATCCATAAATCCCCCCTCCATCGGCTTCCCT
TTCCTCAATCCAGCACCCTGATTCCGATCGAAAAGTCCCCGCAAGAGCAAGCGACC
GATCTCGTGAATCTCCGTCAAGGTATGCAGCCTCGCTTCCTCCTCGCTACCGTTTCA
ATTCTGGAGTAGGTCGTAGAGGATACCATGTTGATTTGACAGAGGGAGTAGATTAG
ATACTTGTAGATCGAAGTGCGGATGTTCCATGGTAGATGATACCATGTTGATTTCG
ATTAGATCGGATTAAATCTTTGTAGATCGAAGTGCGCATGTTCCATGAATTGCCTGT
TACCAGTAGATTCAAGTTTTTCTGTGTTATAGAGGTGGGATCTACTCGTTGAGATGA
TTAGCTCCTAGAGGACACCATGCCGTTTTGGAAAATAGATCAGAACCGTGTAGATC
GATGTGAGCATGTGTTCCTGTAGATCCAAGTTCTTTCGCATGTTACTAGTTGTGATC
TATTGTTTGTGTAATACGCTCTCGATCTATCCGTGTAGATTTCACTCGATTACTGTTA
CTGTGGCTTGATCGTTCATAGTTGTTCGTTAGGTTTGATCGAACAGTGTCTGAACCT
AATTGGATATGTATTCTTGATCTATCAACGTGTAGGTTTCAGTCATGTATTTATGTA
CTCCCTCCGTCCCAAATTAACTGACGTGGATTTTGTATAAGAATCTATACAAATCCA
TGTCAGTTAATTCGGGATGGAGTACCATATTCAATAATTTGTTTATTGCTGTCCACT
TATGTACCATATGTTTGTTGTTCCTCATGTGGATTCTACTAATTATCATTGATTGGTG
ATCTTCTATTTTGCTAGTTTCCTAGCTCAATCTGGTTATTCATGTAGATGTGTTGTTG
AAATCGGAGACCATGCTTGTTATTAGATAGTTTATTGCTTATCAGTTTCATGTTCTG
GTTGATGCAACACATATTCATGTTCGCTATCTGGTTGCTGCTTGATATTCTCTGATTT
ACATTCATTATAAGAATATATTCTGCTCTGGTTGTTGCTTCTCATGACTTTACCTACT
CGGTAGGTGACTTACCTTTTGGTTTACAATTGTCAACTATGCAG (SEQ ID NO:15)

In an embodiment, a promoter operably linked to 5'-UTR and intron can be the *Brachypodium distachyon* ubiquitin 1 (Ubi1) promoter operably linked to a 5'-UTR and intron. In one embodiment the promoter comprises or consists of the sequence of SEQ ID NO: 16:

GGCGTCAGGACTGGCGAAGTCTGGACTCTGCAGGGCCGAACTGCTGAAGACGAAG
CAGAGGAAGAGAAAGGGAAGTGTTCGACTTGTAATTTGTAGGGGTTTTTTTTAGAG
GAACTTGTAATTTGTAGGTGGGCTGGCCTCGTTGGAAAAACGATGCTGGCTGGTTG
GGCTGGGCCGATGTACGCTTGCAAACAACTTGTGGCGGCCCGTTCTGGACGAGCAG
GAGTTTCTTTTTTGTTCTCACTTTTCTGGTCTTCTTTAGTTACGGAGTACCTTTTGTTT
TTTAAAGGAGTTACCTTTTTTTTAGGAATTCTTTAGTTACCTTTCGCTTGCTCTCAAA
AAATATTTAACTTTCGCTTTTTTTCATTTTAATTTTTGCAACTATTTACGAGTTTCAT
GAATGCTTATTTTCCAGCATATCATTATTTGCAAGTATTTTTATGCCGTATGTATTG
GACGAGAGCCATCGGGACTGTTCCAGAGACTGCGTGGTGGGGACGGCTCCCAACC
GCCTTTTCTATCTCTGTTCGCATCCGGTGGCCGACTTGGCTCGCGCGTGAGCCGTGA
CGTAACAGACTTGGTCTCTTCCCCATCTGGCCATCTATAAATTCCCCCATCGATCGA
CCCTCCCTTTCCCCAATCCAGCACCCCCGATCCCGATCGAAAATTCTCCGCAACAGC
AAGCGATCGATCTAGCGAATCCCCGTCAAGGTATGTAGCCTCTCGATTCCTCCTCA
GCCCTGCCCTCGATTTGGTGTACGCGTTGAGATGATGATCTCGTAGATGTCTAGATG
ACACCATGTCGATTTGAAATAGATCAGATCCGTGTAGATCGATGAGCTCCTGTGTA
CCTGTGGATTCAAGTTATTTTCGCATGCTATTGTTGTGATCTACTAGATCTAGTGTG
TGTATTCTATGCTATCGATTTCTCCGTGTAGATTTCACTCGATTACTGTTACTGTGGC
TTGATCGGCCATAGATGTTGGTTAAGGTTTGATCGGTTAGTGTTTGAACCTGCGTGG
ATATCTAGCATCCATCTATTATCGTGTAGGTTTCGAACAAACAAGCACTATTATTGT
ACTGATGGTTCGTCTATGGTTGGTTTTGACCGTTTTAGTGTTGAACGAGCCTTCTGT
ATTTGTTTATTGCTGTCCAGTGATGTACCATGTTCGTTGAGTGTCGGATTATACTAA
TTATTGTTGATTGATAATCTTGTAGTTTGCTTTTCCTAATTTATTTATCGTAGTCCTG
ATTTGCCTCAGCTGTGCCTCACCCGTGCGATGGTCAATCAACTTGTTAGCCCAATCT
GCTTAATCATGTACATTTGTTGTTAGAATCAGAGATCAAGCCAATTAGCTATCTTAT
TGCTTATCTGTTCCATGTTCTGATCGATGTAACAGTCTACACTTTTGCTCTGTGCTAC
TTGATTAAAACATTCTGACTTAAATTCATGATTGGAAGTTTCAGATCTGATTGTTGC
CTTACTTGACTAATATCTATTCATGTGACACCTCTCTGTCTTGGTAACTTACCGCTGT
TTGTTTGTAATTTCTGACTATGCAG (SEQ ID NO:16)

In an embodiment, a promoter operably linked to a 5'-UTR and intron can be the *Setaria italica* ubiquitin 2 (Ubi2) promoter operably linked to a 5'-UTR and intron. In one embodiment the promoter comprises or consists of the sequence of SEQ ID NO: 17:

TGCGTCTGGACGCACAAGTCATAGCATTATCGGCTAAAATTTCTTAATTTCTAAATTA
GTCATATCGGCTAAGAAAGTGGGGAGCACTATCATTTCGTAGAACAAGAACAAGGT
ATCATATATATATATATATATAATATTTAAACTTTGTTAAGTGGAATCAAAGTGCTAG
TATTAATGGAGTTTCATGTGCATTAAATTTTATGTCACATCAGCAATTTTGTTGACTT
GGCAAGGTCATTTAGGGTGTGTTTGGAAGACAGGGGCTATTAGGAGTATTAAACATA
GTCTAATTACAAAACTAATTGCACAACCGCTAAGCTGAATCGCGAGATGGATCTATT
AAGCTTAATTAGTCCATGATTTGACAATGTGGTGCTACAATAACCATTTGCTAATGAT
GGATTACTTAGGTTTAATAGATTCGTCTCGTGATTTAGCCTATGGGTTCTGCTATTAA
TTTTGTAATTAGCTCATATTTAGTTCTTATAATTAGTATCCGAACATCCAATGTGACA
TGCTAAAGTTTAACCCTGGTATCCAAATGAAGTCTTATGAGAGTTTCATCACTCCGGT
GGTATATGTACTTAGGCTCCGTTTTCTTCCACCGACTTATTTTTAGCACCCGTCACATT
GAATGTTTAGATACTAATTAGAAGTATTAAACGTAGACTATTTACAAAATCCATTAC

ATAAGACGAATCTAAACGGCGAGACGAATCTATTAAACCTAATTAGTCCATGATTTG
ACAATGTGTTGCTACAGTAAACATTTGCTAATGATGGATTAATTAGGCTTAATAGATT
CGTCTCGCCGTTTAGCCTCCACTTATGTAATGGGTTTTCTAAACAATCTACGTTTAAT
ACTCCTAATTAGTATCTAAATATTCAATGTGACACGTGCTAAAAATAAGTCAGTGGA
AGGAAGAGAACGTCCCCTTAGTTTTCCATCTTATTAATTGTACGATGAAACTGTGCA
GCCAGATGATTGACAATCGCAATACTTCAACTAGTGGGCCATGCACATCAGCGACGT
GTAACGTCGTGAGTTGCTGTTCCCGTAGAGAAATATCAACTGGTGGGCCACGCACAT
CAGCGTCGTGTAACGTGGACGGAGGAGCCCCGTGACGGCGTCGACATCGAACGGCC
ACCAACCACGGAACCACCCGTCCCCACCTCTCGGAAGCTCCGCTCCACGGCGTCGAC
ATCTAACGGCTACCAGCAGGCGTACGGGTTGGAGTGGACTCCTTGCCTCTTTGCGCT
GGCGGCTTCCGGAAATTGCGTGGCGGAGACGAGGCGGGCTCGTCTCACACGGCACG
GAAGACGTCACGGGTTCCTTCCCCACCTCTCCTCTTCCCCACCGCCATAAATAGCCGA
CCCCCTCGCCTTTCTCCCCAATCTCATCTCGTCTCGTGTTGTTCGGAGCACACCACCC
GCCCCAAATCGTTCTTCCCGCAAGCCTCGGCGATCCTTCACCCGCTTCAAGGTACGGC
GATCGTCTTCCTCCTCTAGATCGGCGTGATCTGCAAGTAGTTGATTTGGTAGATGGTT
AGGATCTGTGCACTGAAGAAATCATGTTAGATCCGCGATGTTTCTGTTCGTAGATGG
CTGGGAGGTGGAATTTTTGTGTAGATCTGATATGTTCTCCTGTTTATCTTGTCACGCT
CCTGCGATTTGTGGGGATTTTAGGTCGTTGATCTGGGAATCGTGGGGTTGCTTCTAGG
CTGTTCGTAGATGAGGTCGTTCTCACGGTTTACTGGATCATTGCCTAGTAGATCAGCT
CGGGCTTTCGTCTTTGTATATGGTGCCCATACTTGCATCTATGATCTGGTTCCGTGGT
GTTACCTAGGTTTCTGCGCCTGATTCGTCCGATCGATTTTGTTAGCATGTGGTAAACG
TTTGGTCATGGTCTGATTTAGATTAGAGTCGAATAGGATGATCTCGATCTAGCTCTTG
GGATTAATATGCATGTGTCACCAATCTGTTCCGTGGTTAAGATGATGAATCTATGCTT
AGTTAATGGGTGTAGATATATATGCTGCTGTTCCTCAATGATGCCGTAGCTTTTACCT
GAGCAGCATGGATCCTCCTGTTACTTAGGTAGATGCACATGCTTATAGATCAAGATA
TGTACTGCTACTGTTGGAATTCTTTAGTATACCTGATGATCATCCATGCTCTTGTTACT
TGTTTTGGTATACTTGGATGATGGCATGCTGCTGCTTTTTGTTGATTTGAGCCCATCC
ATATCTGCATATGTCACATGATTAAGATGATTACGCTGTTTCTGTATGATGCCATAGC
TTTTATGTGAGCAACATGCATCCTCCTGGTTATATGCATTAATAGATGGAAGATATCT
ATTGCTACAATTTGATGATTATTTTGTACATACGATGATCAAGCATGCTCTTCATACT
TTGTTGATATACTTGGATAATGAAATGCTGCTGCACGTTCATTCTATAGCACTAATGA
TGTGATGAACACGCACGACCTGTTTGTGGCATCTGTTTGAATGTGTTGTTGCTGTTCA
CTAGAGACTGTTTTATTAACCTACTGCTAGATACTTACCCTTCTGTCTGTTTATTCTTT
TGCAG (SEQ ID NO:17)

In an embodiment, a promoter operably linked to a 5'-utr and intron can be the *Setaria italica* ubiquitin 2 (ubi2) promoter operably linked to a 5'-utr and intron. In one embodiment the promoter comprises or consists of the sequence of SEQ ID NO: 41:

TGCGTCTGGACGCACAAGTCATAGCATTATCGGCTAAAATTTCTTAATTTCTAAATTA
GTCATATCGGCTAAGAAAGTGGGGAGCACTATCATTTCGTAGAACAAGAACAAGGT
ATCATATATATATATATATATAATATTTAAACTTTGTTAAGTGGAATCAAAGTGCTAG
TATTAATGGAGTTTCATGTGCATTAAATTTTATGTCACATCAGCAATTTTGTTGACTT
GGCAAGGTCATTTAGGGTGTGTTTGGAAGACAGGGGCTATTAGGAGTATTAAACATA
GTCTAATTACAAAACTAATTGCACAACCGCTAAGCTGAATCGCGAGATGGATCTATT
AAGCTTAATTAGTCCATGATTTGACAATGTGGTGCTACAATAACCATTTGCTAATGAT
GGATTACTTAGGTTTAATAGATTCGTCTCGTGATTTAGCCTATGGGTTCTGCTATTAA
TTTTGTAATTAGCTCATATTTAGTTCTTATAATTAGTATCCGAACATCCAATGTGACA
TGCTAAAGTTTAACCCTGGTATCCAAATGAAGTCTTATGAGAGTTTCATCACTCCGGT
GGTATATGTACTTAGGCTCCGTTTTCTTCCACCGACTTATTTTTAGCACCCGTCACATT
GAATGTTTAGATACTAATTAGAAGTATTAAACGTAGACTATTTACAAAATCCATTAC
ATAAGACGAATCTAAACGGCGAGACGAATCTATTAAACCTAATTAGTCCATGATTTG
ACAATGTGTTGCTACAGTAAACATTTGCTAATGATGGATTAATTAGGCTTAATAGATT
CGTCTCGCCGTTTAGCCTCCACTTATGTAATGGGTTTTCTAAACAATCTACGTTTAAT
ACTCCTAATTAGTATCTAAATATTCAATGTGACACGTGCTAAAAATAAGTCAGTGGA
AGGAAGAGAACGTCCCCTTAGTTTTCCATCTTATTAATTGTACGATGAAACTGTGCA
GCCAGATGATTGACAATCGCAATACTTCAACTAGTGGGCCATGCACATCAGCGACGT
GTAACGTCGTGAGTTGCTGTTCCCGTAGAGAAATATCAACTGGTGGGCCACGCACAT
CAGCGTCGTGTAACGTGGACGGAGGAGCCCCGTGACGGCGTCGACATCGAACGGCC
ACCAACCACGGAACCACCCGTCCCCACCTCTCGGAAGCTCCGCTCCACGGCGTCGAC
ATCTAACGGCTACCAGCAGGCGTACGGGTTGGAGTGGACTCCTTGCCTCTTTGCGCT
GGCGGCTTCCGGAAATTGCGTGGCGGAGACGAGGCGGGCTCGTCTCACACGGCACG
GAAGACGTCACGGGTTCCTTCCCCACCTCTCCTCTTCCCCACCGCCATAAATAG (SEQ ID NO: 41)

In an embodiment, a promoter operably linked to a 5'-UTR and intron can be the *Panicum virgatum (Switchgrass)* ubiquitin promoter operably linked to a 5'-UTR and intron. In one embodiment the promoter comprises or consists of the sequence of SEQ ID NO: 39:

TTGAATTTTAATTTCAAATTTTGCAGGGTAGTAGTGGACATCACAATACATATTTAG
AAAAAGTTTTATAATTTTCCTCCGTTAGTTTTCATATAATTTTGAACTCCAACGATT
AATCTATTATTAAATATCCCGATCTATCAAAATAATGATAAAAATTTATGATTAATT
TTTCTAACATGTGTTATGGTGTGTACTATCGTCTTATAAAATTTCAACTTAAAACTC
CACCTATACATGGAGAAATGAAAAAGACGAATTACAGTAGGGAGTAATTTGAACC
AAATGGAATAGTTTGAGGGTAAAATGAACTAAACAATAGTTTAGGAGGTTATTCAG
ATTTTAGTTATAGTTGAGAGGAGTAATTTAGACTTTTTCCTATCTTGAATTGTTGAC
GGCTCTCCTATCGGATATCGGATGGAGTCTTTCAGCCCAACATAACTTCATTCGGGC
CCAAACGTTCGTCCATCCAGCCTAGGGAGAACATTTTGCCCATGATATCTGTTTTTC
TTTTTTTCTATTTTCACTGGTATTATAGGAGGGAAATATACAACGTGTTCACCTTTG
GTTTCATTCTTGTTCCATCTGAATTTATCTAAAACTGTGTTTGAACTTCGTAAGAATT
TTGTTCGATCTGTCCGGTACATCGTGTTGATAGGTGGCCTCCGAGATTCTTCTTTTT
AACCGGCAAAGTAAAATAATCTCAGCTCCAGCCTAACGTCAATTATCAGAGAGAG
AAAAAAATATTTTTTTATGATTGATCGGAAACCAACCGCCTTACGTGTCGATCCTG
GTTCCTGGCCGGCACGGCGGAGGAAAGCGACCGACCTCGCAACGCCGGCGCACGG
CGCCGCCGTGTTGGACTTGGTCTCCCGCGACTCCGTGGGCCTCGGCTTATCGCCGCC

GCTCCATCTCAACCGTCCGCTTGGACACGTGGAAGTTGATCCGTCGCGCACCAGCC
TCGGAGGTAACCTAACTGCCCGTACTATAAATCCGGGATCCGGCCTCTCCAATCCC
CATCGCCACAAGTTCGCGATCTCTCGATTTCACAAATCGCCGAGAAGACCCGAGCA
GAGAAGTTCCCTCCGATCGCCTTGCCAAGGTACTCCTACCTAATCCTCCTTAACTGA
TCTCTCCTCTATCACGTTGGTAATCTTCGAATGATCTGCTGCCTGGCTCGCTGTTCCC
CCTCGTTATGCACTGTTTCCATCACGAGTTTTTTTTTTCATCATCTAATCTATGCGGT
TGCGGAAGAATTGTGGCTAGTGGAGTAGTTTTCTGTGCTTGATCGGTAGATTCGAT
GTGTGGGTGTATGGATGTTTTCTGAAAAGTTGCTGGATTAGTTTACGCTTTCAGGCC
GCAGGTCTGTTCGAAATTGATTATGAAGTCTATATGCTTTGGATCTATCGATTTCCA
GTTTTATTCAGATGTAGGCCAAAAAATTGTCGGCATTTGTGTGGAATTAGTTGGCCT
TTAGGTCTGCACATTCATGGTGACGGCACAGTTGCTGCTGGCTGTTGCGTGGGACG
AGTTATTATAGTTGTTTTTGTTTTTCCCTGATTGATTCACATTTTCAATGATAACTAG
CCTTTGTCACCTAACCAAGTCCAGGTTGATCCTATCTGTGTTCTTCAGCTACCAGTT
TGCATAGATGATGGTGTATTCGATTGCTTTAGTAGGCCTTCTGATTTCACATCTAAT
TCTGTCATGAATATAGATAACTTTACATGCTTTTGATATACTTTATATTTGAACTGTT
CACTGTCCAGCCTATTTTGGATAATTGAGTGCATTGGCTTTTGATGCCTGAATTATT
CACATGTTCCTGGATAATTGACCTGTGTCACCTAGTTGACTGTTTTTTGAGGTGCCA
CCCGTCTGTTCAGCTGATTTGTGTATTCGATTGCTCTAGTTAATCTTTTGATTATGCA
GCTAGTGCTTTGTCATATGTAGCTTTATAGGCTTCTGATGTCCTTGGATATAGTTCA
GTCTACTTGTCAAGTTGCTTTACAAGTAGTAGCTCTGATTCTATTTGGCTTCCTGAG
TCAGAGCTTTGCAAATTGCTTGTTGTTACATTACATCATATTACTTGAATTGCAGTT
ATTTAATGGTTGGATTGTTGCTGTTTACTTCTACATTTTTTGCTGTTTTATATTATAC
TAAAATGTTTGTGTTGCTGCTTTTCAG (SEQ ID NO:39)

In an embodiment, a nucleic acid construct is provided comprising a promoter operably linked to an intron and 5'-UTR. In one embodiment the construct comprises at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:41 or SEQ ID NO:39. In one embodiment, a nucleic acid construct is provided comprising a ubiquitin promoter sequence comprising or consisting of a sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, or SEQ ID NO:39 operably linked to a polylinker. Optionally, the construct can further comprise 3'-UTR that is operably linked to the 3' end of the polylinker. In an embodiment, a gene expression cassette is provided comprising a ubiquitin promoter sequence wherein the promoter sequence comprises or consists of a sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO: 41 or SEQ ID NO:39. operably linked to a non-ubiquitin transgene. Optionally, the construct can further comprise 3'-UTR that is operably linked to the 3' end of the non-ubiquitin transgene. In one embodiment the 3'-UTR sequence consists of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36. In an illustrative embodiment, the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding

transgene, a selectable marker transgene, or combinations thereof. In one embodiment the transgene is an herbicide resistance gene. In one embodiment a vector is provided comprising 1, 2, 3 or 4 promoter sequences independently selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36.

In an embodiment, a gene expression cassette comprises a ubiquitin promoter, a ubiquitin 5'-UTR, a ubiquitin intron, and a ubiquitin 3'-UTR. In an embodiment, a ubiquitin promoter, a ubiquitin 5'-UTR, a ubiquitin intron, and a ubiquitin 3'-UTR can each be independently a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter; *Panicum virgatum Brachypodium distachyon* or *Setaria italica* ubiquitin 5'-UTR; *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin intron; and, a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin 3'-UTR. In an embodiment, a gene expression cassette comprises: a) a promoter, wherein the promoter is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:36; b) a 3'-UTR, wherein the 3'-UTR is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:37; c) a 5' –UTR, wherein the 5' -UTR is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:38; or, d) an intron, wherein the intron is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO: 41 or SEQ ID NO:39.

For example, a gene expression cassette may include both a promoter, an intron, and a 5' -UTR wherein the promoter is a polynucleotide of SEQ ID NO:3, the intron is a polynucleotide of SEQ ID NO:9 or 10, and the 5' -UTR is a polynucleotide of SEQ ID NO:13 or 14. Likewise, a gene expression cassette may include both a promoter, an intron, and a 5' -UTR wherein the promoter is a polynucleotide of SEQ ID NO:2, the intron is a polynucleotide of SEQ ID NO:8, and the 5' -UTR is a polynucleotide of SEQ ID NO:12. Furthermore, a gene expression cassette may include both a promoter, an intron, and a 5' -UTR wherein the promoter is a polynucleotide of SEQ ID NO:3, the intron is a polynucleotide of SEQ ID NO:9 and/or SEQ ID NO:10, and the 5' -UTR is a polynucleotide of SEQ ID NO:13 or 14. In addition, a gene expression cassette may include both a promoter, an intron, and a 5' -UTR wherein the promoter is a polynucleotide of SEQ ID NO:3, the intron is a polynucleotide of SEQ ID NO:9 or 10, and the 5' -UTR is a polynucleotide of SEQ ID NO:13.

For example, a gene expression cassette may include both a promoter, an intron, a 5' –UTR, and a 3'-UTR wherein the promoter is a polynucleotide of SEQ ID NO:3, the intron is a polynucleotide of SEQ ID NO:9 or 10, the 5' -UTR is a polynucleotide of SEQ ID NO:13 or 14, and the 3'-UTR is a polynucleotide of SEQ ID NO:6. Likewise, a gene expression cassette may include both a promoter, an intron, a 5' –UTR, and a 3'-UTR wherein the promoter is a polynucleotide of SEQ ID NO:3, the intron is a polynucleotide of SEQ ID NO:9 or 10, the 5' -UTR is a polynucleotide of SEQ ID NO:13 or 14 and the 3'-UTR is a polynucleotide of SEQ ID NO:6. Furthermore, a gene expression cassette may include both a promoter, an intron, a 5' –UTR, and a 3'-UTR wherein the promoter is a polynucleotide of SEQ ID NO:3, the intron is a polynucleotide of SEQ ID NO:9 and/or SEQ ID NO:10, the 5' -UTR is a polynucleotide of SEQ ID NO:13 or 14, and the 3'-UTR is a polynucleotide of SEQ ID NO:6. In addition, a gene expression cassette may include both a promoter, an intron, a 5' –UTR, and a 3'-UTR wherein the promoter is a polynucleotide of SEQ ID NO:35, the intron is a polynucleotide of SEQ ID NO:37, the 5' -UTR is a polynucleotide of SEQ ID NO:38, and the 3'-UTR is a polynucleotide of SEQ ID NO:36.

In addition, a gene expression cassette may include both a promoter, and a 3'-UTR wherein the promoter is a polynucleotide of SEQ ID NO:3 and a 3'-UTR of SEQ ID NO:6. In an embodiment, a gene expression cassette may include both a promoter and a 3'-UTR wherein the promoter is a polynucleotide of SEQ ID NO:3 and a 3'-UTR of SEQ ID NO:5. In an embodiment, a gene expression cassette may include both a promoter and a 3'-UTR wherein the promoter is a polynucleotide of SEQ ID NO:3 and a 3'-UTR of SEQ ID NO:6. In an embodiment, a gene expression cassette may include both a promoter and a 3' -UTR wherein the promoter is a polynucleotide of SEQ ID NO:35 and a 3' -UTR of SEQ ID NO:36.

In an embodiment, a gene expression cassette comprises a ubiquitin promoter, ubiquitin 5' -UTR, and a ubiquitin 3'-UTR that are operably linked to a non-ubiquitin transgene. In an embodiment, a gene expression cassette comprises a ubiquitin promoter, a ubiquitin intron, ubiquitin 5' -UTR, and a ubiquitin 3'-UTR that are operably linked to a non-ubiquitin transgene.

A promoter, an intron, a 5' -UTR, and 3'-UTR can be operably linked to different transgenes within a gene expression cassette when a gene expression cassette includes one or more transgenes. In an illustrative embodiment, a gene expression cassette comprises a ubiquitin promoter that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water use efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof. In an illustrative embodiment, a gene expression

cassette comprises a ubiquitin promoter, an intron, and a 5' -UTR that are operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water use efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof. In an illustrative embodiment, a gene expression cassette comprises a ubiquitin 3'-UTR that is operably linked to a transgene, wherein the transgene encodes for a gene product that enhances insecticidal resistance, herbicide tolerance, nitrogen use efficiency, water us efficiency, nutritional quality or combinations thereof.

A ubiquitin intron and a 5' -UTR can be operably linked to different promoters within a gene expression cassette. In an illustrative embodiment, the promoters originate from a plant (e.g., *Zea mays* ubiquitin 1 promoter), a virus (e.g., Cassava vein mosaic virus promoter) or a bacteria (e.g., *Agrobacterium tumefaciens* delta mas). In an illustrative embodiment, a gene expression cassette comprises a ubiquitin promoter that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water use efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof.

In an embodiment, a vector comprises a gene expression cassette as disclosed herein. In an embodiment, a vector can be a plasmid, a cosmid, a bacterial artificial chromosome (BAC), a bacteriophage, a virus, or an excised polynucleotide fragment for use in direct transformation or gene targeting such as a donor DNA.

In accordance with one embodiment a nucleic acid vector is provided comprising a recombinant gene cassette wherein the recombinant gene cassette comprises a ubiquitin based promoter operably linked to a polylinker sequence, a non-ubiquitin transgene or combination thereof. In one embodiment the recombinant gene cassette comprises a ubiquitin based promoter operably linked to a non-ubiquitin transgene. In one embodiment the recombinant gene cassette comprises a ubiquitin based promoter as disclosed herein operably linked to a polylinker sequence. The polylinker is operably linked to the ubiquitin based promoter in a manner such that insertion of a coding sequence into one of the restriction sites of the polylinker will operably link the coding sequence allowing for expression of the coding sequence when the vector is transfected into a host cell.

In accordance with one embodiment the ubiquitin based promoter comprises SEQ ID NO: 3 or a sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 3. In accordance with one embodiment the promoter sequence has a total length of no more than 1.5, 2, 2.5, 3 or 4 kb. In

accordance with one embodiment the ubiquitin based promoter consists of SEQ ID NO: 3 or a 1064 bp sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 3.

In accordance with one embodiment a nucleic acid vector is provided comprising a gene cassette that consists of SEQ ID NO: 17, a non-ubiquitin transgene and a 3'-UTR, wherein SEQ ID NO: 17 is operably linked to the 5' end of the non-ubiquitin transgene and the 3'-UTR is operably linked to the 3' end of the non-ubiquitin transgene. In a further embodiment the 3' untranslated sequence comprises SEQ ID NO: 6 or a sequence that has 90, 95, 99 or 100% sequence identity with SEQ ID NO: 6. In accordance with one embodiment a nucleic acid vector is provided comprising a gene cassette that consists of SEQ ID NO: 17, or a 2600 bp sequence that has 90, 95, or 99% sequence identity with SEQ ID NO: 17, a non-ubiquitin transgene and a 3'-UTR, wherein SEQ ID NO: 17 is operably linked to the 5' end of the non-ubiquitin transgene and the 3'-UTR is operably linked to the 3' end of the non-ubiquitin transgene. In a further embodiment the 3' untranslated sequence comprises SEQ ID NO: 6 or a sequence that has 90, 95, 99 or 100% sequence identity with SEQ ID NO: 6. I a further embodiment the 3' untranslated sequence consists of SEQ ID NO: 6,or a 1032 bp sequence that has 90, 95, or 99% sequence identity with SEQ ID NO: 6.

In accordance with one embodiment the nucleic acid vector further comprises a sequence encoding a selectable maker. In accordance with one embodiment the recombinant gene cassette is operably linked to an Agrobacterium T-DNA border. In accordance with one embodiment the recombinant gene cassette further comprises a first and second T-DNA border, wherein first T-DNA border is operably linked to one end of the gene construct, and said second T-DNA border is operably linked to the other end of the gene construct. The first and second Agrobacterium T-DNA borders can be independently selected from T-DNA border sequences originating from bacterial strains selected from the group consisting of a nopaline synthesizing Agrobacterium T-DNA border, an ocotopine synthesizing Agrobacterium T-DNA border, a succinamopine synthesizing Agrobacterium T-DNA border, or any combination thereof. In one embodiment an Agrobacterium strain selected from the group consisting of a nopaline synthesizing strain, a mannopine synthesizing strain, a succinamopine synthesizing strain, or an octopine synthesizing strain is provided, wherein said strain comprises a plasmid wherein the plasmid comprises a transgene operably linked to a sequence selected from SEQ ID NO: 3, SEQ ID NO: 17 or a sequence having 90, 95, or 99% sequence identity with SEQ ID NO: 3 or SEQ ID NO: 17.

Transgenes of interest and suitable for use in the present disclosed constructs include, but are not limited to, coding sequences that confer (1) resistance to pests or disease, (2) resistance to herbicides, and (3) value added traits as disclosed in WO2013116700 (DGT-28), US20110107455

(DSM-2), U.S. Pat. Nos. 8,283,522 (AAD-12); 7,838,733 (AAD-1); 5,188,960; 5,691,308; 6,096,708; and 6,573,240 (Cry1F); U.S. Pat. Nos. 6,114,138; 5,710,020; and 6,251,656 (Cry1Ac); U.S. Pat. Nos. 6,127,180; 6,624,145 and 6,340,593 (Cry34Ab1); U.S. Pat. Nos. 6,083,499; 6,548,291 and 6,340,593 (Cry35Ab1), the disclosures of which are incorporated herein. In accordance with one embodiment the transgene encodes a selectable marker or a gene product conferring insecticidal resistance, herbicide tolerance, nitrogen use efficiency, water use efficiency, or nutritional quality.

In accordance with one embodiment a nucleic acid vector is provided comprising a gene cassette wherein the gene cassette comprises a promoter region operably linked to the 5' end of a transgene wherein the 3' end of the transgene is linked to a 3' untranslated region. In one embodiment the promoter region comprises SEQ ID NO: 3 or a sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 3. In accordance with one embodiment the promoter region consists of SEQ ID NO: 3 or SEQ ID NO: 17. In one embodiment the 3' untranslated sequence comprises SEQ ID NO: 6 or a sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 6, and in one embodiment the 3' untranslated sequence consists of SEQ ID NO: 6 or a 1032 bp sequence having 90, 95 or 99% sequence identity with SEQ ID NO: 6.

In accordance with one embodiment a nucleic acid vector is provided comprising a gene cassette wherein the gene cassette comprises a promoter region operably linked to the 5' end of a 5' untranslated sequence, wherein the 3' end of the 5' untranslated sequence is operably linked to the 5' end of the transgene wherein the 3' end of the transgene is linked to a 3' untranslated region. In one embodiment the promoter region comprises or consists of SEQ ID NO: 3 or a sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 3. In one embodiment the promoter region consists of SEQ ID NO: 3 or a 1032 bp sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 3. In accordance with one embodiment the 5' untranslated sequence comprises or consists of SEQ ID NO: 13 or a sequence that has 90% sequence identity with SEQ ID NO: 13. In accordance with one embodiment the 5' untranslated sequence comprises or consists of SEQ ID NO: 14 or a sequence that has 90% sequence identity with SEQ ID NO: 14. In accordance with one embodiment the 5' untranslated sequence consists of SEQ ID NO: 13 or a 261 bp sequence that has 90% sequence identity with SEQ ID NO: 13. In accordance with one embodiment the 5' untranslated sequence consists of SEQ ID NO: 14 or a 113 bp sequence that has 90% sequence identity with SEQ ID NO: 14. In one embodiment the 3' untranslated sequence comprises or consists of SEQ ID NO: 6 or a sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 6. In one embodiment the 3' untranslated sequence consists of SEQ ID NO: 6 or a 1032 bp sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 6. In a further embodiment

the nucleic acid vector further comprises a ubiquitin intron inserted between the 5' untranslated region and the transgene, and operably linked to the promoter and transgene. In one embodiment the ubiquitin intron comprises or consists of SEQ ID NO: 9 or 10 or a sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 9 or 10. In one embodiment the ubiquitin intron consists of SEQ ID NO: 9 or a 48 bp sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 9. In one embodiment the ubiquitin intron consists of SEQ ID NO: 10 or a 1114 bp sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 10

In accordance with one embodiment a nucleic acid vector is provided comprising a gene cassette wherein the gene cassette comprises a promoter region operably linked to the 5' end of a transgene wherein the 3' end of the transgene is linked to a 3' untranslated region. In one embodiment the promoter region comprises SEQ ID NO: 40 or a sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 40.

TGCGTCTGGACGCACAAGTCATAGCATTATCGGCTAAAATTTCTTAATTTCTAAATT
AGTCATATCGGCTAAGAAAGTGGGGAGCACTATCATTTCGTAGAACAAGAACAAG
GTATCATATATATATATATATATAATATTTAAACTTTGTTAAGTGGAATCAAAGTGC
TAGTATTAATGGAGTTTCATGTGCATTAAATTTTATGTCACATCAGCAATTTTGTTG
ACTTGGCAAGGTCATTTAGGGTGTGTTTGGAAGACAGGGGCTATTAGGAGTATTAA
ACATAGTCTAATTACAAAACTAATTGCACAACCGCTAAGCTGAATCGCGAGATGGA
TCTATTAAGCTTAATTAGTCCATGATTTGACAATGTGGTGCTACAATAACCATTTGC
TAATGATGGATTACTTAGGTTTAATAGATTCGTCTCGTGATTTAGCCTATGGGTTCT
GCTATTAATTTTGTAATTAGCTCATATTTAGTTCTTATAATTAGTATCCGAACATCC
AATGTGACATGCTAAAGTTTAACCCTGGTATCCAAATGAAGTCTTATGAGAGTTTC
ATCACTCCGGTGGTATATGTACTTAGGCTCCGTTTTCTTCCACCGACTTATTTTTAGC
ACCCGTCACATTGAATGTTTAGATACTAATTAGAAGTATTAAACGTAGACTATTTAC
AAAATCCATTACATAAGACGAATCTAAACGGCGAGACGAATCTATTAAACCTAATT
AGTCCATGATTTGACAATGTGTTGCTACAGTAAACATTTGCTAATGATGGATTAATT
AGGCTTAATAGATTCGTCTCGCCGTTTAGCCTCCACTTATGTAATGGGTTTTCTAAA
CAATCTACGTTTAATACTCCTAATTAGTATCTAAATATTCAATGTGACACGTGCTAA
AAATAAGTCAGTGGAAGGAAGAGAACGTCCCCTTAGTTTTCCATCTTATTAATTGT
ACGATGAAACTGTGCAGCCAGATGATTGACAATCGCAATACTTCAACTAGTGGGCC
ATGCACATCAGCGACGTGTAACGTCGTGAGTTGCTGTTCCCGTAGCCGACCCCCTC
GCCTTTCTCCCCAATCTCATCTCGTCTCGTGTTGTTCGGAGCACACCACCCGCCCCA
AATCGTTCTTCCCGCAAGCCTCGGCGATCCTTCACCCGCTTCAAG (SEQ ID NO: 40).

In one embodiment the promoter region comprises SEQ ID NO: 42 or a sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 42.

TGCGTCTGGACGCACAAGTCATAGCATTATCGGCTAAAATTTCTTAATTTCTAAATT
AGTCATATCGGCTAAGAAAGTGGGGAGCACTATCATTTCGTAGAACAAGAACAAG
GTATCATATATATATATATATATAATATTTAAACTTTGTTAAGTGGAATCAAAGTGC
TAGTATTAATGGAGTTTCATGTGCATTAAATTTTATGTCACATCAGCAATTTTGTTG
ACTTGGCAAGGTCATTTAGGGTGTGTTTGGAAGACAGGGGCTATTAGGAGTATTAA
ACATAGTCTAATTACAAAACTAATTGCACAACCGCTAAGCTGAATCGCGAGATGGA
TCTATTAAGCTTAATTAGTCCATGATTTGACAATGTGGTGCTACAATAACCATTTGC
TAATGATGGATTACTTAGGTTTAATAGATTCGTCTCGTGATTTAGCCTATGGGTTCT
GCTATTAATTTTGTAATTAGCTCATATTTAGTTCTTATAATTAGTATCCGAACATCC
AATGTGACATGCTAAAGTTTAACCCTGGTATCCAAATGAAGTCTTATGAGAGTTTC
ATCACTCCGGTGGTATATGTACTTAGGCTCCGTTTTCTTCCACCGACTTATTTTTAGC
ACCCGTCACATTGAATGTTTAGATACTAATTAGAAGTATTAAACGTAGACTATTTAC
AAAATCCATTACATAAGACGAATCTAAACGGCGAGACGAATCTATTAAACCTAATT
AGTCCATGATTTGACAATGTGTTGCTACAGTAAACATTTGCTAATGATGGATTAATT
AGGCTTAATAGATTCGTCTCGCCGTTTAGCCTCCACTTATGTAATGGGTTTTCTAAA
CAATCTACGTTTAATACTCCTAATTAGTATCTAAATATTCAATGTGACACGTGCTAA
AAATAAGTCAGTGGAAGGAAGAGAACGTCCCCTTAGTTTTCCATCTTATTAATTGT
ACGATGAAACTGTGCAGCCAGATGATTGACAATCGCAATACTTCAACTAGTGGGCC
ATGCACATCAGCGACGTGTAACGTCGTGAGTTGCTGTTCCCGTAGAGAAATATCAA
CTGGTGGGCCACGCACATCAGCGTCGTGTAACGTGGACGGAGGAGCCCCGTGACG
GCGTCGACATCGAACGGCCACCAACCACGGAACCACCCGTCCCCACCTCTCGGAAG
CTCCGCTCCACGGCGTCGACATCTAACGGCTACCAGCAGGCGTACGGGTTGGAGTG
GACTCCTTGCCTCTTTGCGCTGGCGGCTTCCGGAAATTGCGTGGCGGAGACGAGGC
GGGCTCGTCTCACACGGCACGGAAGAC (SEQ ID NO: 42)

In accordance with one embodiment the promoter region consists of SEQ ID NO: 40 or a 1177 bp sequence having 90, 95 or 99% sequence identity with SEQ ID NO: 40. In accordance with one embodiment the promoter region consists of SEQ ID NO: 40. In accordance with one embodiment the promoter region consists of SEQ ID NO: 42 or a 1325 bp sequence having 90, 95 or 99% sequence identity with SEQ ID NO: 42. In accordance with one embodiment the promoter region consists of SEQ ID NO: 42. In one embodiment the 3' untranslated sequence consists of SEQ ID NO: 6 or a 1032 bp sequence that has 90, 95 or 99% sequence identity with SEQ ID NO: 6, and in one embodiment the 3' untranslated sequence consists of SEQ ID NO: 6.

In an embodiment, a cell or plant is provided comprising a gene expression cassette as disclosed herein. In an embodiment, a cell or plant comprises a vector comprising a gene expression cassette as disclosed herein. In an embodiment, a vector can be a plasmid, a cosmid, a bacterial artificial chromosome (BAC), a bacteriophage, or a virus. Thereby, a cell or plant comprising a gene expression cassette as disclosed herein is a transgenic cell or transgenic plant, respectively. In an embodiment, a transgenic plant can be a monocotyledonous plant. In an embodiment, a transgenic monocotyledonous plant can be, but is not limited to maize, wheat, rice, sorghum, oats, rye, bananas, sugar cane, and millet. In an embodiment, a transgenic plant can be a dicotyledonous plant. In an embodiment, a transgenic dicotyledonous plant can be, but is not limited to soybean, cotton, sunflower, and canola. An embodiment also includes a transgenic seed from a transgenic plant as disclosed herein.

In an embodiment, a gene expression cassette includes two or more transgenes. The two or more transgenes may not be operably linked to the same promoter, intron, or 5'-UTR or 3'-UTR as disclosed herein. In an embodiment, a gene expression cassette includes one or more transgenes. In an embodiment with one or more transgenes, at least one transgene is operably linked to a promoter, intron, 5'-UTR, or 3'-UTR or the subject disclosure.

Selectable Markers

Various selectable markers also described as reporter genes can be incorporated into a chosen expression vector to allow for identification and selectable of transformed plants ("transformants"). Many methods are available to confirm expression of selectable 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 visual observation of other proteins such as reporter genes encoding β-glucuronidase (GUS), luciferase, green fluorescent protein (GFP), yellow fluorescent protein (YFP), 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, the content of which is incorporated herein by reference in its entirety).

Selectable marker genes are utilized for 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 has been obtained by using genes coding for mutant target enzymes, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Genes and mutants for EPSPS are well known, and further described below. Resistance to glufosinate ammonium, bromoxynil, and 2,4-dichlorophenoxyacetate (2,4-D) have been obtained by using bacterial genes encoding *pat* or *DSM-2*, a *nitrilase*, an *aad*-1, or an *aad*-12 gene, which detoxifies the respective herbicides.

In an embodiment, 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 are well known. Glyphosate resistance genes include mutant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) and *dgt-28* 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 bar genes from *Streptomyces* species, including *Streptomyces hygroscopicus* and *Streptomyces viridichromogenes*, and pyridinoxy or phenoxy proprionic acids and cyclohexones (ACCase inhibitor-encoding genes). Exemplary genes conferring resistance to cyclohexanediones and/or aryloxyphenoxypropanoic acid (including Haloxyfop, Diclofop, Fenoxyprop, Fluazifop, Quizalofop) include genes of acetyl coenzyme A carboxylase (ACCase)--Acc1-S1, Acc1-S2 and Acc1-S3. In an embodiment, herbicides can inhibit photosynthesis, including triazine (psbA and 1s+ genes) or benzonitrile (nitrilase gene).

In an embodiment, selectable marker genes include, but are not limited to genes encoding: neomycin phosphotransferase II; cyanamide hydratase; aspartate kinase; dihydrodipicolinate synthase; tryptophan decarboxylase; dihydrodipicolinate synthase and desensitized aspartate kinase; bar gene; tryptophan decarboxylase; neomycin phosphotransferase (NEO); hygromycin phosphotransferase (HPT or HYG); dihydrofolate reductase (DHFR); phosphinothricin acetyltransferase; 2,2-dichloropropionic acid dehalogenase; acetohydroxyacid synthase; 5-enolpyruvyl-shikimate-phosphate synthase (aroA); haloarylnitrilase; acetyl-coenzyme A carboxylase; dihydropteroate synthase (sul I); and 32 kD photosystem II polypeptide (psbA).

An embodiment also includes genes encoding resistance to: chloramphenicol; methotrexate; hygromycin; spectinomycin; bromoxynil; glyphosate; and phosphinothricin.

The above list of selectable marker genes is not meant to be limiting. Any reporter or selectable marker gene are encompassed by the present invention.

Selectable marker genes are synthesized for optimal expression in a plant. For example, in an embodiment, a coding sequence of a gene has been modified by codon optimization to enhance expression in plants. A selectable marker gene can be optimized for expression in a particular plant species or alternatively can be modified for optimal expression in dicotyledonous or monocotyledonous plants. 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. In an embodiment, a selectable marker gene is designed to be expressed in plants at a higher level resulting in higher transformation efficiency. Methods for plant optimization of genes are well known. Guidance regarding the optimization and production of synthetic DNA sequences can be found in, for example, WO2013016546, WO2011146524, WO1997013402, US Patent No. 6166302, and US Patent No. 5380831, herein incorporated by reference.

Transformation

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); micro-projectile 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).

A DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as agitation with silicon carbide fibers (*See, e.g.*, U.S. Patents 5,302,523 and 5,464,765), or the DNA constructs can be introduced directly to plant tissue using biolistic methods, such as DNA particle bombardment (see, e.g., Klein et al. (1987) Nature 327:70-73). Alternatively, the DNA construct can be introduced into the plant cell via nanoparticle transformation (see, e.g., US Patent Publication No. 20090104700, which is incorporated herein by reference in its entirety).

In addition, gene transfer may be achieved using non-*Agrobacterium* bacteria or viruses such as *Rhizobium* sp. NGR234, *Sinorhizoboium meliloti*, *Mesorhizobium loti*, potato virus X, cauliflower mosaic virus and cassava vein mosaic virus and/or tobacco mosaic virus, See, e.g., Chung et al. (2006) Trends Plant Sci. 11(1):1-4.

Through the application of transformation techniques, cells of virtually any plant species may be stably transformed, and these cells may be developed into transgenic plants by well-known techniques. 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 soy bean 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, a 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 marker gene with the transformation vector used to generate the transformant. In an illustrative embodiment, a 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 exposure to a selective agent, or cells that have been scored positive in a screening assay, may be cultured in media that supports regeneration of plants. In an embodiment, any suitable plant tissue culture 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 a desired nucleic acid comprising constructs provided in regenerating plants, a variety of assays may be performed. Such assays may include: 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, western blots, and/or LC-MS MS spectrophotometry) or by enzymatic function; plant part assays, such as leaf or root assays; and/or analysis of the phenotype of the whole regenerated plant.

Transgenic 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. Primers may be used in conjunction with primers described herein. 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. In an embodiment, oligonucleotide primers specific for the gene target are employed in PCR amplifications.

Method of Expressing a Transgene

In an embodiment, a method of expressing at least one transgene in a plant comprises growing a plant comprising a ubiquitin promoter operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant comprising growing a plant comprising a ubiquitin 5'-UTR operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant comprising growing a plant comprising a ubiquitin intron operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant comprising growing a plant comprising a ubiquitin promoter, a ubiquitin 5' –UTR, and a ubiquitin intron operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant comprising growing a plant comprising a ubiquitin 3'-UTR operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprising culturing a plant tissue or plant cell comprising a ubiquitin promoter

operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprising culturing a plant tissue or plant cell comprising a ubiquitin 5'-UTR operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprising culturing a plant tissue or plant cell comprising a ubiquitin intron operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprising culturing a plant tissue or plant cell comprising a ubiquitin promoter, a ubiquitin 5' –UTR, and a ubiquitin intron operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprising culturing a plant tissue or plant cell comprising a ubiquitin 3'-UTR operably linked to at least one transgene.

In an embodiment, a method of expressing at least one transgene in a plant comprises growing a plant comprising a gene expression cassette comprising a ubiquitin promoter operably linked to at least one transgene. In one embodiment the ubiquitin promoter consists of a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:35, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:39 or a sequence that has 90, 95 or 995 sequence identity with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:35, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:39. In an embodiment, a method of expressing at least one transgene in a plant comprises growing a plant comprising a gene expression cassette comprising a ubiquitin intron operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant comprises growing a plant comprising a gene expression cassette comprising a ubiquitin 5' -UTR operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant comprises growing a plant comprising a gene expression cassette comprising a ubiquitin promoter, a ubiquitin 5' –UTR, and a ubiquitin intron operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant comprises growing a plant comprising a gene expression cassette comprising a ubiquitin 3'-UTR operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprises culturing a plant tissue or plant cell comprising a gene expression cassette a ubiquitin promoter operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprises culturing a plant tissue or plant cell comprising a gene expression cassette a ubiquitin intron operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprises culturing a plant tissue

or plant cell comprising a gene expression cassette a ubiquitin 5' -UTR operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprises culturing a plant tissue or plant cell comprising a gene expression cassette a ubiquitin promoter, a ubiquitin 5' -UTR, and a ubiquitin intron operably linked to at least one transgene. In an embodiment, a method of expressing at least one transgene in a plant tissue or plant cell comprises culturing a plant tissue or plant cell comprising a gene expression cassette comprising a ubiquitin 3'-UTR operably linked to at least one transgene.

Transgenic Plants

In an embodiment, a plant, plant tissue, or plant cell comprises a ubiquitin promoter. In an embodiment, a ubiquitin promoter can be a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter. In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprises a promoter, wherein the promoter is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:35 wherein the promoter is operably linked to a non-ubiquitin transgene. In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:35, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:39 or a sequence that has 90, 95 or 995 sequence identity with a sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:35, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:39 that is operably linked to a non-ubiquitin transgene. In an illustrative embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a ubiquitin promoter that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof.

In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a 3'-UTR. In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a ubiquitin 3'-UTR. In an embodiment, the ubiquitin 3'-UTR is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin 3'-UTR. In an embodiment, a 3'-UTR can be the *Brachypodium distachyon* ubiquitin1 C (Ubi1C) 3'-UTR, *Brachypodium distachyon* ubiquitin1 3'-UTR, or *Setaria italica* ubiquitin 3'-UTR.

In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising an intron, wherein the intron is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:37. In an embodiment, a gene expression cassette comprises a ubiquitin intron that is operably linked to a promoter, wherein the promoter is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter, or a promoter that originates from a plant (e.g., *Zea mays* ubiquitin 1 promoter), a virus (e.g., Cassava vein mosaic virus promoter) or a bacteria (e.g., *Agrobacterium tumefaciens* delta mas). In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a ubiquitin intron that is operably linked to a transgene. In an illustrative embodiment, a plant, plant tissue, or plant cell comprising a gene expression cassette comprising a ubiquitin intron that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof.

In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a 5'-UTR, wherein the 5'-UTR is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:38. In an embodiment, a gene expression cassette comprises a ubiquitin intron that is operably linked to a promoter, wherein the promoter is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter, or a promoter that originates from a plant (e.g., *Zea mays* ubiquitin 1 promoter), a virus (e.g., Cassava vein mosaic virus promoter) or a bacteria (e.g., *Agrobacterium tumefaciens* delta mas). In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a ubiquitin 5'-UTR that is operably linked to a transgene. In an illustrative embodiment, a plant, plant tissue, or plant cell comprising a gene expression cassette comprising a ubiquitin 5'-UTR that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof.

In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a ubiquitin promoter and a ubiquitin 3'-UTR. In an embodiment, a plant, plant tissue, or plant cell comprises a ubiquitin promoter and 3'-UTR can each be independently a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter and a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter. In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a) a promoter,

wherein the promoter is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35 and b) a 3'-UTR, wherein the 3'-UTR is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, or 100% identical to SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:36.

In an embodiment, a plant, plant tissue, or plant cell comprises a gene expression cassette comprising a ubiquitin promoter, ubiquitin 5'-UTR, ubiquitin intron, and a ubiquitin 3'-UTR that are operably linked to a transgene. The promoter, intron, 5' -UTR, and 3'-UTR can be operably linked to different transgenes within a gene expression cassette when a gene expression cassette includes two or more transgenes. In an illustrative embodiment, a gene expression cassette comprises a ubiquitin promoter that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof. In an illustrative embodiment, a gene expression cassette comprises a ubiquitin intron that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof. In an embodiment, a gene expression cassette comprises a ubiquitin intron that is operably linked to a promoter, wherein the promoter is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter, or a promoter that originates from a plant (e.g., *Zea mays* ubiquitin 1 promoter), a virus (e.g., Cassava vein mosaic virus promoter) or a bacteria (e.g., *Agrobacterium tumefaciens* delta mas). In an illustrative embodiment, a gene expression cassette comprises a ubiquitin 5' -UTR that is operably linked to a transgene, wherein the transgene can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof. In an embodiment, a gene expression cassette comprises a ubiquitin 5' -UTR that is operably linked to a promoter, wherein the promoter is a *Panicum virgatum, Brachypodium distachyon* or *Setaria italica* ubiquitin promoter, or a promoter that originates from a plant (e.g., *Zea mays* ubiquitin 1 promoter), a virus (e.g., Cassava vein mosaic virus promoter) or a bacteria (e.g., *Agrobacterium tumefaciens* delta mas). In an illustrative embodiment, a gene expression cassette comprises a ubiquitin 3'-UTR that is operably linked to a transgene, wherein the 3'-UTR can be an insecticidal resistance transgene, an herbicide tolerance transgene, a nitrogen use efficiency transgene, a water us efficiency

transgene, a nutritional quality transgene, a DNA binding transgene, a selectable marker transgene, or combinations thereof.

In an embodiment, a plant, plant tissue, or plant cell comprises a vector comprising a ubiquitin promoter, 5'-UTR, intron, and/or 3'-UTR as disclosed herein. In an embodiment, a plant, plant tissue, or plant cell comprises a vector comprising a ubiquitin promoter, 5'-UTR, intron, and/or 3'-UTR as disclosed herein operably linked to a non-ubiquitin transgene. In an embodiment, a plant, plant tissue, or plant cell comprises a vector comprising a gene expression cassette as disclosed herein. In an embodiment, a vector can be a plasmid, a cosmid, a bacterial artificial chromosome (BAC), a bacteriophage, or a virus.

In accordance with one embodiment a plant, plant tissue, or plant cell is provided wherein the plant, plant tissue, or plant cell comprises a non-endogenous ubiquitin derived promoter sequence operably linked to a transgene, wherein the ubiquitin derived promoter sequence comprises a sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35 or a sequence having 90. 95, 98 or 99% sequence identity with SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:35. In one embodiment a plant, plant tissue, or plant cell is provided wherein the plant, plant tissue, or plant cell comprises SEQ ID NO: 3, or a sequence that has 90% sequence identity with SEQ ID NO: 3 operably linked to a non-ubiquitin transgene. In one embodiment the plant, plant tissue, or plant cell is a dicotyledonous or monocotyledonous plant or a cell or tissue derived from a dicotyledonous or monocotyledonous plant. In one embodiment the plant is selected from the group consisting of maize, wheat, rice, sorghum, oats, rye, bananas, sugar cane, soybean, cotton, sunflower, and canola. In one embodiment the plant is Zea mays. In accordance with one embodiment the plant, plant tissue, or plant cell comprises SEQ ID NO: 3, SEQ ID NO: 17 or a sequence having 90. 95, 98 or 99% sequence identity with SEQ ID NO: 3 or SEQ ID NO: 17 operably linked to a non-ubiquitin transgene. In one embodiment the plant, plant tissue, or plant cell comprises a promoter operably linked to a transgene wherein the promoter consists of SEQ ID NO: 3, SEQ ID NO: 17 or a sequence having 90. 95, 98 or 99% sequence identity with SEQ ID NO: 3 or SEQ ID NO: 17. In accordance with one embodiment the gene construct comprising non-endogenous ubiquitin derived promoter sequence operably linked to a transgene is incorporated into the genome of the plant, plant tissue, or plant cell.

In one embodiment a non-Setaria plant, plant tissue, or plant cell is provided comprising SEQ ID NO: 3, or a sequence that has 90, 95, 98 or 99% sequence identity with SEQ ID NO: 3, operably linked to a transgene. In accordance with one embodiment the non-Setaria plant, plant tissue, or plant cell is a dicotyledonous or monocotyledonous plant or plant cell or tissue derived from a dicotyledonous or monocotyledonous plant. In one embodiment the plant is selected from

the group consisting of maize, wheat, rice, sorghum, oats, rye, bananas, sugar cane, soybean, cotton, sunflower, and canola. In one embodiment the plant is *Zea mays*. In accordance with one embodiment the promoter sequence operably linked to a transgene is incorporated into the genome of the plant, plant tissue, or plant cell. In one embodiment the plant, plant tissue, or plant cell further comprises a 5' untranslated sequence comprising SEQ ID NO: 13 or a sequence that has 90% sequence identity with SEQ ID NO: 13, wherein the 5' untranslated sequence is inserted between, and operably linked to, said promoter and said transgene. In one embodiment the plant, plant tissue, or plant cell further comprises a 5' untranslated sequence comprising SEQ ID NO: 14 or a sequence that has 90% sequence identity with SEQ ID NO: 14, wherein the 5' untranslated sequence is inserted between, and operably linked to, said promoter and said transgene. In a further embodiment the plant, plant tissue, or plant cell further comprises an intron sequence inserted after the 5' untranslated sequence. In one embodiment the intron sequence is an intron sequence isolated from a ubiquitin gene of *Panicum virgatum, Brachypodium distachyon*, or *Setaria italica*. In one embodiment the sequence comprises or consists of SEQ ID NO: 9. In one embodiment the sequence comprises or consists of SEQ ID NO: 10.

In one embodiment a non-Setaria plant, plant tissue, or plant cell is provided that comprises SEQ ID NO: 3, or a sequence that has 90. 95, 98 or 99% sequence identity with SEQ ID NO: 3, operably linked to the 5' end of a transgene and a 3' untranslated sequence comprising SEQ ID NO: 6 or a sequence that has 90% sequence identity with SEQ ID NO: 6, wherein the 3' untranslated sequence is operably linked to said transgene. In accordance with one embodiment the non-Setaria plant, plant tissue, or plant cell is a dicotyledonous or monocotyledonous plant or is a plant issue or cell derived from a dicotyledonous or monocotyledonous plant. In one embodiment the plant is selected from the group consisting of maize, wheat, rice, sorghum, oats, rye, bananas, sugar cane, soybean, cotton, sunflower, and canola. In one embodiment the plant is Zea mays. In accordance with one embodiment the promoter sequence operably linked to a transgene is incorporated into the genome of the plant, plant tissue, or plant cell. In one embodiment the plant, plant tissue, or plant cell further comprises a 5' untranslated sequence comprising SEQ ID NO: 13 or 14 or a sequence that has 90% sequence identity with SEQ ID NO: 13 or 14, wherein the 5' untranslated sequence is inserted between, and operably linked to, said promoter and said transgene. In a further embodiment the plant, plant tissue, or plant cell further comprises an intron sequence inserted after the 5' untranslated sequence. In one embodiment the intron sequence is an intron sequence isolated from a ubiquitin gene of *Panicum virgatum, Brachypodium distachyon*, or *Setaria italica*. In one embodiment the 5' untranslated sequence

consists of SEQ ID NO: 13. In one embodiment the 5' untranslated sequence consists of SEQ ID NO: 13.

In one embodiment a non-Setaria plant, plant tissue, or plant cell is provided that comprises SEQ ID NO: 17, or a sequence having 90% sequence identity with SEQ ID NO: 17 operably linked to a transgene. In one embodiment a non-Setaria plant, plant tissue, or plant cell is provided that comprises SEQ ID NO: 40, or a sequence having 90% sequence identity with SEQ ID NO: 40 operably linked to a transgene. In one embodiment a non-Setaria plant, plant tissue, or plant cell is provided that comprises SEQ ID NO: 41, or a sequence having 90% sequence identity with SEQ ID NO: 41 operably linked to a transgene. In one embodiment a non-Setaria plant, plant tissue, or plant cell is provided that comprises SEQ ID NO: 42, or a sequence having 90% sequence identity with SEQ ID NO: 42 operably linked to a transgene. In one embodiment a non-Setaria plant, plant tissue, or plant cell is provided that comprises a promoter operably linked to a transgene, wherein the promoter consists of SEQ ID NO: 17, or a sequence having 90% sequence identity with SEQ ID NO: 17. In a further embodiment non-Setaria plant, plant tissue, or plant cell further comprises a 3' untranslated sequence of a ubiquitin gene of *Panicum virgatum, Brachypodium distachyon*, or *Setaria italica*. In one embodiment the 3' untranslated sequence comprises or consists of SEQ ID NO: 6 or a sequence that has 90% sequence identity with SEQ ID NO: 6, wherein the 3' untranslated sequence is operably linked to 3' end of the transgene.

In an embodiment, a plant, plant tissue, or plant cell according to the methods disclosed herein can be a monocotyledonous plant. The monocotyledonous plant, plant tissue, or plant cell can be, but not limited to corn, rice, wheat, sugarcane, barley, rye, sorghum, orchids, bamboo, banana, cattails, lilies, oat, onion, millet, and triticale.

In an embodiment, a plant, plant tissue, or plant cell according to the methods disclosed herein can be a dicotyledonous plant. The dicotyledonous plant, plant tissue, or plant cell can be, but not limited to rapeseed, canola, indian mustard, ethiopian mustard, soybean, sunflower, and cotton.

With regard to the production of genetically modified plants, methods for the genetic engineering of plants are well known in the art. For instance, numerous methods for plant transformation have been developed, including biological and physical transformation protocols for dicotyledonous plants as well as monocotyledonous plants (*e.g.*, Goto-Fumiyuki *et al.*, *Nature Biotech 17*:282-286 (1999); Miki *et al.*, Methods in Plant Molecular Biology and Biotechnology, Glick, B. R. and Thompson, J. E. Eds., CRC Press, Inc., Boca Raton, pp. 67-88 (1993)). In addition, vectors and *in vitro* culture methods for plant cell or tissue transformation and regeneration of plants are available, for example, in Gruber *et al.*, Methods in Plant

Molecular Biology and Biotechnology, Glick, B. R. and Thompson, J. E. Eds., CRC Press, Inc., Boca Raton, pp. 89-119 (1993).

One of skill in the art will recognize that after the exogenous sequence is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

A transformed plant cell, callus, tissue or plant may be identified and isolated by selecting or screening the engineered plant material for traits encoded by the marker genes present on the transforming DNA. For instance, selection can be performed by growing the engineered plant material on media containing an inhibitory amount of the antibiotic or herbicide to which the transforming gene construct confers resistance. Further, transformed cells can also be identified by screening for the activities of any visible marker genes (e.g., the yfp, gfp, β-glucuronidase, luciferase, B or C1 genes) that may be present on the recombinant nucleic acid constructs. Such selection and screening methodologies are well known to those skilled in the art.

Physical and biochemical methods also may be used to identify plant or plant cell transformants containing inserted gene constructs. These methods include but are not limited to: 1) Southern analysis or PCR amplification for detecting and determining the structure of the recombinant DNA insert; 2) Northern blot, S1 RNase protection, primer-extension or reverse transcriptase-PCR amplification for detecting and examining RNA transcripts of the gene constructs; 3) enzymatic assays for detecting enzyme or ribozyme activity, where such gene products are encoded by the gene construct; 4) Next Generation Sequencing analysis; 5) protein gel electrophoresis, Western blot techniques, immunoprecipitation, or enzyme-linked immunoassays (ELISA), where the gene construct products are proteins. Additional techniques, such as in situ hybridization, enzyme staining, and immunostaining, also may be used to detect the presence or expression of the recombinant construct in specific plant organs and tissues. The methods for doing all these assays are well known to those skilled in the art.

Effects of gene manipulation using the methods disclosed herein can be observed by, for example, northern blots of the RNA (e.g., mRNA) isolated from the tissues of interest. Typically, if the mRNA is present or the amount of mRNA has increased, it can be assumed that the corresponding transgene is being expressed. Other methods of measuring gene and/or encoded polypeptide activity can be used. Different types of enzymatic assays can be used, depending on the substrate used and the method of detecting the increase or decrease of a reaction product or by-product. In addition, the levels of polypeptide expressed can be

measured immunochemically, i.e., ELISA, RIA, EIA and other antibody based assays well known to those of skill in the art, such as by electrophoretic detection assays (either with staining or western blotting). As one non-limiting example, the detection of the AAD-1 (aryloxyalkanoate dioxygenase; see WO 2005/107437) and PAT (phosphinothricin-N-acetyl-transferase), EC 2.3.1.183) proteins using an ELISA assay is described in U.S. Patent Publication No. 20090093366 which is herein incorporated by reference in its entirety. The transgene may be selectively expressed in some cell types or tissues of the plant or at some developmental stages, or the transgene may be expressed in substantially all plant tissues, substantially along its entire life cycle. However, any combinatorial expression mode is also applicable.

The present disclosure also encompasses seeds of the transgenic plants described above wherein the seed has the transgene or gene construct. The present disclosure further encompasses the progeny, clones, cell lines or cells of the transgenic plants described above wherein said progeny, clone, cell line or cell has the transgene or gene construct.

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.

EXAMPLE 1

Transformation of *Agrobacterium tumefaciens*

The binary expression vectors were transformed into *Agrobacterium tumefaciens* strain DAt13192 (RecA minus ternary strain) (Int'l. Pat. Pub. No. WO2012016222). Bacterial colonies were isolated, and binary plasmid DNA was isolated and confirmed *via* restriction enzyme digestion.

Corn Transformation

***Agrobacterium* Culture Initiation.** *Agrobacterium* cultures were streaked from glycerol stocks onto *Agrobacterium* (AB) minimal medium (as disclosed in WO 2013090734, the disclosure of which is incorporated herein by reference) and incubated at 20°C in the dark for 3 days. *Agrobacterium* cultures were then streaked onto a plate of YEP (see WO 2013090734) medium and incubated at 20°C in the dark for 1 day.

On the day of the experiment, a mixture of inoculation medium (see WO 2013090734) and acetosyringone were prepared in a volume appropriate to the number of bacterial strains comprising plant transformation constructs in the experiment. Inoculation medium was pipetted into a sterile, disposable 250 ml flask. Next, a 1 M stock solution of acetosyringone in 100% dimethyl sulfoxide was

added to the flask containing inoculation medium in a volume appropriate to make a final acetosyringone concentration of 200 µM. The required volumes of Inoculation medium and 1 M acetosyringone stock solution are listed in TABLE 1.

TABLE 1: The amount of inoculation medium/acetosyringone mixture to make according to the number of constructs being prepared

Number of constructs to prepare	Inoculation medium (mL)	1M acetosyringone stock (µL)
1	50	10
2	100	20
3	150	30
4	200	40
5	250	50

For each construct, 1-2 loops of *Agrobacterium* from the YEP plate were suspended in 15 ml of the inoculation medium/acetosyringone mixture inside a sterile, disposable 50 ml centrifuge tube, and the optical density of the solution at 600 nm (OD_{600}) was measured in a spectrophotometer. The suspension was then diluted down to 0.25-0.35 OD_{600} using additional inoculation medium/acetosyringone mixture. The tube of *Agrobacterium* suspension was then placed horizontally on a platform shaker set at about 75 rpm at room temperature and incubated between 1 and 4 hours before use.

Ear sterilization and embryo isolation. Ears from *Zea mays* cultivar B104 were harvested 10-12 days post pollination. Harvested ears were de-husked and surface-sterilized by immersion in a 20% solution of commercial bleach (Ultra Clorox® Germicidal Bleach, 6.15% sodium hypochlorite) and two drops of Tween® 20, for 20 minutes, followed by three rinses in sterile, deionized water inside a laminar flow hood. Immature zygotic embryos (1.8–2.2 mm long) were aseptically excised from each ear and distributed into one or more micro-centrifuge tubes containing 2.0 ml of *Agrobacterium* suspension into which 2 µl of 10% Break-Thru® S233 surfactant had been added.

***Agrobacterium* co-cultivation.** Upon completion of the embryo isolation activity, the tube of embryos was closed and placed on a rocker platform for 5 minutes. The contents of the tube were then poured out onto a plate of co-cultivation medium, and the liquid *Agrobacterium* suspension was removed with a sterile, disposable transfer pipette. The co-cultivation plate containing embryos was placed at the back of the laminar flow hood with the

lid ajar for 30 minutes; after which time the embryos were oriented with the scutellum facing up using a microscope. The co-cultivation plate with embryos was then returned to the back of the laminar flow hood with the lid ajar for a further 15 minutes. The plate was then closed, sealed with 3M® Micropore® tape, and placed in an incubator at 25°C with 24 hours/day light at approximately 60 μmol m^{-2} s^{-1} light intensity

Callus Selection and Regeneration of Transgenic Events. Following the co-cultivation period, embryos were transferred to Resting medium (see WO 2013090734). No more than 36 embryos were moved to each plate. The plates were placed in clear boxes and incubated at 27°C with 24 hours/day light at approximately 50 μmol m^{-2} s^{-1} light intensity for 7-10 days. Callused embryos were then transferred onto Selection I medium (see WO 2013090734). No more than 18 callused embryos were moved to each plate of Selection I. The plates were placed in clear boxes and incubated at 27 °C with 24 hours/day light at approximately 50 μmol m^{-2} s^{-1} light intensity for 7 days. Callused embryos were then transferred to Selection II medium (see WO 2013090734). No more than 12 callused embryos were moved to each plate of Selection II media. The plates were placed in clear boxes and incubated at 27°C with 24 hours/day light at approximately 50 μmol m^{-2} s^{-1} light intensity for 14 days.

At this stage resistant calli were moved to Pre-Regeneration medium (see WO 2013090734). No more than 9 calli were moved to each plate of Pre-Regeneration media. The plates were placed in clear boxes and incubated at 27 °C with 24 hours/day light at approximately 50 μmol m^{-2} s^{-1} light intensity for 7 days. Regenerating calli were then transferred to Regeneration medium in Phytatrays™ (see WO 2013090734). and incubated at 28°C with 16 hours light/8 hours dark per day at approximately 150 μmol m^{-2} s^{-1} light intensity for 7-14 days or until shoots develop. No more than 5 calli were placed in each Phytatray™. Small shoots with primary roots were then isolated and transferred to Shoot Elongation medium (see WO 2013090734). Rooted plantlets about 6 cm or taller were transplanted into soil and moved out to a growth chamber for hardening off.

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

Transfer and Establishment of T_0 Plants in the Greenhouse. Transgenic plants were transferred on a regular basis to the greenhouse. Plants were transplanted from Phytatrays™ to small pots (T. O. Plastics, 3.5" SVD, 700022C) filled with growing media (Premier Tech

Horticulture, ProMix BX, 0581 P) and covered with humidomes to help acclimate the plants. Plants were placed in a Conviron growth chamber (28 °C/24 °C, 16-hour photoperiod, 50-70% RH, 200 µmol m^{-2} s^{-1} light intensity) until reaching V3-V4 stage. This aided in acclimating the plants to soil and harsher temperatures. Plants were then moved to the greenhouse (Light Exposure Type: Photo or Assimilation; High Light Limit: 1200 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR); 16-hour day length; 27 °C Day/24 °C Night) and transplanted from the small pots to 5.5 inch pots. Approximately 1-2 weeks after transplanting to larger pots plants were sampled for bioassay. One plant per event was assayed.

Example 2: Identification of the Promoters

The maize ubiquitin coding sequence was BLASTx searched in the Phytozome (Goodstein et al., 2012) database using *Brachypodium distachyon* and *Setaria italica* as target genomes.

Maize Ubiquitin (ZM Ubi1) Coding Sequence

ATGCAGATCTTTGTGAAAACCCTGACTGGCAAGACTATCACCCTCGAGGTGGAGTCGTCTGAC
ACCATTGACAACGTTAAGGCCAAGATCCAGGACAAGGAGGGCATCCCCCCAGACCAGCAGCG
GCTCATCTTTGCTGGCAAACAGCTTGAGGACGGGCGCACGCTTGCTGACTACAACATCCAGAA
GGAGAGCACCCTCCACCTTGTGCTCCGTCTCAGGGGAGGCATGCAGATCTTTGTGAAAACCCT
GACCGGCAAGACTATCACCCTCGAGGTGGAGTCCTCTGACACCATTGACAACGTCAAGGCCAA
GATCCAGGACAAGGAGGGCATCCCTCCAGACCAGCAGCGGCTCATCTTTGCTGGGAAGCAGC
TTGAGGACGGGCGCACGCTTGCCGACTACAACATCCAGAAGGAGAGCACCCTCCACTTGGTG
CTGCGCCTCAGGGGAGGCATGCAGATCTTCGTGAAGACCCTGACCGGCAAGACTATCACCCTC
GAGGTGGAGTCTTCAGACACCATCGACAACGTCAAGGCCAAGATCCAGGACAAGGAGGGCAT
TCCCCCAGACCAGCAGCGGCTCATCTTTGCTGGAAAGCAGCTTGAGGACGGGCGCACGCTTGC
CGACTACAACATCCAGAAGGAGAGCACCCTCCACTTGGTGCTGCGCCTCAGGGGAGGCATGC
AGATCTTCGTGAAGACCCTGACCGGCAAGACTATCACCCTCGAGGTGGAGTCTTCAGACACCA
TCGACAATGTCAAGGCCAAGATCCAGGACAAGGAGGGCATCCCACCGGACCAGCAGCGTTTG
ATCTTCGCTGGCAAGCAGCTGGAGGATGGCCGCACCCTTGCGGATTACAACATCCAGAAGGA
GAGCACCCTCCACCTGGTGCTCCGTCTCAGGGGTGGTATGCAGATCTTTGTGAAGACACTCAC
TGGCAAGACAATCACCCTTGAGGTGGAGTCTTCGGATACCATTGACAATGTCAAGGCCAAGAT
CCAGGACAAGGAGGGCATCCCACCCGACCAGCAGCGCCTCATCTTCGCCGGCAAGCAGCTGG
AGGATGGCCGCACCCTGGCGGATTACAACATCCAGAAGGAGAGCACTCTCCACCTGGTGCTCC
GCCTCAGGGGTGGCATGCAGATTTTTGTGAAGACATTGACTGGCAAGACCATCACCTTGGAG
GTGGAGAGCTCTGACACCATTGACAATGTGAAGGCCAAGATCCAGGACAAGGAGGGCATTCC
CCCAGACCAGCAGCGTCTGATCTTTGCGGGCAAGCAGCTGGAGGATGGCCGCACTCTCGCGG
ACTACAACATCCAGAAGGAGAGCACCCTTCACCTTGTTCTCCGCCTCAGAGGTGGTATGCAGA
TCTTTGTAAAGACCCTGACTGGAAAAACCATAACCCTGGAGGTTGAGAGCTCGGACACCATCG
ACAATGTGAAGGCGAAGATCCAGGACAAGGAGGGCATCCCCCCGGACCAGCAGCGTCTGATC
TTCGCCGGCAAACAGCTGGAGGATGGCCGCACCCTAGCAGACTACAACATCCAAAAGGAGAG
CACCCTCCACCTTGTGCTCCGTCTCCGTGGTGGTCAG**TAA** (SEQ ID NO:18)

The protein alignments are shown in Figure 1. Two sequences that aligned with the *Zea mays* Ubiquitin 1 protein were identified from *Brachypodium distachyon*. Only one sequence that aligned with the *Zea mays* Ubiquitin 1 protein was identified each from *Setaria italic* and *Panicum virgatum*. An approximately 2 kb DNA sequence upstream from a predicted translational start site (ATG) was determined to be the beginning of the putative promoter sequence and used for expression characterization. The polynucleotide sequence alignments of the novel promoters that were isolated from *Panicum virgatum, Brachypodium distachyon* and *Setaria italica* were aligned to the ZM Ubi1 promoter and found to share low levels of sequence similarity across the 2 kb DNA region (Figs. 2A-C).

The UBI coding sequence and putative promoter for the *Panicum virgatum, Brachypodium distachyon* and *Setaria italica* ubiquitin genes are indicated in Figs 35-38.

Example 3: Vector Construction

The four promoter sequences were commercially synthesized and incorporated into plasmid vectors as depicted in Fig. 3 (pDAB113091), Fig. 4 (pDAB113092), Fig. 5 (pDAB113066) and Fig. 22 (pDAB118238). Similarly four 3'UTR/transcription termination sequences were commercially synthesized and incorporated into plasmid vectors as depicted in Fig. 23 (pDAB118237), Fig. 24 (pDAB118207), Fig. 25 (pDAB118208) and Fig. 26 (pDAB118209). . The sequences were flanked by 15-18 nucleotide homology fragments on both ends for seamless cloning (GeneArt® Seamless Cloning and Assembly Kit, Invitrogen, Carlsbad, CA) and type II restriction enzyme sites inserted for the isolation of promoter fragments. Seamless cloning compatible *Zea mays* Ubi1 promoter (Christensen and Quail (1996) Transgenic Research. 5; 213-218; Christensen et al., (1992) Plant Molecular Biology. 18; 675-689) or *Oryzae sativa* Actin promoter (McElroy et al., (1990) Plant Cell. 2; 163-71), and PhiYFP (Shagin et al., (2004) Mol Biol Evol. 21; 841-50) coding sequence comprising the ST-LS1 intron (Vancanneyt et al., (1990) Mol Gen Genet. 220; 245-50), and St PinII or native 3'-UTR (An et al., 1989 Plant Cell. 1; 115-22.) fragments were obtained using PCR or typeII restriction enzymes. Finally, the promoter :: PhiYFP :: St PinII 3'-UTR fragments were assembled using seamless cloning to create transient expression vectors (Fig. 6, pDAB113103; Fig. 7, pDAB113104; Fig. 8, pDAB113105; Fig. 9, pDAB113106; and, Fig. 10, pDAB113107; Fig. 27, pDAB120403; Fig. 28, pDAB118234, Fig. 29, pDAB118235; and Fig. 30, pDAB118236) for transient expression testing. These transient expression vectors were integrated into a binary vector containing the Zm Ubi 1 promoter and AAD-1 coding sequence (International Patent Publication No. 2005107437) and Zm Lip

3'UTR (Paek *et al.*, (1998) Molecules and Cells, 8(3): 336-342). The resulting binaries were confirmed via restriction enzyme digestion and sequencing reaction (Fig. 12, pDAB113117; Fig. 13, pDAB113118; Fig. 14, pDAB113119; Fig. 15, pDAB113120; Fig. 16, pDAB113121; Fig. 31, pDAB120400; Fig. 32, pDAB120404; Fig. 33, pDAB120401; and, Fig. 34, pDAB120402).

Example 4: Transient Expression Testing

Transient expression was tested using particle bombardment of immature maize (B104) embryos. Forty embryos were used per treatment in a Petri plate for bombardment. YFP image analysis was done after overnight incubation of particle bombardment. Figure 19 shows YFP expression levels obtained from the novel promoters. The data show that YFP expression levels obtained from the novel promoters (pDAB113103, pDAB113104, and pDAB113105) is comparable to the YFP expression levels obtained from the ZM Ubi1 promoter (pDAB113106) and the OS Act 1 promoter (pDAB113107) as visually observed under the microscope. Plant tissues were imaged on a Leica EL6000 – mercury metal halide™ microscope. Confocal and Differential Interference Contrast (DIC) images were captured using Chroma 42003- ZsYellow 1™ filters.

Example 5: Transgene Copy Number Estimation Using Real Time TaqMan® PCR

The stable integration of the *yfp* transgene within the genome of the transgenic *Z. mays* plants was confirmed via a hydrolysis probe assay. Stably-transformed transgenic *Z. mays* plantlets that developed from the callus were obtained and analyzed to identify events that contained a low copy number (1-2 copies) of full-length T-strand inserts. Identified plantlets were advanced to the green house and grown.

The Roche Light Cycler480™ system was used to determine the transgene copy number. The method utilized a biplex TaqMan® reaction that employed 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 were determined by measuring the intensity of *yfp*-specific fluorescence, relative to the *invertase*-specific fluorescence, as compared to known copy number standards.

A *yfp* gene-specific DNA fragment was amplified with one TaqMan® primer/probe set containing a probe labeled with FAM™ fluorescent dye, and *invertase* was amplified with a second TaqMan® primer/probe set containing a probe labeled with HEX™ fluorescence (TABLE 2). The PCR reaction mixture was prepared as set forth in TABLE 3, and the gene-specific DNA fragments were amplified according to the conditions set forth in TABLE 4.

Copy number and zygosity of the samples were 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.

	Primer/Probe	Sequence
PhiYFP v3	Forward Primer	(SEQ ID NO:28) CGTGTTGGGAAAGAACTTGGA
PhiYFP v3	Reverse Primer	(SEQ ID NO:29) CCGTGGTTGGCTTGGTCT
PhiYFP v3	Probe	(SEQ ID NO:30) 5'FAM/ CACTCCCCACTGCCT /MGB_BHQ_1/3'
Invertase	Forward Primer	(SEQ ID NO:31) TGGCGGACGACGACTTGT
Invertase	Reverse Primer	(SEQ ID NO:32) AAAGTTTGGAGGCTGCCGT
Invertase	Probe	(SEQ ID NO:33) 5'HEX/ CGAGCAGACCGCCGTGTACTT /3BHQ_1/3'

(synthesized by Integrated DNA Technologies, Coralville, IA).

TABLE 3: Taqman® PCR reaction mixture.

Component	**Working Concentration**	**Final Concentration**	**Volume (µl)**
Water	-	-	0.5
Roche LightCyler 480 Probes Master Mix	2X	1X	5
PhiYFP v3 F	10 µM	400nM	0.4
PhiYFP v3 R	10 µM	400nM	0.4
PhiYFP v3 Probe-FAM	5 µM	200nM	0.4
Invertase F	10 µM	400nM	0.4
Invertase R	10 µM	400nM	0.4
Invertase Probe – Hex	5 µM	200nM	0.4
Polyvinylpyrrolidone (PVP)	10%	0.1%	0.1
Genomic DNA template	Diluted BioCel DNA (~5nglul)	~10ng/uL	2
Total reaction volume	**-**	**-**	**10.0**

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

	58	35 seconds	
	72	1 second	
Step-3	40	seconds	1

Standards were created by diluting the vector, pDAB108706, 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 were prepared. One and two copy dilutions of the pDAB108706 mixed with the *Z. mays* B104 gDNA standard were validated against a control *Z. mays* event that was known to be hemizygous, and a control *Z. mays* event that was known to be homozygous (*Z. mays* event 278; *see* PCT International Patent Publication No. WO 2011/022469 A2). A TaqMan® biplex assay that utilizes oligonucleotides specific to th*e AAD1* gene and oligonucleotides specific to the endogenous *Z. mays* reference gene, *invertase,* was 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 was prepared as set forth in TABLE 3**,** and the specific fragments were amplified according to the conditions set forth in TABLE 4.

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). Results from the transgene copy number analysis of transgenic plants obtained *via* transformation with different promoter constructs are shown in TABLE 5. Only plants with 1-2 copies of the *yfp* transgene were transferred to the greenhouse for further expression analyses.

TABLE 5: Transgene copy number estimation of the transgenic plants obtained from promoter construct described herein and control constructs.

Construct	**Number of Positive Events**	**1-2 Copies of *yfp***

pDAB113117	32	17
pDAB113118	26	13
pDAB113119	30	16
pDAB113120	43	10
pDAB113121	36	19

EXAMPLE 6: Expression of Genes Operably Linked to Ubiquitin Promoters

Protein Extraction

T_0 plants were sampled at V4-5 using a leaf ELISA assays. Sample were collected in 96-well collection tube plate, and 4 leaf disks (paper hole punch size) were taken for each sample. Two 4.5mm BBs and 200 µL extraction buffer [1x PBS supplemented with 0.05% Tween®-20 and 0.05% BSA (Millipore Probumin®, EMD Millipore Corp., Billerica, MA)] were added to each tube. For AAD1 extraction, the concentration of BSA was increased to 0.5%. Plates were processed in a KLECO bead mill at full speed for 3 minutes. Additional 200 µL of extraction buffer was added to each tube followed by inversion to mix. Plates were spun for 5 minutes at 3000 rpm. Supernatant was transferred to corresponding wells in a deep well 96 stored on ice.

YFP and AAD1 ELISA Procedure

Nunc® 96-well Maxi-Sorp Plates (Thermo Fisher Scientific Inc., Rockford, IL) were used for ELISA. Plates were coated with mouse monoclonal anti-YFP capture antibody (OriGene Technologies Inc., Rockville, MD). The antibody was diluted in PBS (1 µg/mL) and 150 µL of diluted PBS was added per well. The plates were incubated overnight at 4°C. The overnight plates were kept at room temperature for 20-30 minutes before washing 4x with 350 µL of wash buffer [1x PBS supplemented with 0.05% Tween®-20 (Sigma-Aldrich, St. Louis, MO)]. Plates were blocked with 200 µL per well of blocking buffer [1x PBS supplemented with 0.05% Tween®-20 plus 0.5% BSA (Millipore Probumin®)] for a minimum of 1 hr at +37°C followed by 4x washing with 350 µL of wash buffer (Tomtec QuadraWash™ 2, Tomtec, Inc., Hamden, CT).

For the YFP ELISA, Evrogen recombinant Phi-YFP 1mg/mL (Axxora LLC, Farmingdale, NY) was used as a standard. A 5-parameter fit standard curve (between the 1 ng/ml and 0.125 ng/ml Standards) was used to ensure all data fall in the linear portion of the

curve. 100 µL of standard or sample was added to the well. A minimum 1:4 dilution of sample in the Assay Buffer was used. Plates were incubated for 1hr at RT on plate shaker (250 rpm; Titer Plate shaker) followed by 4x washing with 350 µL of wash buffer (Tomtec QuadraWash™ 2). About 100 µL of 1 µg/mL Evrogen rabbit polyclonal anti-PhiYFP primary antibody (Axxora) was added to each well. Plates were incubated for 1 hr at room temperature on a plate shaker at 250 rpm followed by 4x washing with 350 µL of wash buffer (Tomtec QuadraWash™ 2). Next, 100 µL of anti-rabbit IgG HRP secondary antibody (Thermo Scientific) diluted 1:5000 in Blocking/Assay buffer, which was added to each well. Plates were incubated for 1 hr at room temperature on plate shaker at 250 rpm followed by 4x washes with 350 µL of wash buffer (Tomtec QuadraWash™ 2). 100 µL of Pierce 1 Step Ultra TMB ELISA (Thermo Scientific) substrate was added in the well with gentle shaking for 10 minutes. Reaction was stopped by adding 50 µL of 0.4N H_2SO_4. Absorbance was read at 450 nm with a 650 nm reference filter.

AAD1 expression levels were determined by ELISAs using kits from Acadia BioSciences (Portland, ME). The ELISAs were performed using multiple dilutions of the extracts and using the reagents and instructions provided by the supplier. The protein levels were normalized using total soluble protein assay, performed using the 660 nm protein assay reagent supplied by Thermo Scientific and following the supplier's instructions.

EXAMPLE 7: Whole Plant YFP Image Analysis exemplifying Stable Expression of Genes Operably Linked to Ubiquitin Promoters

Whole plants that contained a low copy number of the binary plasmid were grown in a greenhouse. Plant tissues were imaged on a Leica EL6000 – mercury metal halide™ microscope. Confocal and Differential Interference Contrast (DIC) images were captured using Chroma 42003- ZsYellow 1™ filters. Representative examples of stable expression of YFP in callus and root tissue of transgenic T_0 maize plants obtained from *Z. mays* embryos transformed with the *Brachypodium distachyon* Ubiquitin1 C, *Brachypodium distachyon* Ubiquitin1, and *Setaria italica* ubiquitin 2 promoters described herein are presented in FIG. 20 to FIG. 21, respectively. The promoters drove robust expression of the *yfp* coding sequences both in callus (FIG. 20) and root (FIG. 21) plant tissues.

EXAMPLE 8: Whole Plant T_0 Stable Expression of Genes Operably Linked to Ubiquitin Promoters

Additional data was produced from an ELISA analysis of the expressed YFP protein. The ELISA analysis further confirmed that the novel promoters drove robust expression of a transgene. The quantitative measurements of YFP protein obtained from transgenic plants comprising novel promoter constructs are shown in FIG. 17 and TABLE 6. The data show that expression of YFP protein in the plants containing the novel promoters (pDAB113117, pDAB113118, and pDAB113119) is several fold higher that YFP expression obtained from the Os Act1 (Rice Actin1) promoter (pDAB113120). Comparatively, FIG. 18 and TABLE 7 show that similar level of AAD1 expression was obtained from all the constructs. This is expected because AAD1 is driven by the Zm Ubi1 promoter for all of the constructs.

TABLE 6: Cross Species Ubiquitin Promoter T_0 Leaf YFP expression

Construct	Mean (ng/mg TSP)	Statistical significance
pDAB113121	144.00173	A
pDAB113118	92.37256	AB
pDAB113119	65.30393	B
pDAB113117	55.24345	B
pDAB113120	12.77181	B

Levels not connected by same letter are significantly different

TABLE 7: Cross Species Ubiquitin Promoter T_0 Leaf AAD1 expression

Construct	Mean (ng/mg TSP)	Statistical significance
pDAB113121	119.06932	A
pDAB113118	109.19796	A
pDAB113119	96.29021	A
pDAB113117	85.40412	A
pDAB113120	83.81594	A

Levels not connected by same letter are significantly different

EXAMPLE 9: Whole Plant T_1 Stable Expression of Genes Operable Linked to Ubiquitin Promoters and 3'UTRs

T_0 single transgene copy plants were backcrossed to wild type B104 corn plants to obtain T_1 seed. Hemizygous T_1 plants were used for analysis. Five events per construct and 5-10 plants per event for V4 and V12 leaf expression. Three events per construct and 3 plants per event were used for the other tissue type expression. Zygosity analysis was done for AAD1/YFP.

The quantitative measurements of YFP protein obtained from leaf tissue of T_1 transgenic plants comprising novel promoter constructs are shown in TABLE 8. The data confirmed the T_0 leaf expression results and further showed that consistent high expression of YFP protein was obtained in the V4, V12 and R3 leaf tissue of the plants containing the novel promoters (pDAB113117, pDAB113118, and pDAB113119). TABLE 8 also shows that there was several fold increase in the expression of YFP protein when this novel promoters were used in combination with their native 3'UTRs (pDAB120400, pDAB120401, and pDAB120402) instead of PinII 3'UTR (pDAB113117, pDAB113118, and pDAB113119). YFP protein expression was detected from the plants containing construct pDAB120404 confirming that novel promoter and 3'UTR used in this construct drive expression of a transgene.

TABLE 8: Cross Species Ubiquitin Promoter and 3'UTR T_1 Leaf Expression

		Mean YFP (ng/mg TSP)		
Construct	Event	V4 Leaf	V12 Leaf	R3 Leaf
pDAB113117	pDAB113117[1]-006	44.0	169.4	2108.3
pDAB113117	pDAB113117[1]-007	44.8	181.4	2582.7
pDAB113117	pDAB113117[1]-008	79.6	322.8	4096.3
pDAB113117	pDAB113117[1]-019	74.3	369.1	3420.3
pDAB113117	pDAB113117[1]-028	34.2	168.2	2164.1
pDAB113118	pDAB113118[1]-005	33.6	148.8	2094.7
pDAB113118	pDAB113118[1]-007	54.9	180.1	2171.4
pDAB113118	pDAB113118[1]-010		138.0	2748.2
pDAB113118	pDAB113118[1]-023	46.2	156.6	2216.8
pDAB113118	pDAB113118[1]-025	41.7	132.9	2071.4
pDAB113119	pDAB113119[1]-001	133.1	436.0	6744.0
pDAB113119	pDAB113119[1]-005	49.2	138.6	1772.9
pDAB113119	pDAB113119[1]-011	54.6	133.9	1415.5
pDAB113119	pDAB113119[1]-013		129.1	1807.7
pDAB113119	pDAB113119[1]-028	38.5	129.9	1632.8
pDAB113120	pDAB113120[1]-005	9.8	69.6	493.5
pDAB113120	pDAB113120[1]-010	24.3	74.5	638.3
pDAB113120	pDAB113120[1]-014	17.2	79.7	552.4
pDAB113120	pDAB113120[1]-023	13.2	55.4	372.2
pDAB113120	pDAB113120[1]-032	12.5	69.6	233.7

pDAB113121	pDAB113121[1]-008	327.9		
pDAB113121	pDAB113121[1]-011	166.2	271.2	4472.6
pDAB113121	pDAB113121[1]-018	128.2	362.0	7116.3
pDAB113121	pDAB113121[1]-023	112.2	309.1	6813.7
pDAB113121	pDAB113121[1]-026	118.7	311.7	6300.7
pDAB120400	pDAB120400[1]-001	640.8182		
pDAB120400	pDAB120400[1]-002	339.24463		
pDAB120400	pDAB120400[1]-004	943.96511		
pDAB120400	pDAB120400[1]-007	1653.7402		
pDAB120400	pDAB120400[1]-024	466.01906		
pDAB120401	pDAB120401[1]-001	833.04373		
pDAB120401	pDAB120401[1]-011	471.9103		
pDAB120401	pDAB120401[1]-019	795.08285		
pDAB120401	pDAB120401[1]-022	721.58288		
pDAB120401	pDAB120401[1]-025	696.94286		
pDAB120402	pDAB120402[1]-010	750.82185		
pDAB120402	pDAB120402[1]-011	619.38603		
pDAB120402	pDAB120402[1]-014	618.98144		
pDAB120402	pDAB120402[1]-030	625.84385		
pDAB120404	pDAB120404[1]-003	44.088479		
pDAB120404	pDAB120404[1]-013	47.464389		
pDAB120404	pDAB120404[1]-014	52.204801		
pDAB120404	pDAB120404[1]-016	45.397854		
pDAB120404	pDAB120404[1]-020	46.913279		

High YFP protein expression was found in different tissue types including cob, husk, kernel, pollen, root, silk and stem sampled from the transgenic corn plants containing novel Ubiquitin Promoters driving *YFP* (Table 9). These data demonstrate that the novel promoters and 3'UTRs claimed here drive high constitutive expression of transgene in plants and would be useful for biotechnological applications.

TABLE 9: Cross Species Ubiquitin Promoter T1 Expression in Different Tissue Type

		Mean YFP (ng/mg TSP)						
Construct	**Event**	**Cob**	**Husk**	**Kernel**	**Pollen**	**V12 Root**	**Silk**	**Stem**
pDAB113117	pDAB113117[1]-006	3452.1	1164.3	1341.2	397.1	2292.7	1405.0	7279.8
pDAB113117	pDAB113117[1]-007	2519.6	954.7	1410.9	414.3	2245.6	1974.3	6179.0
pDAB113117	pDAB113117[1]-019	8362.3	2280.8	2829.6	749.5	7112.4	4790.2	13044.1
pDAB113118	pDAB113118[1]-005	2801.6	620.9	886.3	782.2	1136.1	636.7	1953.6
pDAB113118	pDAB113118[1]-007	2339.2	524.9	725.1	376.1	1495.6	1271.0	2806.9
pDAB113118	pDAB113118[1]-023	1302.1	491.8	716.8	435.3	1193.0	829.1	1522.9
pDAB113119	pDAB113119[1]-011				399.7	1025.9	942.2	2475.6
pDAB113119	pDAB113119[1]-013	2238.1	572.0	1050.5	438.1	1311.2	539.7	2235.2
pDAB113119	pDAB113119[1]-028	2013.9	536.4	1061.4	450.0	1166.3	826.9	1912.5
pDAB113120	pDAB113120[1]-005	1166.8	310.5	514.0	1704.1	169.7	322.1	739.3
pDAB113120	pDAB113120[1]-023	1096.4	531.9	845.8	1433.9	268.4	572.2	877.9
pDAB113120	pDAB113120[1]-032	1344.1	587.8	985.1	1252.3	187.6	472.4	694.0
pDAB113121	pDAB113121[1]-011	6779.1	2942.3	3452.6	2022.6	5834.0	2881.7	7445.5
pDAB113121	pDAB113121[1]-023	4830.0	2689.8	1913.7	1641.8	2547.7	2453.8	8295.9
pDAB113121	pDAB113121[1]-026	8186.2	3889.3	4432.3	1432.0	2521.9	2182.5	7760.7

All references, including publications, patents, and patent applications, cited herein are hereby incorporated by reference to the extent they are not inconsistent with the explicit details of this disclosure, and are so incorporated to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein. The references discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior 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.

WHAT IS CLAIMED IS:

1. A nucleic acid vector comprising a promoter operably linked to

i) a polylinker sequence;

ii) a non-ubiquitin transgene or

iii) a combination of i) and ii), wherein said promoter comprises SEQ ID NO: 3 or a sequence that has 90% sequence identity with SEQ ID NO: 3.

2. The nucleic acid vector of claim 1 wherein said promoter is less than 3kb in length.

3. The nucleic acid vector of claim 1 wherein said promoter consists of SEQ ID NO: 3 or a sequence that has 90% sequence identity with SEQ ID NO: 3.

4. The nucleic acid vector of any one of claims 1-3 further comprising a sequence encoding a selectable maker.

5. The nucleic acid vector of claim 4 wherein said promoter is operably linked to a transgene.

6. The nucleic acid vector of claim 5 wherein the transgene encodes a selectable marker or a gene product conferring insecticidal resistance, herbicide tolerance, nitrogen use efficiency, water use efficiency, or nutritional quality.

7. The nucleic acid vector of any of claims 1-3, or 5 further comprising a 3' untranslated sequence comprising SEQ ID NO: 6 or a sequence that has 90% sequence identity with SEQ ID NO: 6, wherein the 3' untranslated sequence is operably linked to said polylinker or said transgene.

8. The nucleic acid vector of any of claims 1-3, or 5 further comprising a 5' untranslated sequence comprising SEQ ID NO: 13 or a sequence that has 90% sequence identity with SEQ ID NO: 13, wherein the 5' untranslated sequence is inserted between, and operably linked to, said promoter sequence and said polylinker or transgene.

9. The nucleic acid vector of any of claims 1-3, or 5 further comprising a 5' untranslated sequence comprising SEQ ID NO: 14 or a sequence that has 90% sequence identity with SEQ ID NO: 14, wherein the 5' untranslated sequence is inserted between, and operably linked to, said promoter sequence and said polylinker or transgene.

10. The nucleic acid vector of claim 8 or 9 further comprising an intron sequence inserted after the 5' untranslated sequence.

11. The nucleic acid vector of claim 10 wherein the intron sequence comprises SEQ ID NO: 9 or SEQ ID NO: 10.

12. The nucleic acid vector of claim 1 wherein the promoter consists of a sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42 and a sequence having 90% sequence identity with SEQ ID NO: 17 SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42 wherein said promoter is operably linked to a transgene.

13. The nucleic acid vector of claim 1 wherein the promoter consists of a sequence SEQ ID NO: 40 or SEQ ID NO: 42 wherein said promoter is operably linked to a transgene.

14. The nucleic acid vector of claim 1 wherein the promoter consists of SEQ ID NO: 17 or a sequence having 90% sequence identity with SEQ ID NO: 17 and said promoter is operably linked to a transgene.

15. The nucleic acid vector of claim 12, 13 or 14 further comprising a 3' untranslated sequence comprising SEQ ID NO: 6 or a sequence that has 90% sequence identity with SEQ ID NO: 6, wherein the 3' untranslated sequence is operably linked to said transgene.

16. A non-Setaria plant comprising SEQ ID NO: 3, or a sequence that has 90% sequence identity with SEQ ID NO: 3 operably linked to a transgene.

17. The plant of claim 16 wherein said plant is selected from the group consisting of maize, wheat, rice, sorghum, oats, rye, bananas, sugar cane, soybean, cotton, sunflower, and canola.

18. The plant of claim 16 wherein said plant is *Zea mays*.

19. The plant of any one of claims 16-18 wherein the transgene is inserted into the genome of said plant.

20. The plant of claim 16 further comprising a 5' untranslated sequence comprising SEQ ID NO: 13 or a sequence that has 90% sequence identity with SEQ ID NO: 13, wherein the 5' untranslated sequence is inserted between, and operably linked to, said promoter and said transgene.

21. The plant of claim 16 further comprising a 5' untranslated sequence comprising SEQ ID NO: 14 or a sequence that has 90% sequence identity with SEQ ID NO: 14, wherein the 5' untranslated sequence is inserted between, and operably linked to, said promoter and said transgene.

22. The plant of claim 20 or 21 further comprising an intron sequence inserted after the 5' untranslated sequence.

23. The plant of claim 22 wherein the intron sequence comprises SEQ ID NO: 9 or SEQ ID NO: 10.

24. The plant of claim 20 further comprising a 3' untranslated sequence comprising SEQ ID NO: 6 or a sequence that has 90% sequence identity with SEQ ID NO: 6, wherein the 3' untranslated sequence is operably linked to said transgene

25. The plant of claim 16 wherein the promoter consists of SEQ ID NO: 40, SEQ ID NO: 42 or a sequence having 90% sequence identity with SEQ ID NO: 40 or SEQ ID NO:42 wherein said promoter is operably linked to a transgene.

26. The plant of claim 16 wherein the promoter consists of SEQ ID NO: 17, SEQ ID NO: 41 or a sequence having 90% sequence identity with SEQ ID NO: 17 of SEQ ID NO: 41 wherein said promoter is operably linked to a transgene.

27. The plant of claim 25 or 26 further comprising a 3' untranslated sequence comprising SEQ ID NO: 6 or a sequence that has 90% sequence identity with SEQ ID NO: 6, wherein the 3' untranslated sequence is operably linked to said transgene.

28. A nucleic acid vector comprising a transcription terminator operably linked to

i) a polylinker sequence;

ii) a non-ubiquitin transgene or

iii) a combination of i) and ii), wherein said transcription terminator comprises SEQ ID NO: 6 or a sequence that has 90% sequence identity with SEQ ID NO: 6.

29. The nucleic acid vector of claim 28 wherein said transcription terminator is less than 1kb in length.

30. The nucleic acid vector of claim 29 wherein said transcription terminator consists of the 3'UTR sequence of SEQ ID NO: 6.

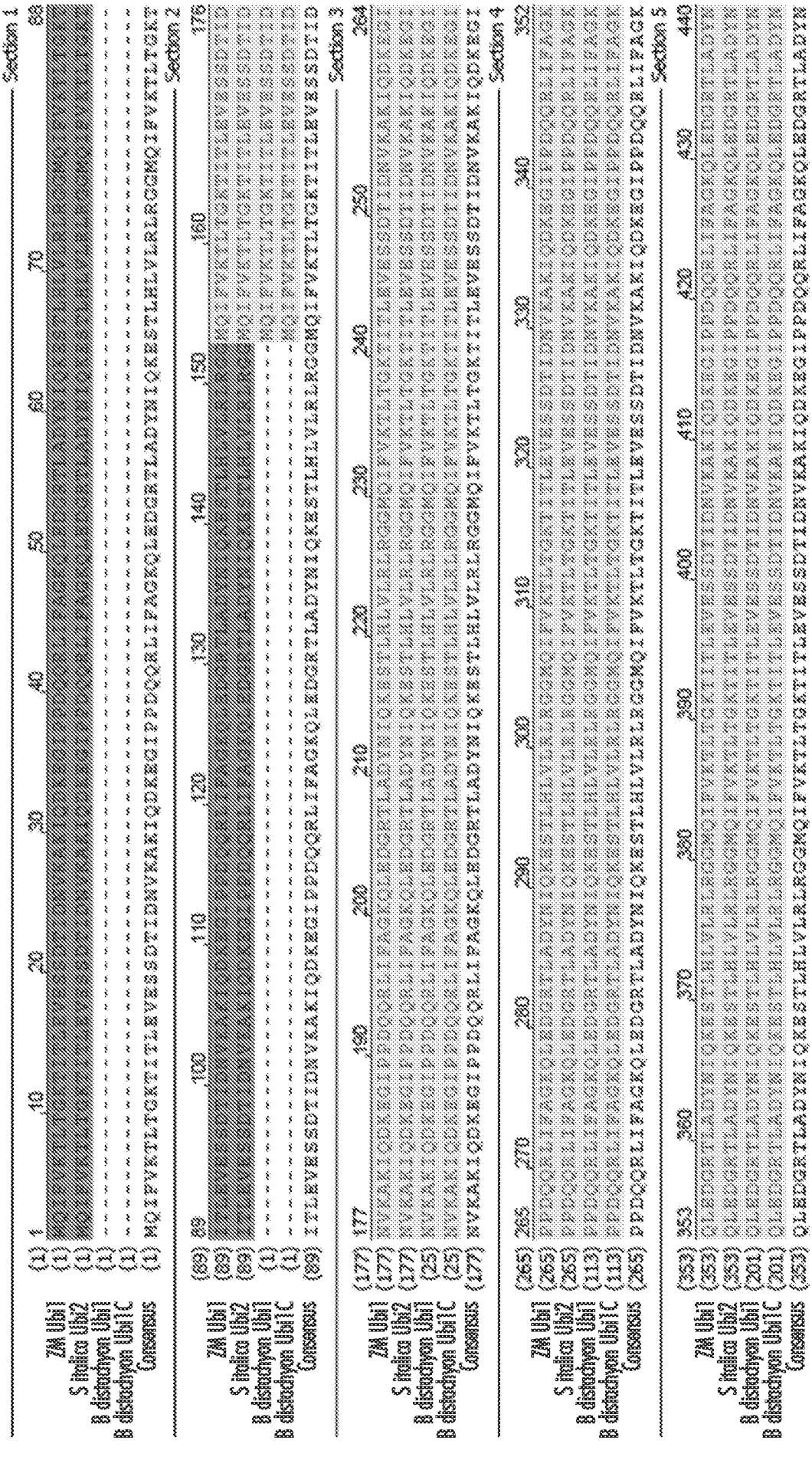
Section 1
Section 2
Section 3
Section 4
Section 5
ZM Ubi1
S italica Ubi2
B distachyon Ubi1
B distachyon Ubi1C
Consensus

FIG. 1

Section 6
441
452
460
470
480
490
500
510
Section 7
ZM Ubi1
S italica Ubi2
B distachyon Ubi1
B distachyon Ubi1C
Consensus
529
534

FIG. 1 cont.

Section 1
Section 2
Section 3
Section 4
Section 5
Section 6
Zm-Ubi1
B distachyon Ubi1
B distachyon Ubi1-C
B indica Ubi2

FIG. 2

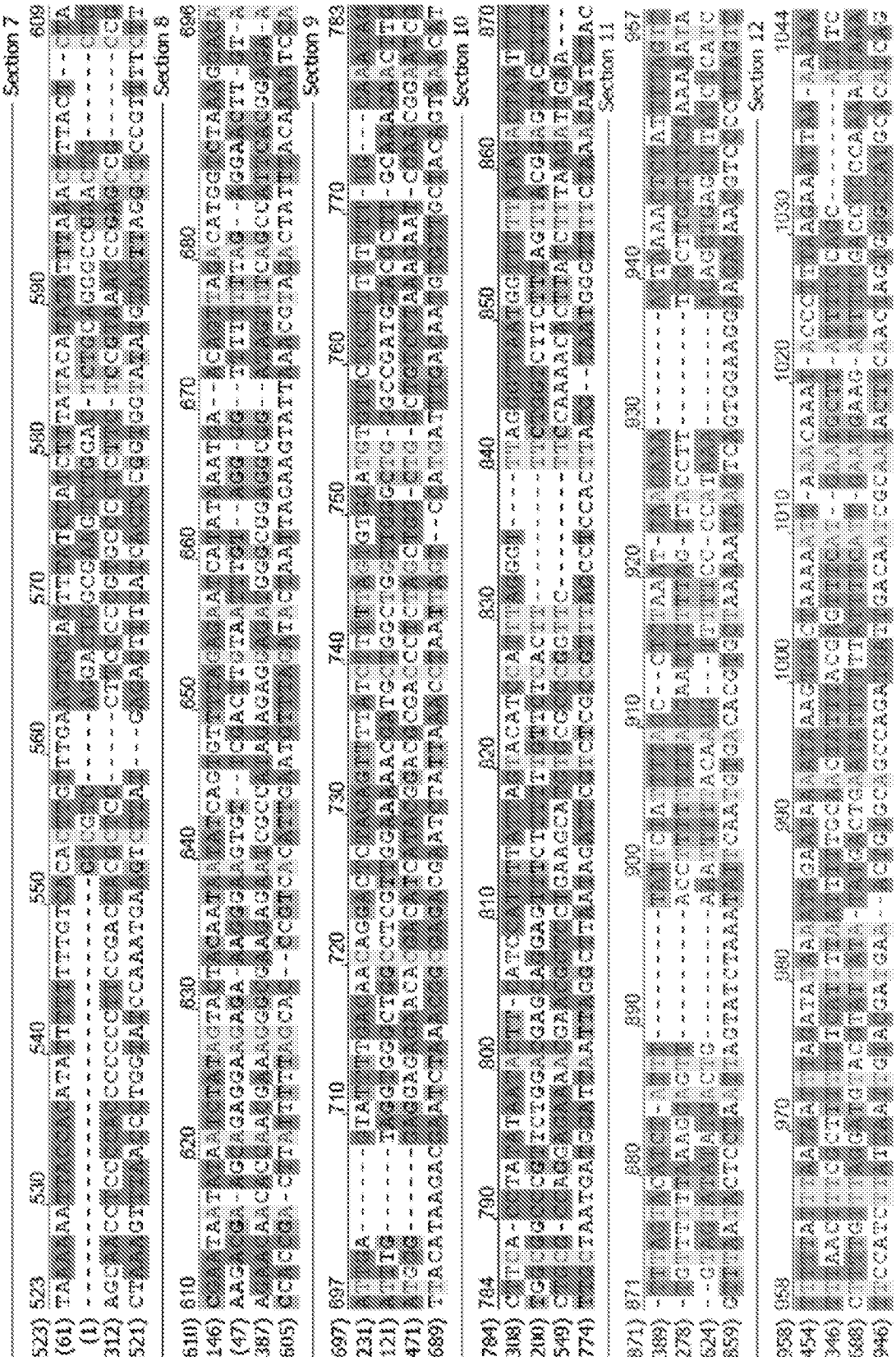

FIG. 2 cont.

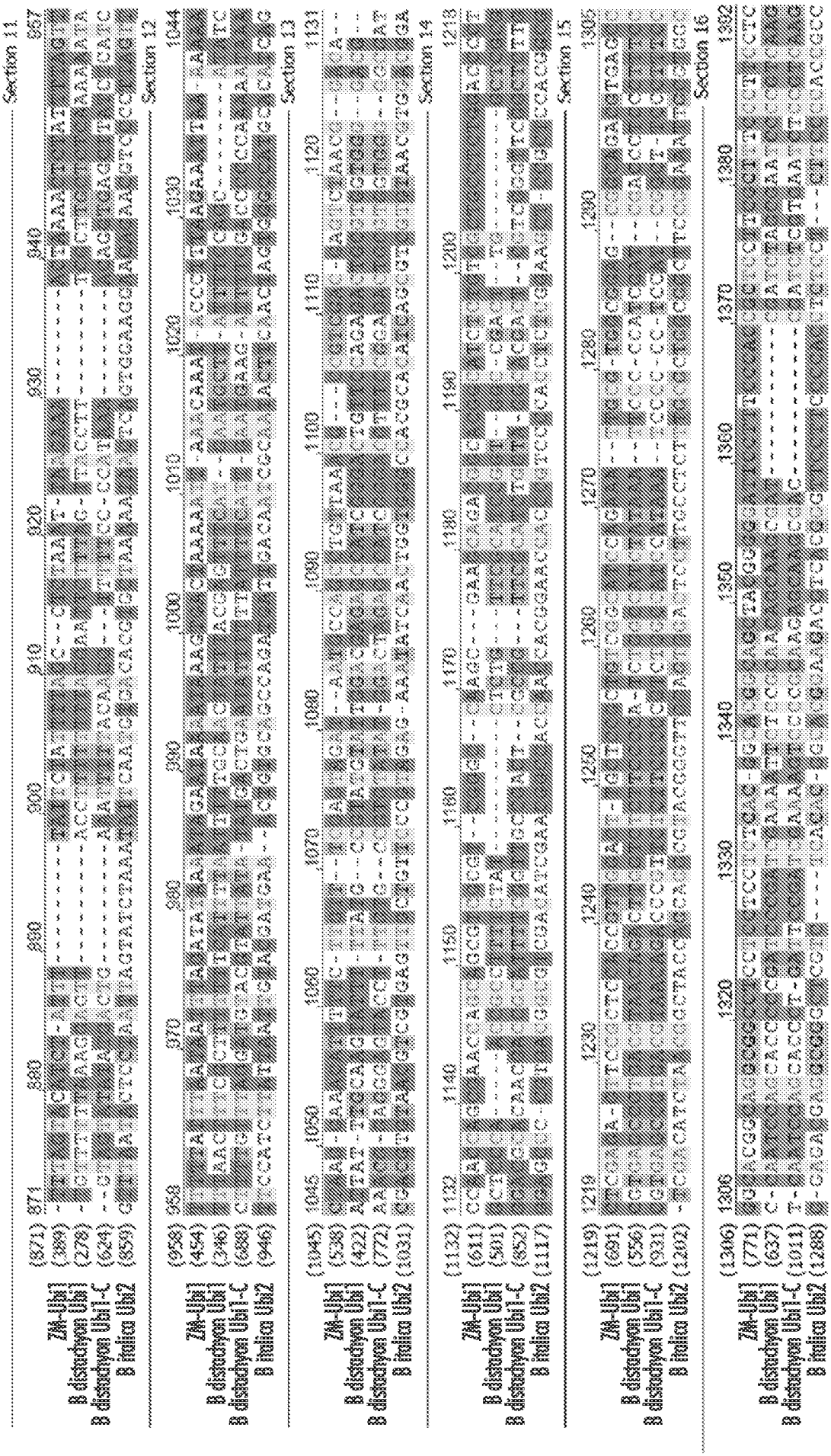

FIG. 2 cont.

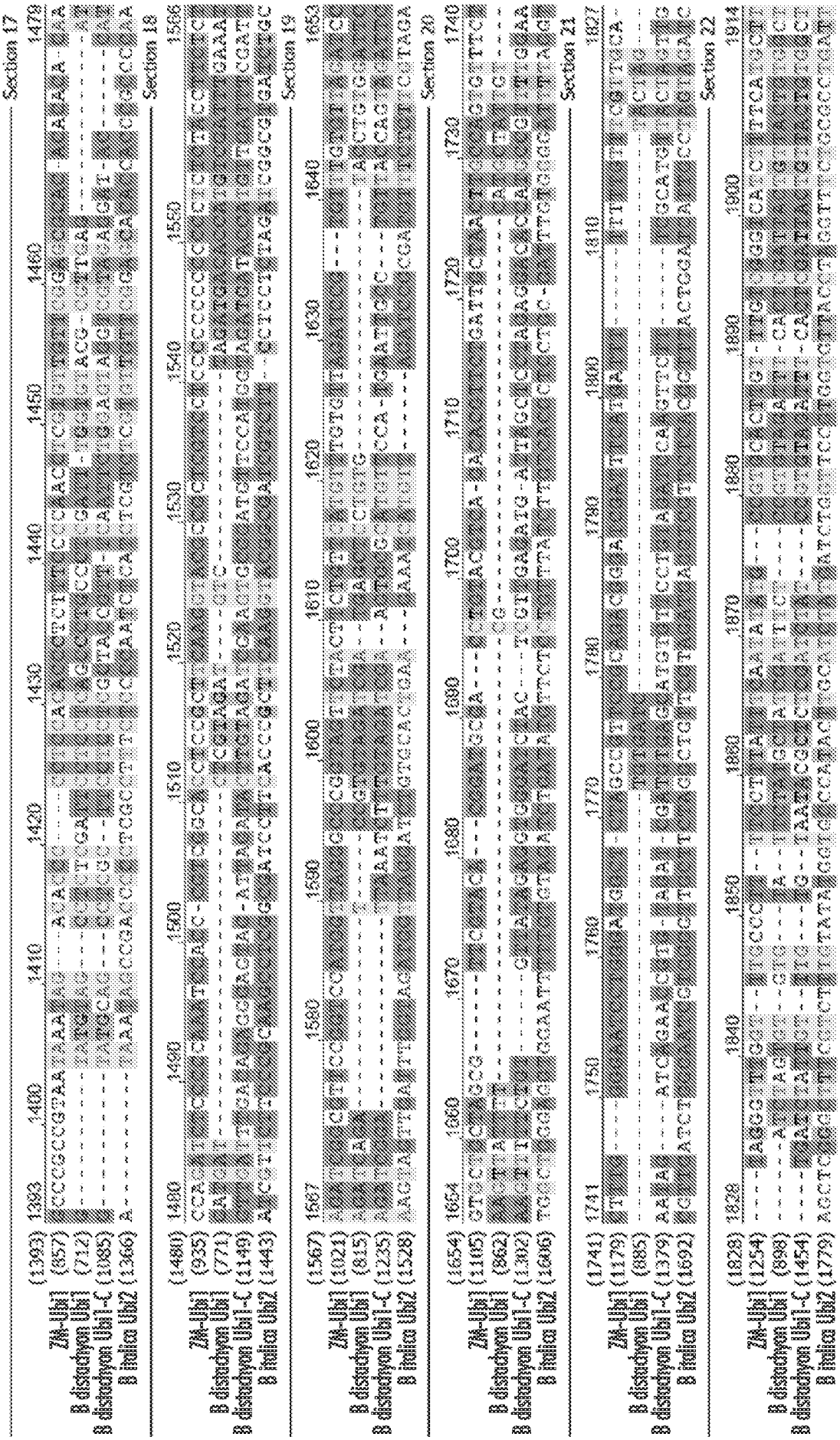

FIG. 2 cont.

Section 23
Section 24
Section 25
Section 26
Section 27
Section 28
ZM-Ubi1
B distachyon Ubi
B distachyon Ubi1-C
B indica Ubi2

FIG. 2 cont.

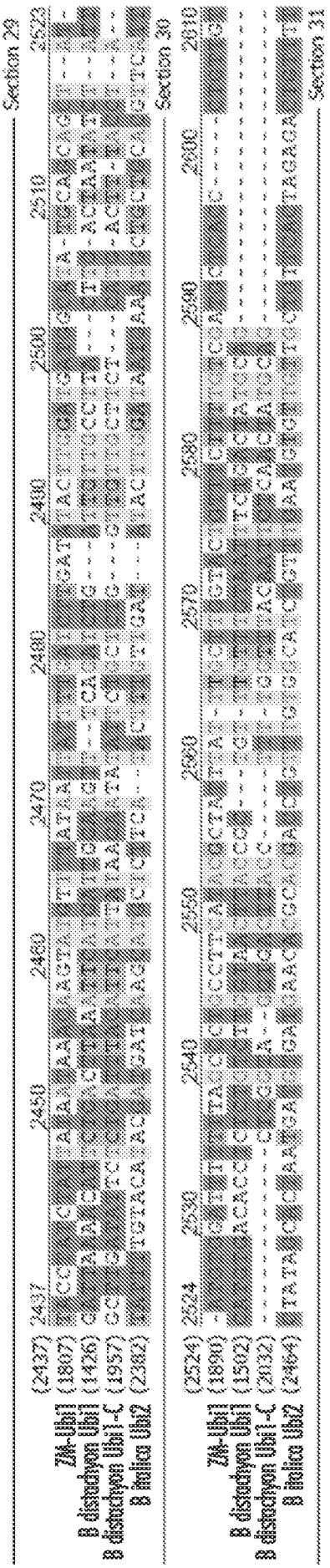
Section 29
Section 30
Section 31
ZM-Ubi1
B distachyon Ubi1
B distachyon Ubi1-C
B indica Ubi2

FIG. 2 cont.

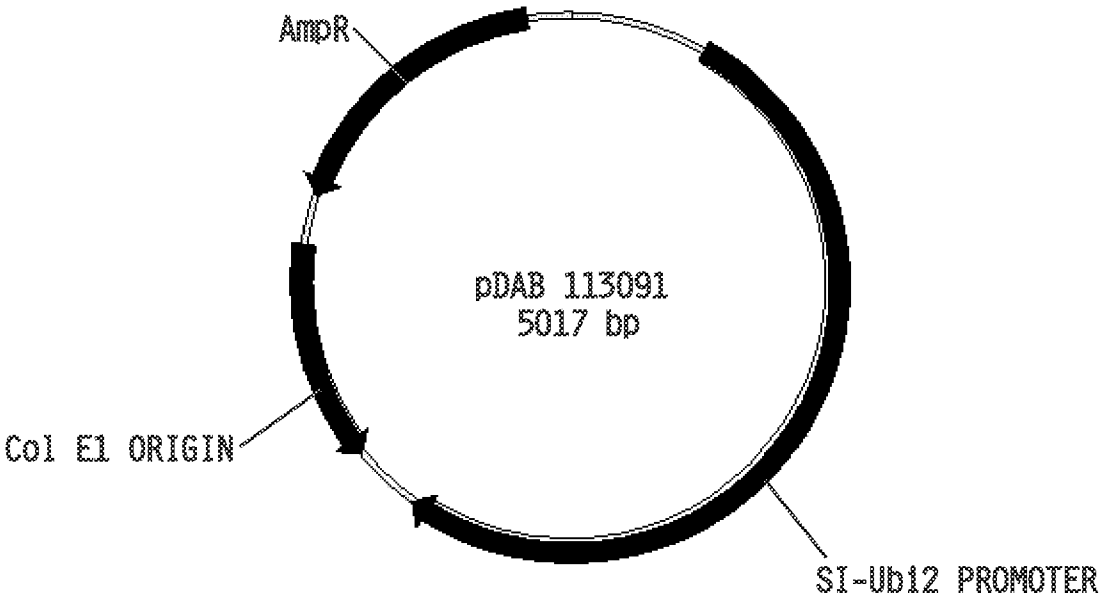
AmpR
pDAB 113091
5017 bp
Col E1 ORIGIN
SI-Ub12 PROMOTER

FIG. 3

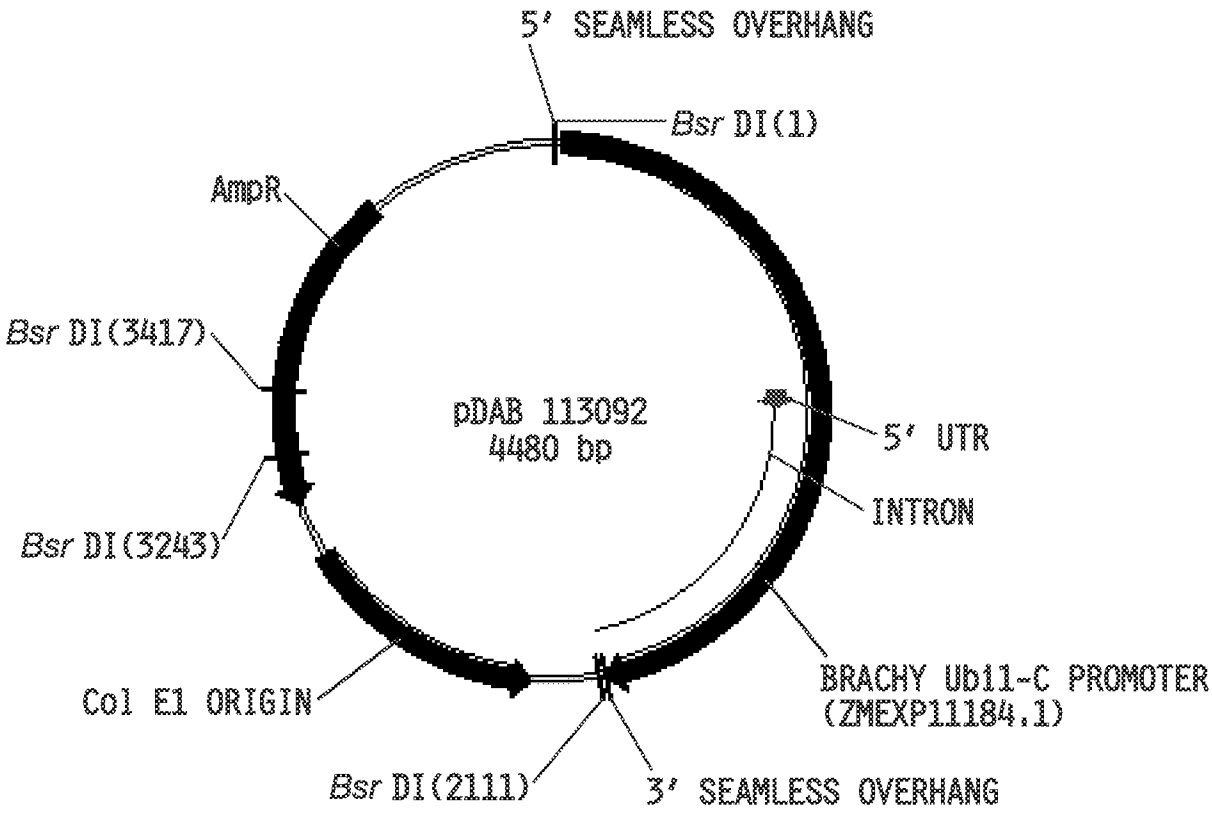
5′ SEAMLESS OVERHANG
Bsr DI(1)
AmpR
Bsr DI(3417)
pDAB 113092
4480 bp
5′ UTR
INTRON
Bsr DI(3243)
BRACHY Ub11-C PROMOTER
(ZMEXP11184.1)
Col E1 ORIGIN
Bsr DI(2111)
3′ SEAMLESS OVERHANG

FIG. 4

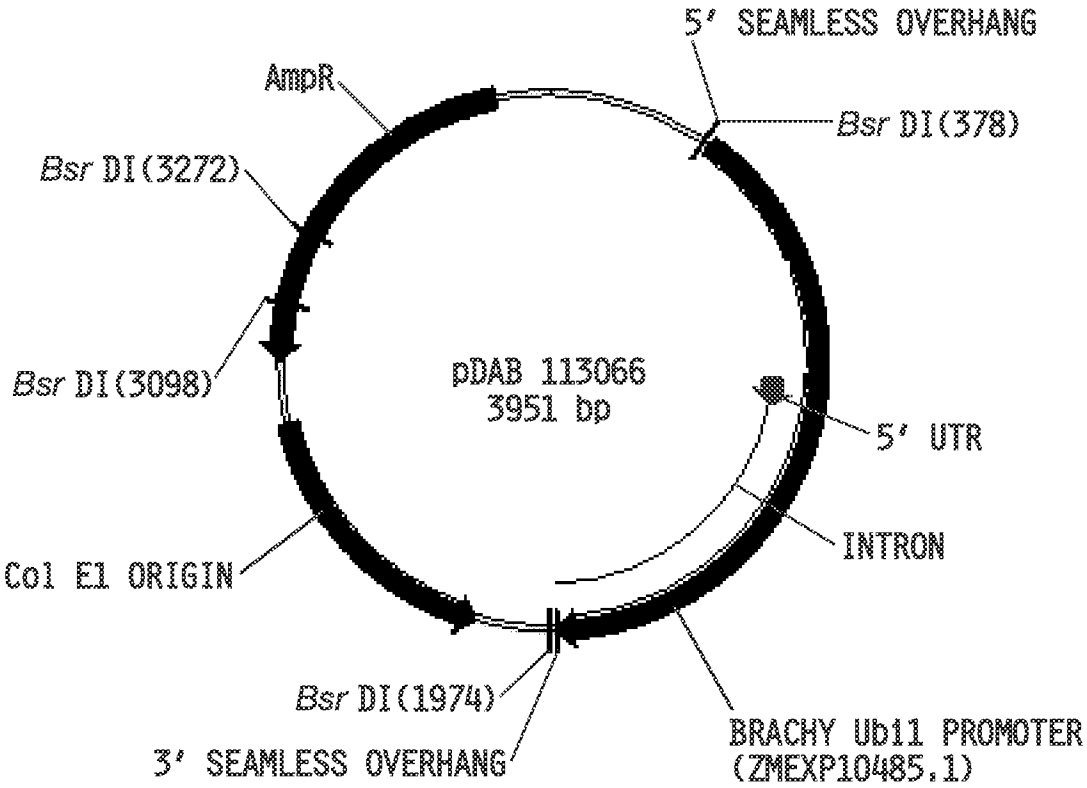
5′ SEAMLESS OVERHANG
AmpR
Bsr DI(378)
Bsr DI(3272)
pDAB 113066
3951 bp
Bsr DI(3098)
5′ UTR
INTRON
Col E1 ORIGIN
Bsr DI(1974)
BRACHY Ub11 PROMOTER
(ZMEXP10485.1)
3′ SEAMLESS OVERHANG

FIG. 5

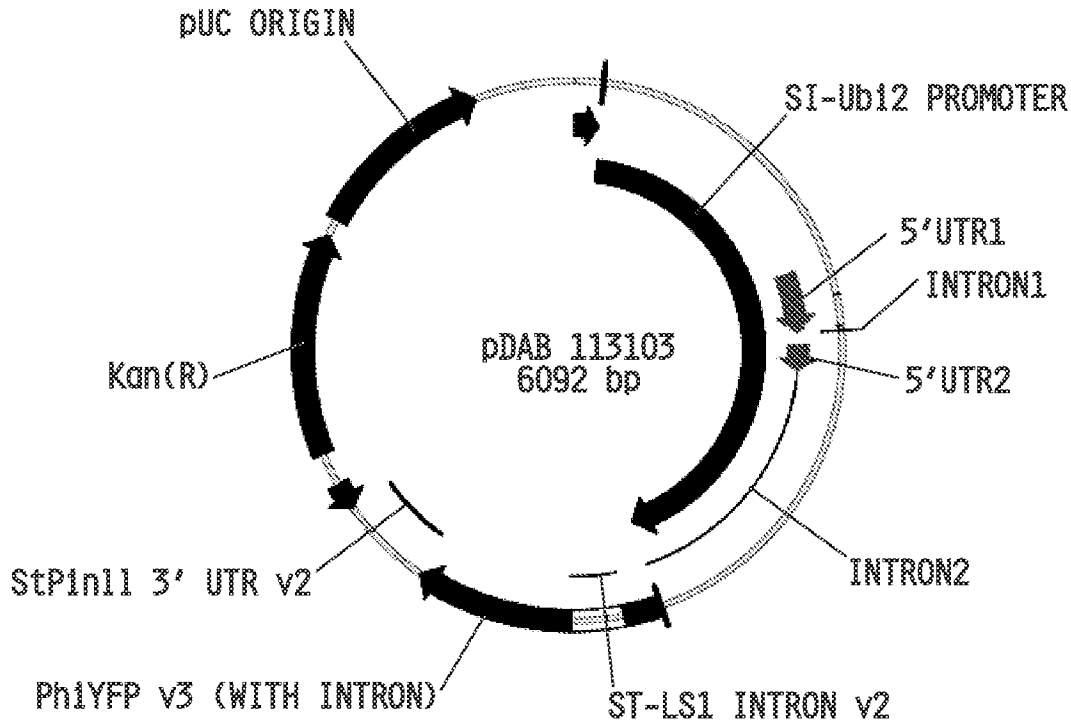
pUC ORIGIN
SI-Ub12 PROMOTER
5′UTR1
INTRON1
pDAB 113103
6092 bp
Kan(R)
5′UTR2
INTRON2
StPinII 3′ UTR v2
PhiYFP v3 (WITH INTRON)
ST-LS1 INTRON v2

FIG. 6

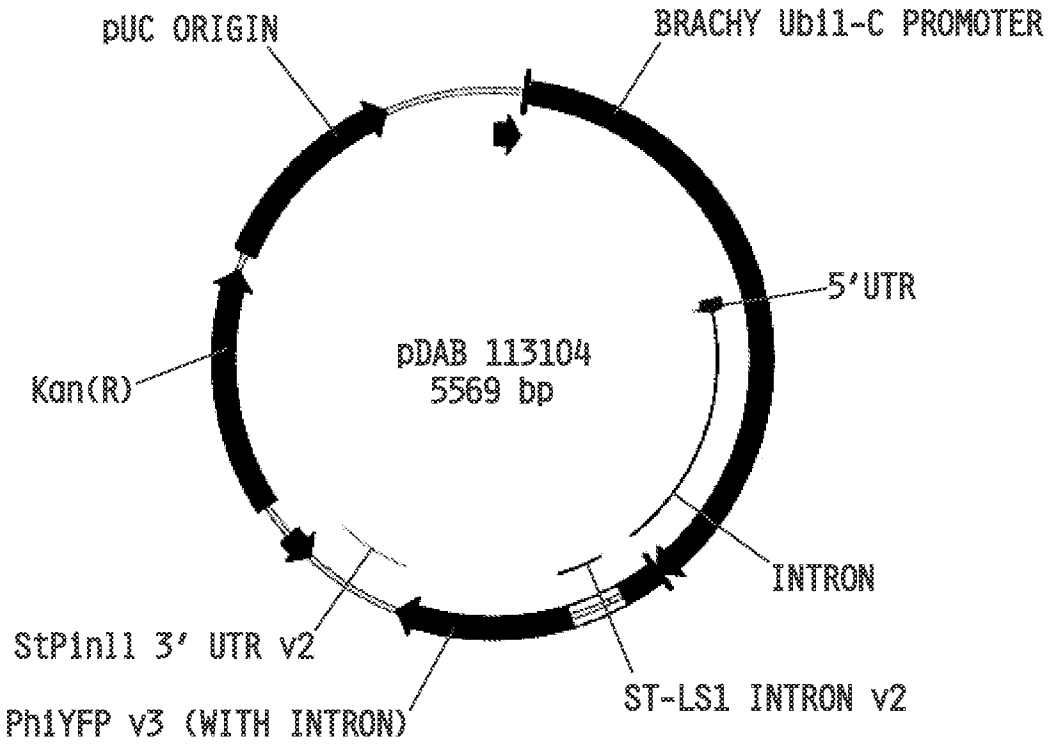
pUC ORIGIN
BRACHY Ub11-C PROMOTER
5'UTR
pDAB 113104
5569 bp
Kan(R)
INTRON
StPinI1 3' UTR v2
ST-LS1 INTRON v2
PhlYFP v3 (WITH INTRON)

FIG. 7

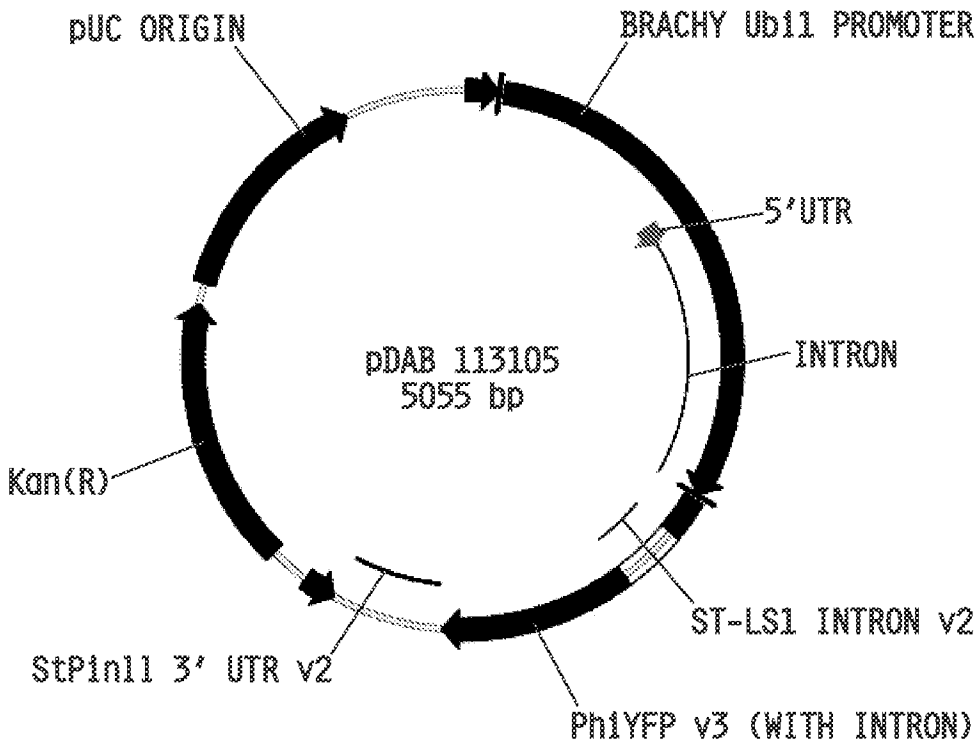
pUC ORIGIN
BRACHY Ub11 PROMOTER
5'UTR
pDAB 113105
5055 bp
INTRON
Kan(R)
ST-LS1 INTRON v2
StPinI1 3' UTR v2
PhlYFP v3 (WITH INTRON)

FIG. 8

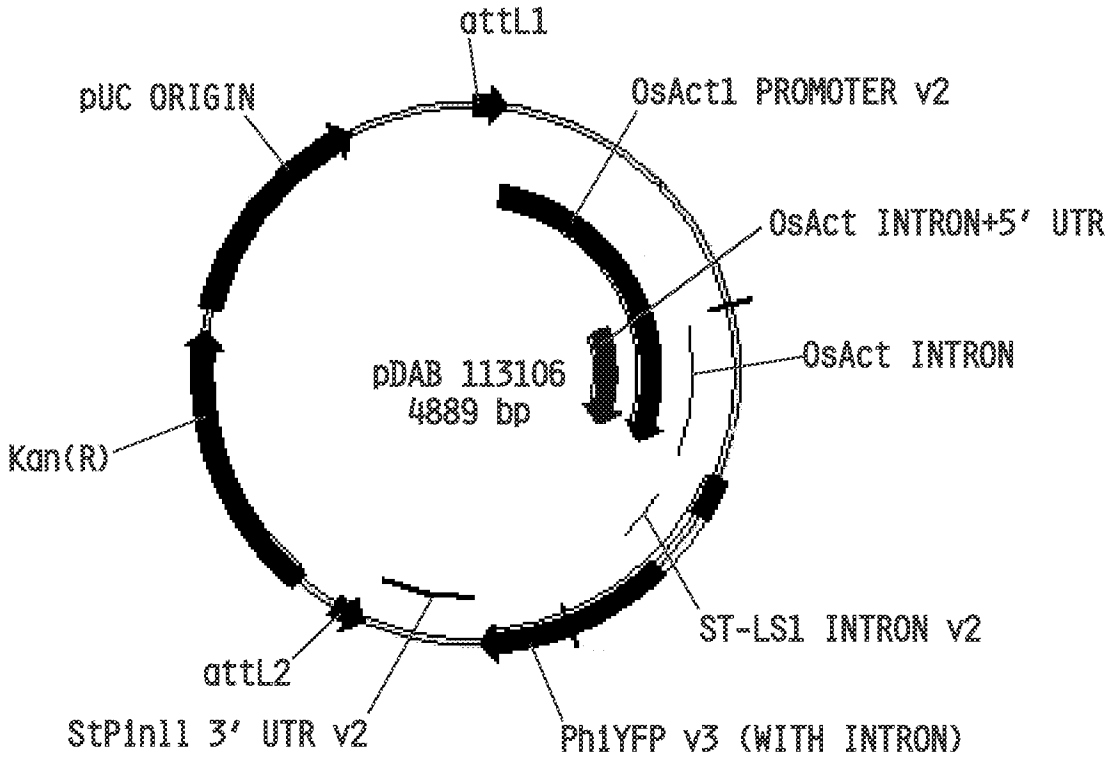
attL1
pUC ORIGIN
OsAct1 PROMOTER v2
OsAct INTRON+5' UTR
OsAct INTRON
pDAB 113106
4889 bp
Kan(R)
ST-LS1 INTRON v2
attL2
StPin11 3' UTR v2
Ph1YFP v3 (WITH INTRON)

FIG. 9

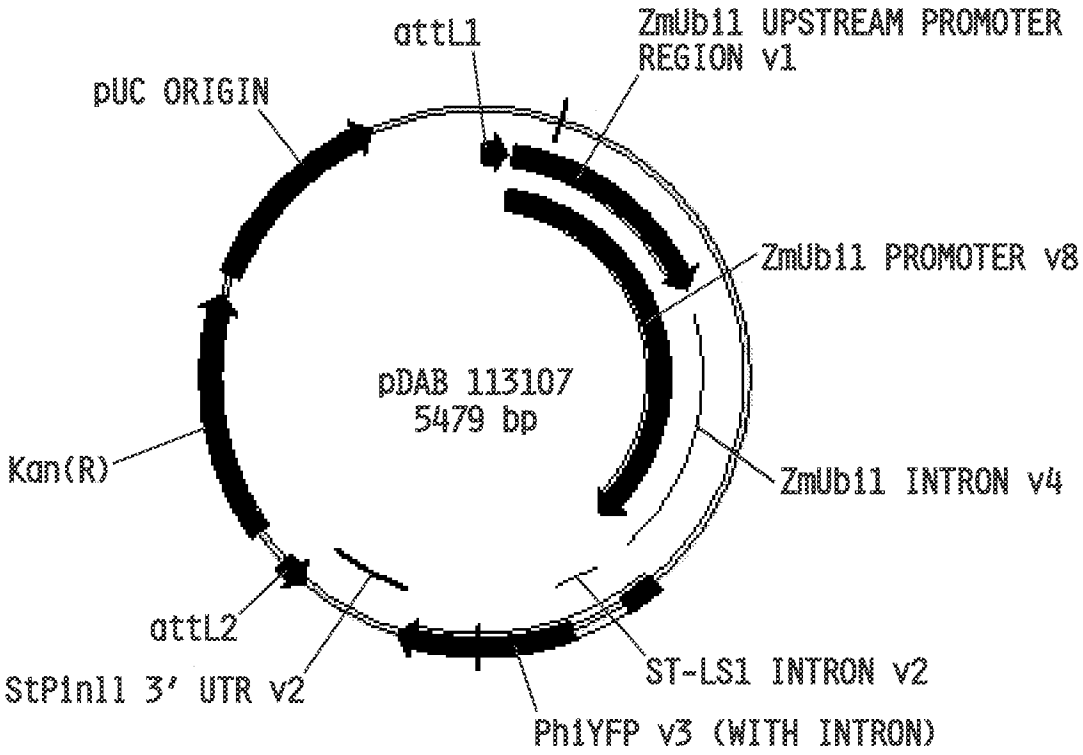
attL1
ZmUb11 UPSTREAM PROMOTER REGION v1
pUC ORIGIN
ZmUb11 PROMOTER v8
pDAB 113107
5479 bp
Kan(R)
ZmUb11 INTRON v4
attL2
ST-LS1 INTRON v2
StPin11 3' UTR v2
Ph1YFP v3 (WITH INTRON)

FIG. 10

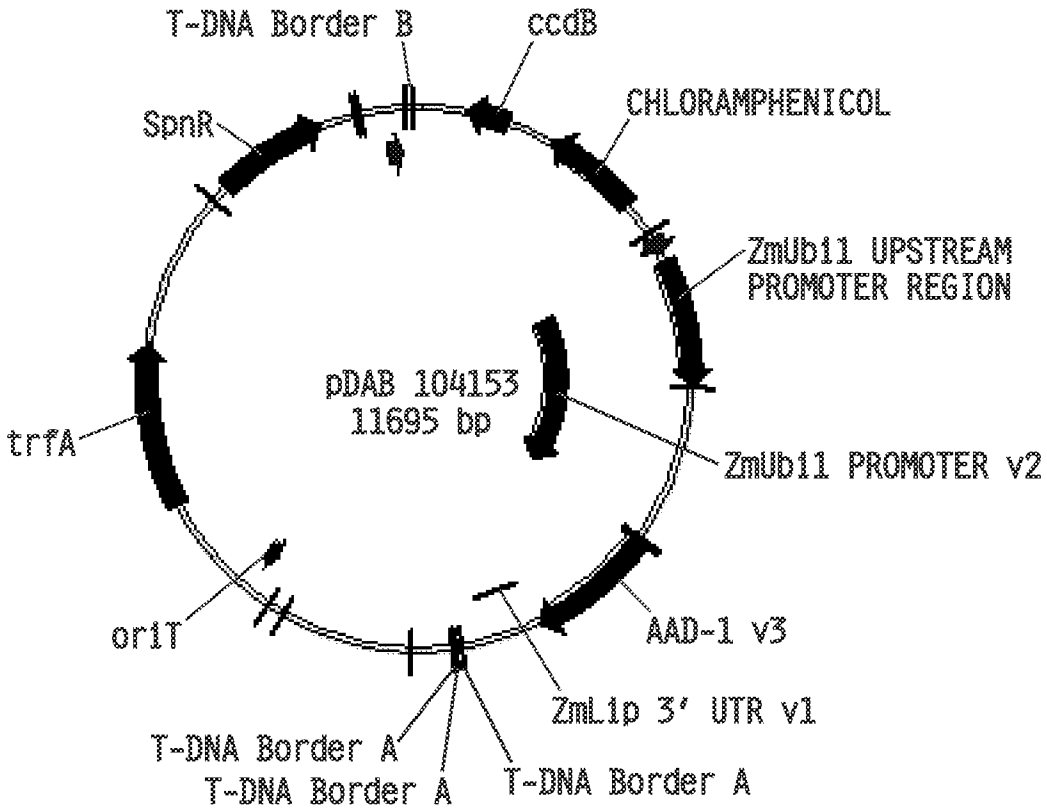
T-DNA Border B
ccdB
CHLORAMPHENICOL
SpnR
ZmUb11 UPSTREAM PROMOTER REGION
pDAB 104153
11695 bp
ZmUb11 PROMOTER v2
trfA
AAD-1 v3
oriT
ZmL1p 3' UTR v1
T-DNA Border A
T-DNA Border A
T-DNA Border A

FIG. 11

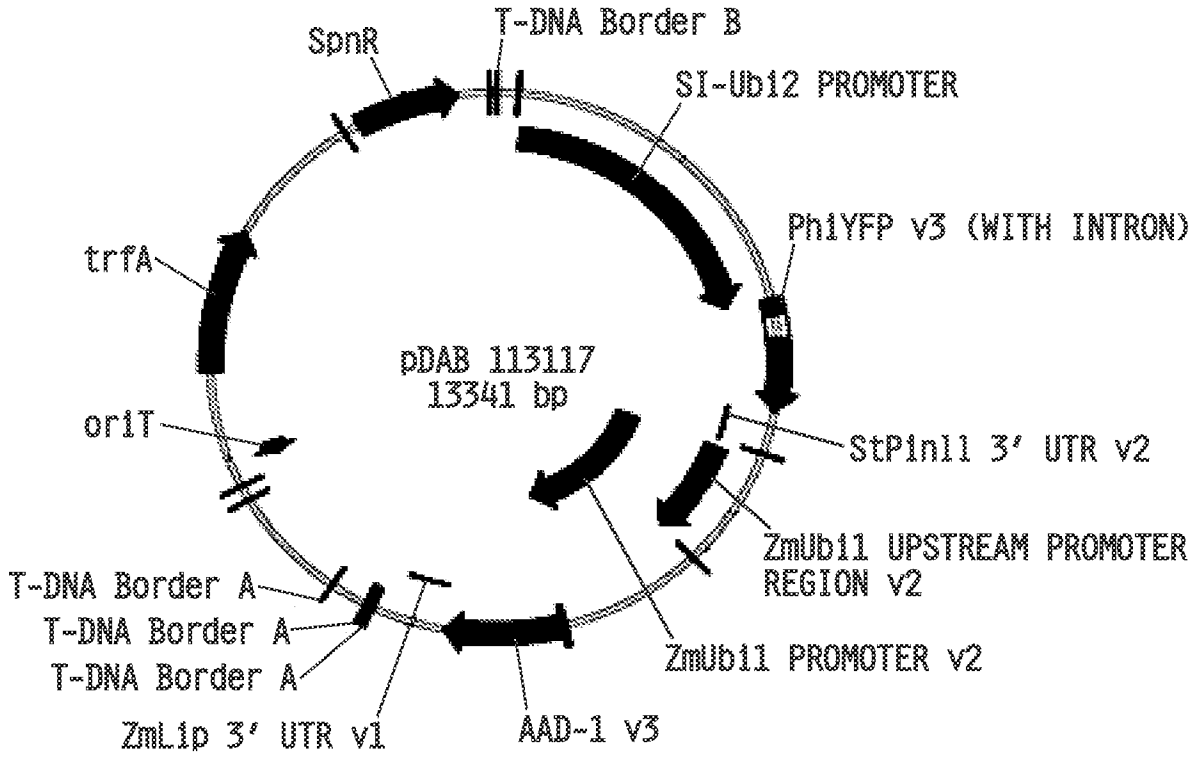
SpnR
T-DNA Border B
SI-Ub12 PROMOTER
PhiYFP v3 (WITH INTRON)
trfA
pDAB 113117
13341 bp
oriT
StPin11 3' UTR v2
ZmUb11 UPSTREAM PROMOTER REGION v2
T-DNA Border A
T-DNA Border A
T-DNA Border A
ZmUb11 PROMOTER v2
ZmL1p 3' UTR v1
AAD-1 v3

FIG. 12

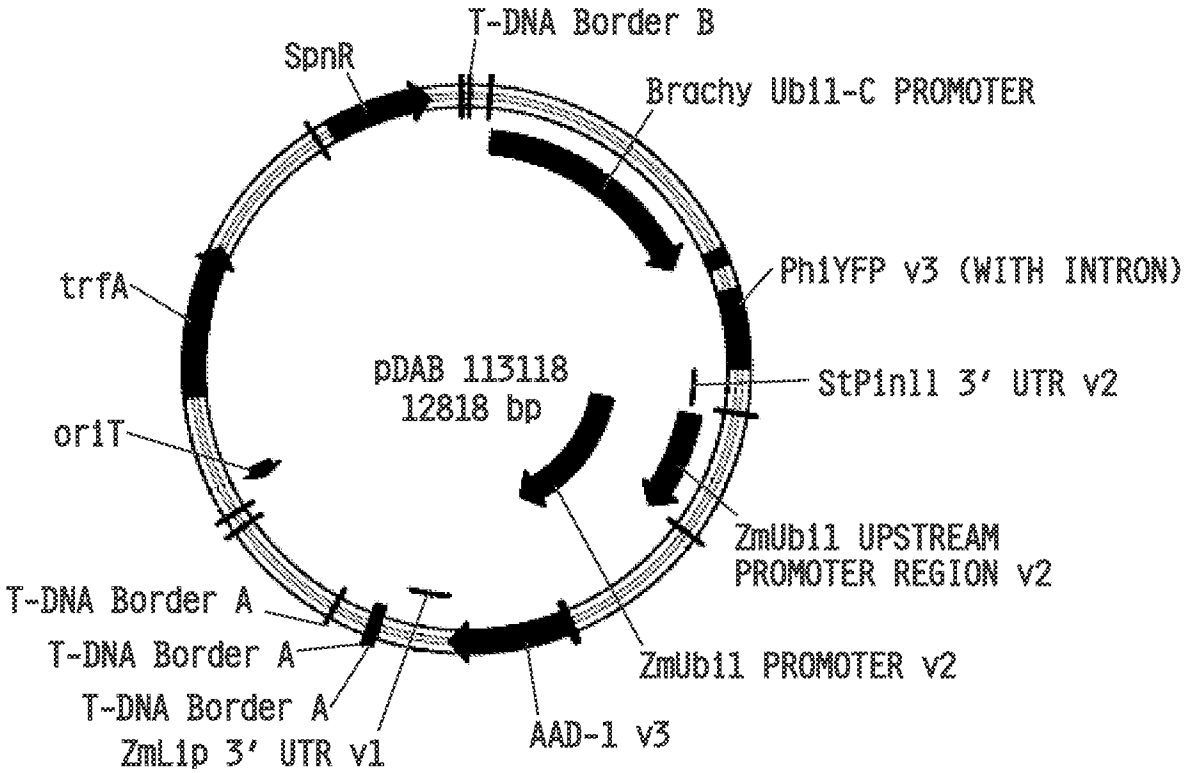
T-DNA Border B
SpnR
Brachy Ub11-C PROMOTER
PhlYFP v3 (WITH INTRON)
trfA
pDAB 113118
12818 bp
StPinII 3′ UTR v2
oriT
ZmUb11 UPSTREAM
PROMOTER REGION v2
T-DNA Border A
T-DNA Border A
T-DNA Border A
ZmUb11 PROMOTER v2
ZmL1p 3′ UTR v1
AAD-1 v3

FIG. 13

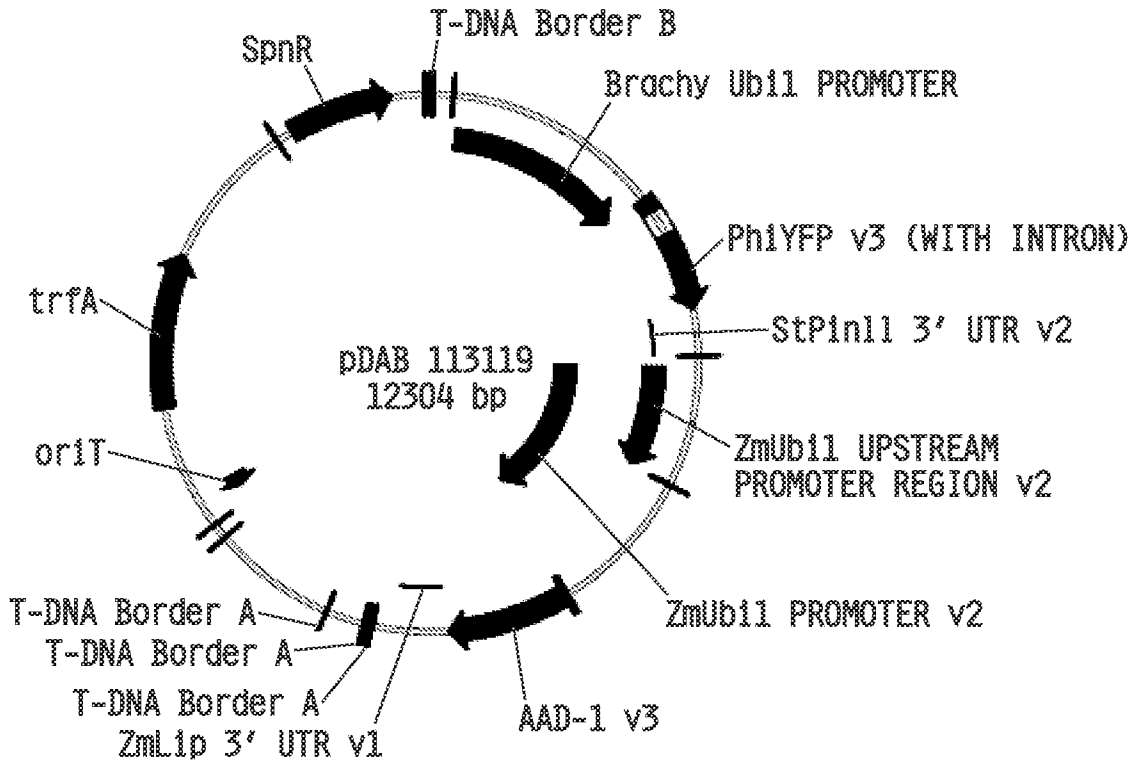
T-DNA Border B
SpnR
Brachy Ub11 PROMOTER
PhlYFP v3 (WITH INTRON)
trfA
pDAB 113119
12304 bp
StPinII 3′ UTR v2
oriT
ZmUb11 UPSTREAM
PROMOTER REGION v2
T-DNA Border A
T-DNA Border A
T-DNA Border A
ZmUb11 PROMOTER v2
ZmL1p 3′ UTR v1
AAD-1 v3

FIG. 14

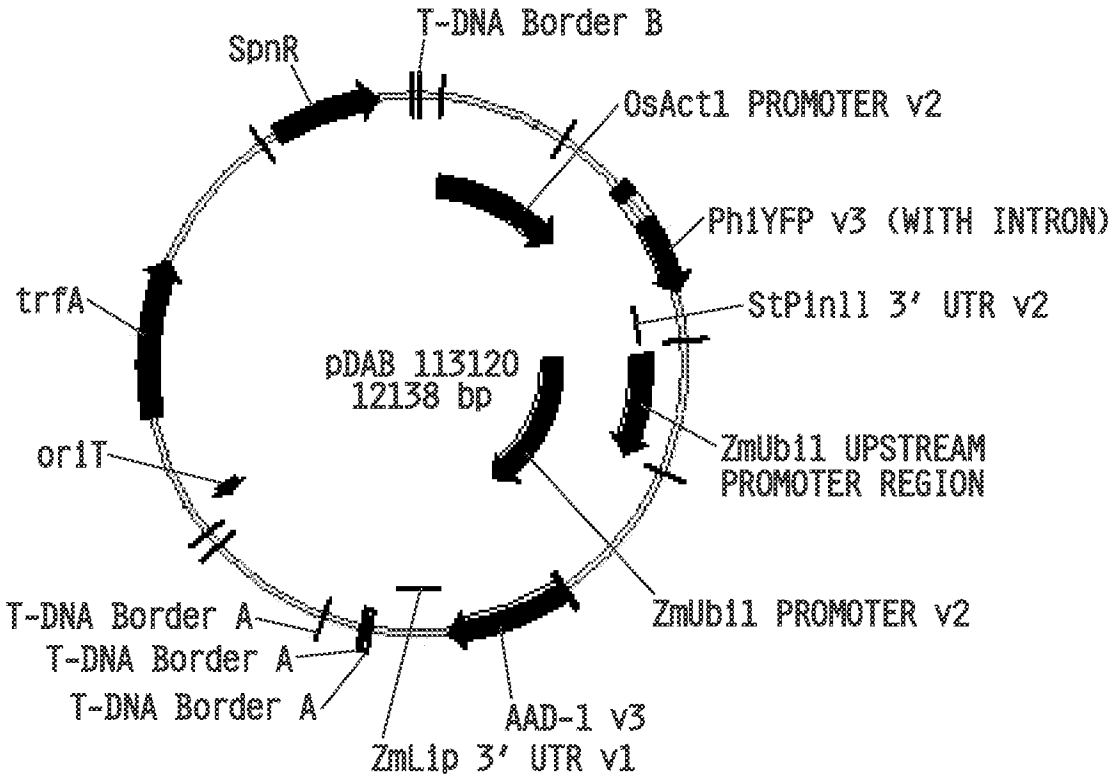
T-DNA Border B
SpnR
OsAct1 PROMOTER v2
Ph1YFP v3 (WITH INTRON)
StPinII 3' UTR v2
trfA
pDAB 113120
12138 bp
ori1T
ZmUb11 UPSTREAM
PROMOTER REGION
ZmUb11 PROMOTER v2
T-DNA Border A
T-DNA Border A
T-DNA Border A
AAD-1 v3
ZmLip 3' UTR v1

FIG. 15

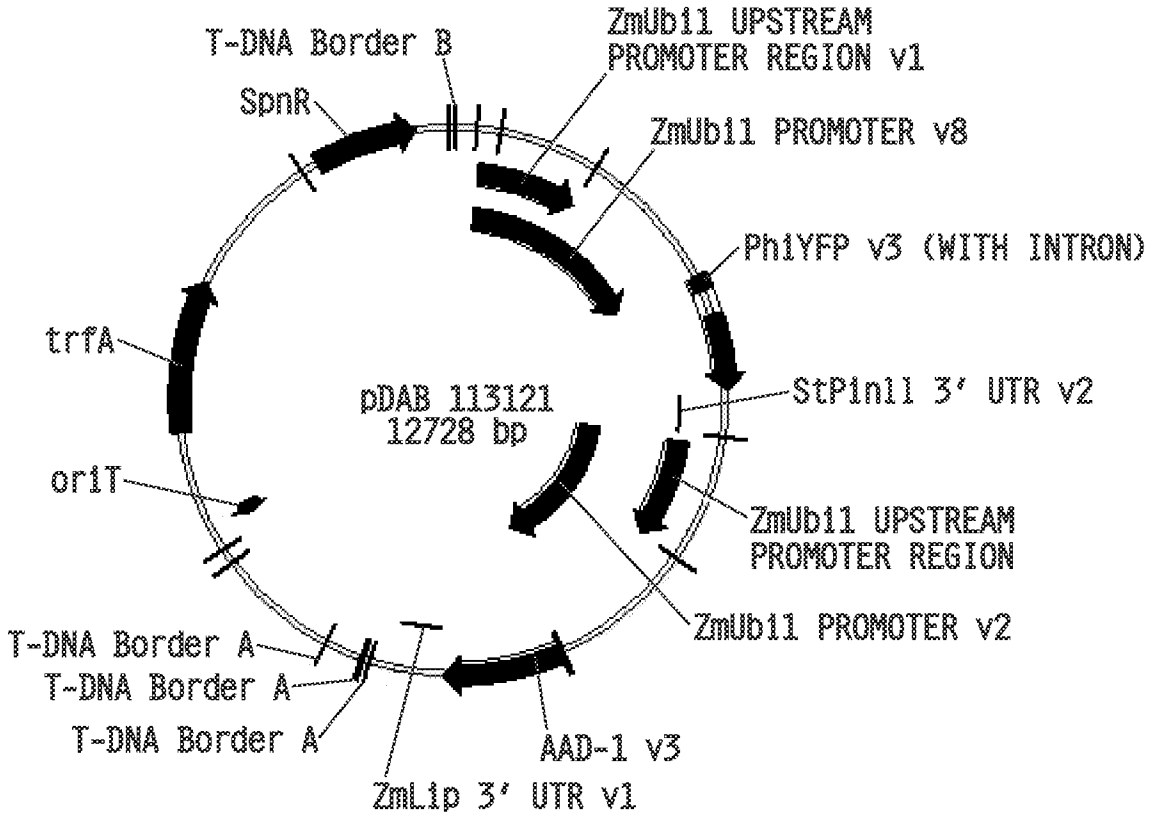
T-DNA Border B
ZmUb11 UPSTREAM
PROMOTER REGION v1
SpnR
ZmUb11 PROMOTER v8
Ph1YFP v3 (WITH INTRON)
trfA
pDAB 113121
12728 bp
StPinII 3' UTR v2
ori1T
ZmUb11 UPSTREAM
PROMOTER REGION
ZmUb11 PROMOTER v2
T-DNA Border A
T-DNA Border A
T-DNA Border A
AAD-1 v3
ZmLip 3' UTR v1

FIG. 16

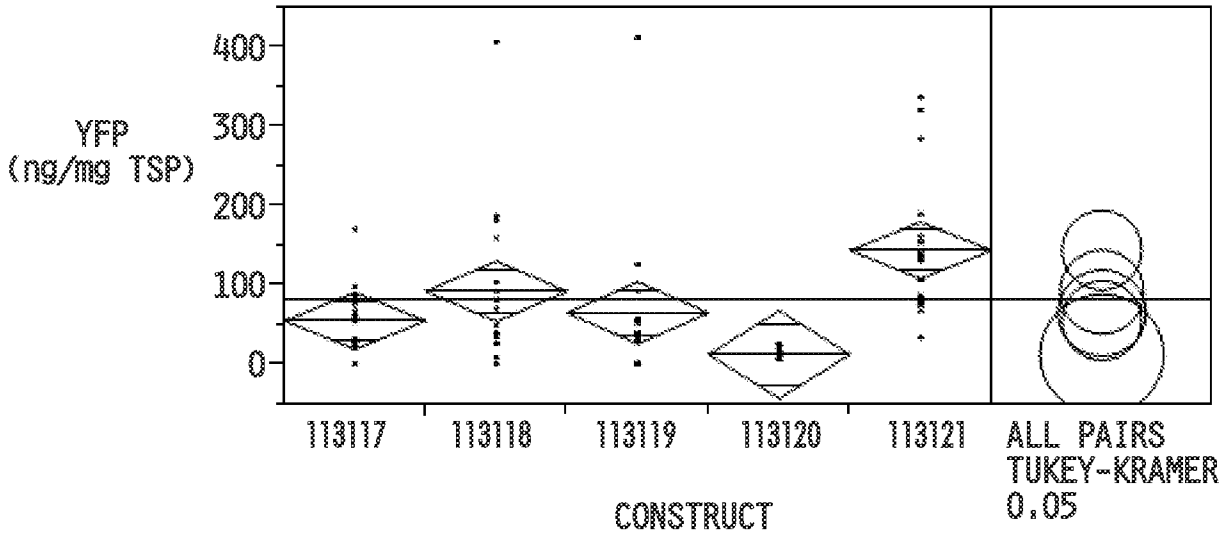
YFP
(ng/mg TSP)
400
300
200
100
0
113117
113118
113119
113120
113121
ALL PAIRS
TUKEY-KRAMER
0.05
CONSTRUCT

FIG. 17

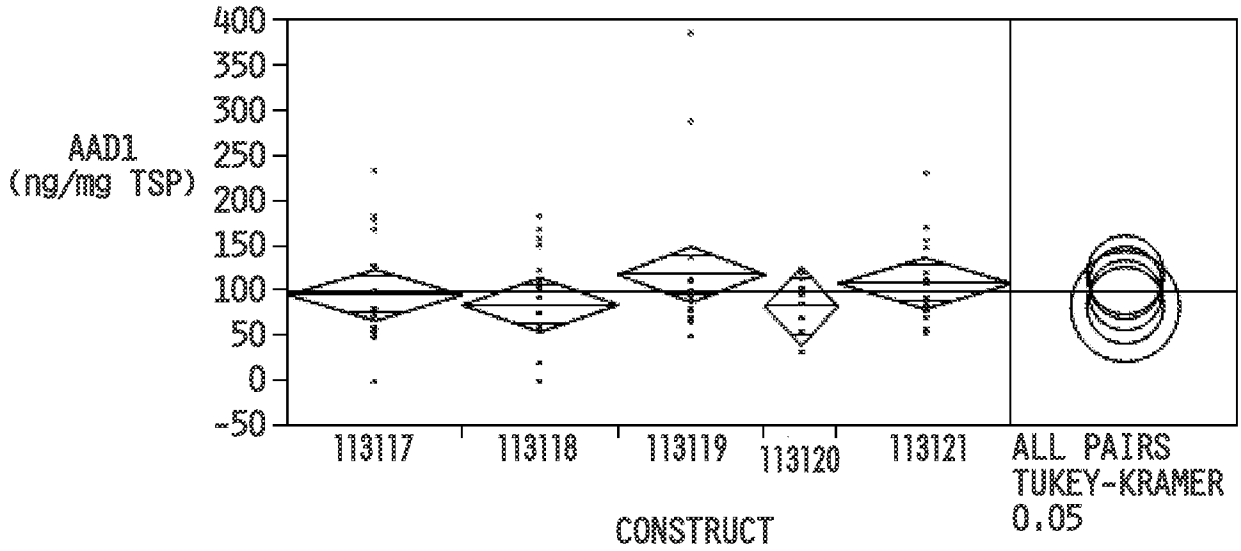
AAD1
(ng/mg TSP)
400
350
300
250
200
150
100
50
0
-50
113117
113118
113119
113120
113121
ALL PAIRS
TUKEY-KRAMER
0.05
CONSTRUCT

FIG. 18

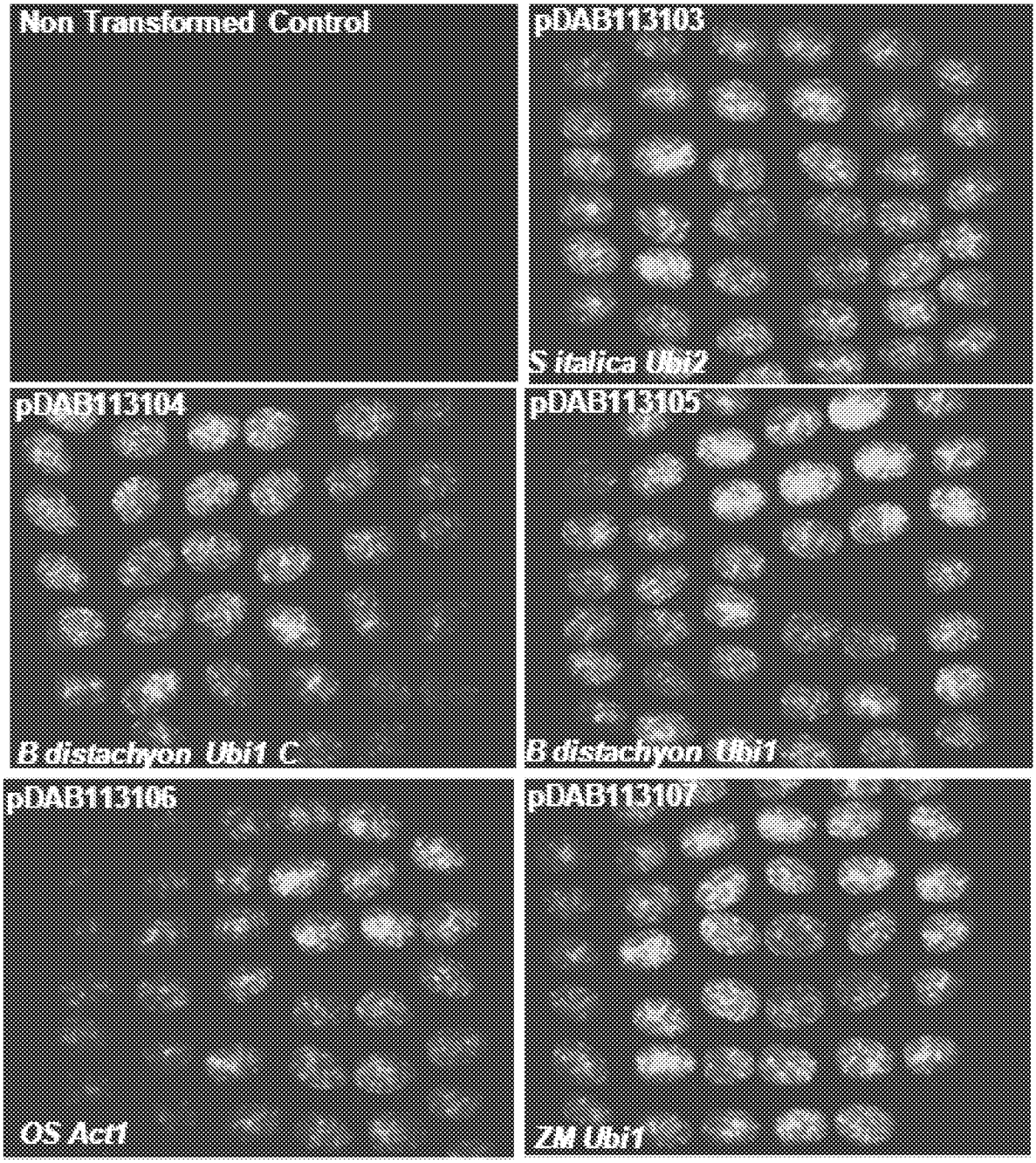
Non Transformed Control
pDAB113103
S italica Ubi2
pDAB113104
B distachyon Ubi1 C
pDAB113105
B distachyon Ubi1
pDAB113106
OS Act1
pDAB113107
ZM Ubi1

FIG. 19

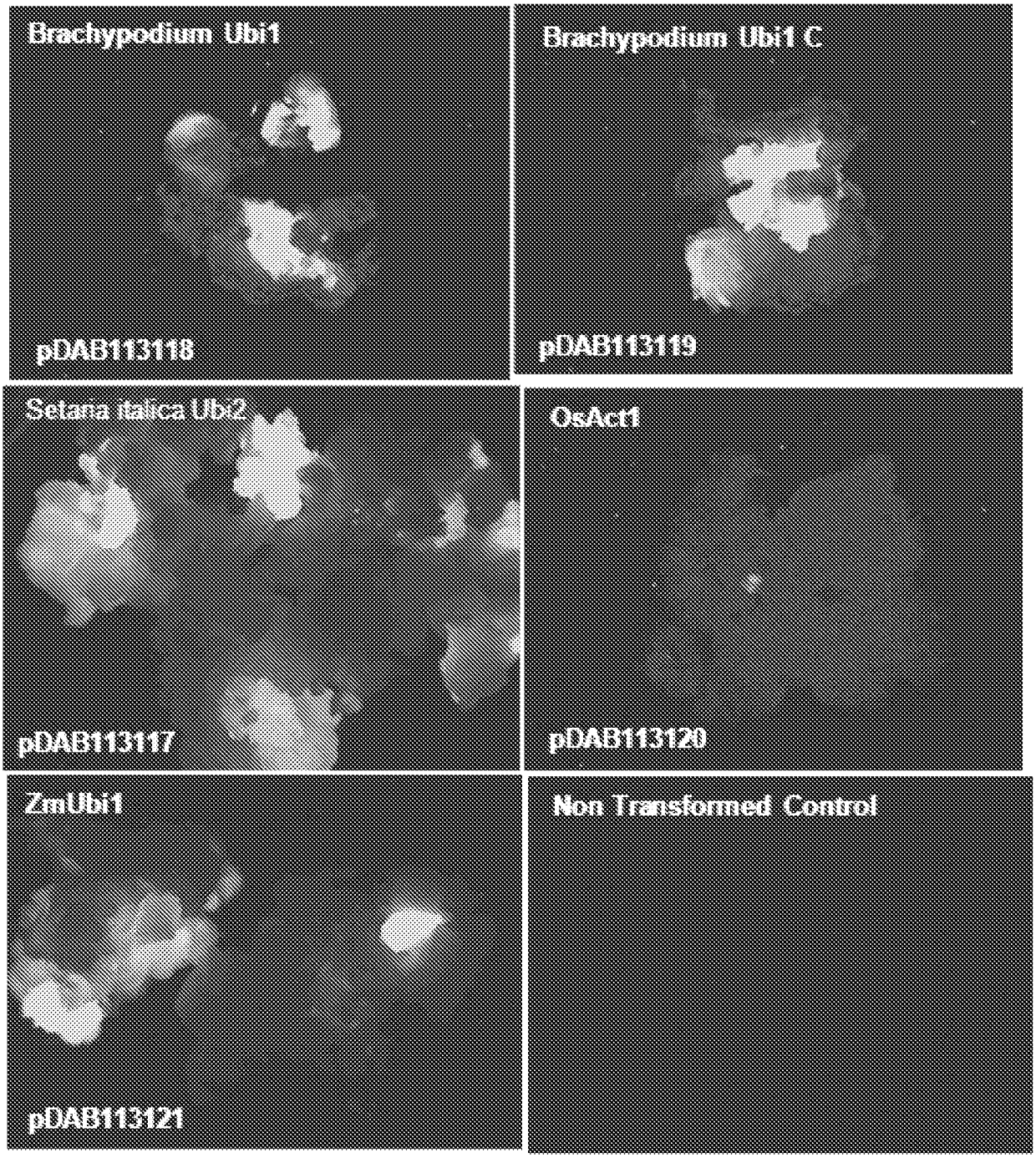
Brachypodium Ubi1
pDAB113118
Brachypodium Ubi1 C
pDAB113119
Setaria italica Ubi2
pDAB113117
OsAct1
pDAB113120
ZmUbi1
pDAB113121
Non Transformed Control

FIG. 20

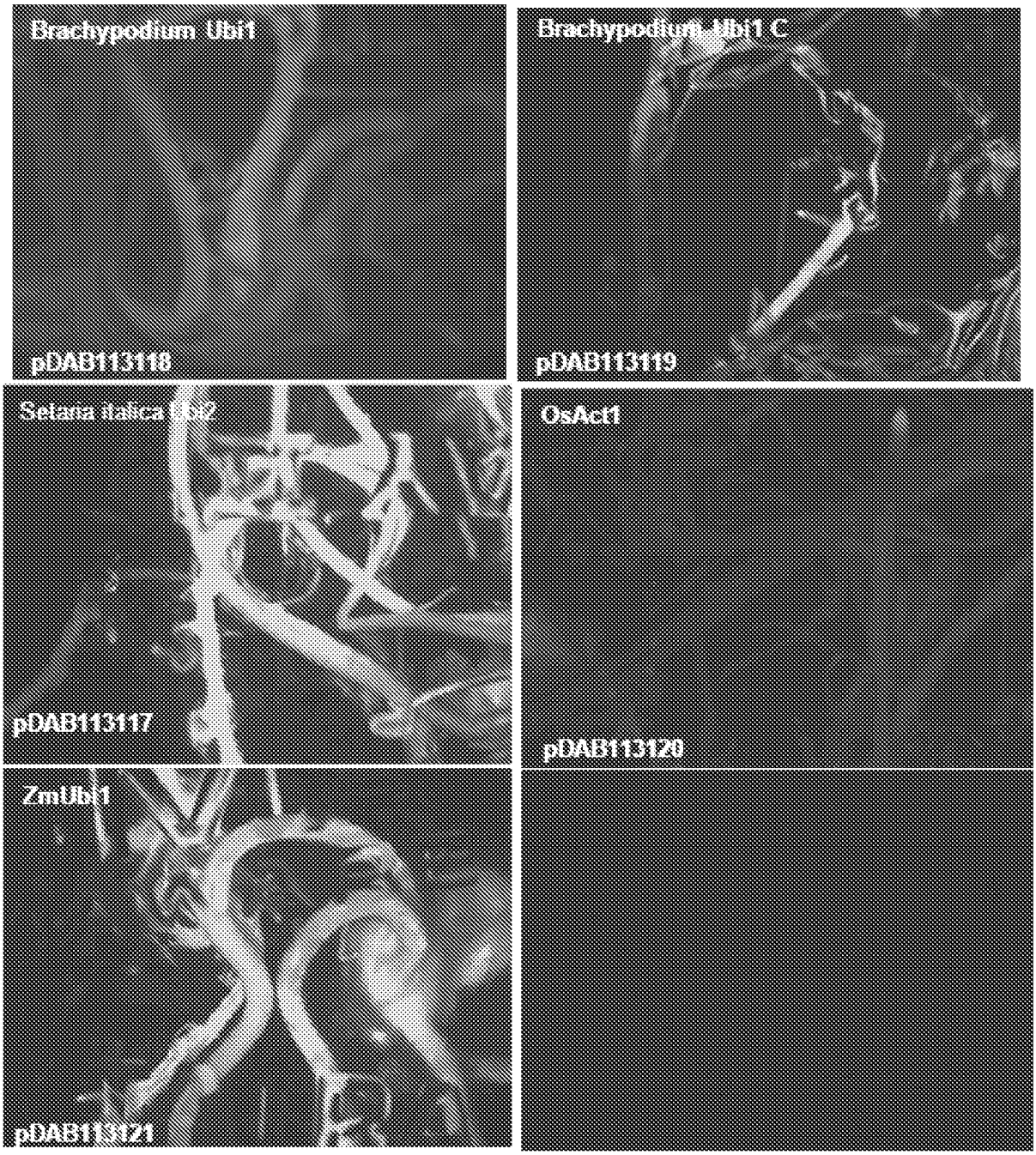
Brachypodium Ubi1
pDAB113118
Brachypodium Ubi1 C
pDAB113119
Setaria italica Ubi2
pDAB113117
OsAct1
pDAB113120
ZmUbi1
pDAB113121

FIG. 21

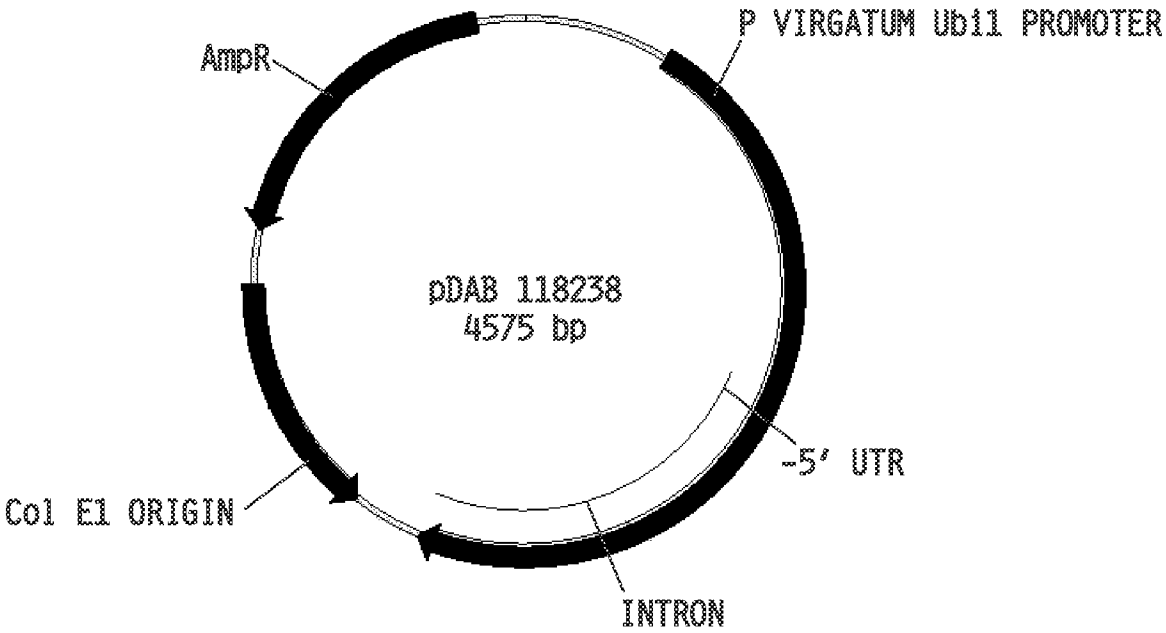
P VIRGATUM Ub11 PROMOTER
AmpR
pDAB 118238
4575 bp
5' UTR
Col E1 ORIGIN
INTRON

FIG. 22

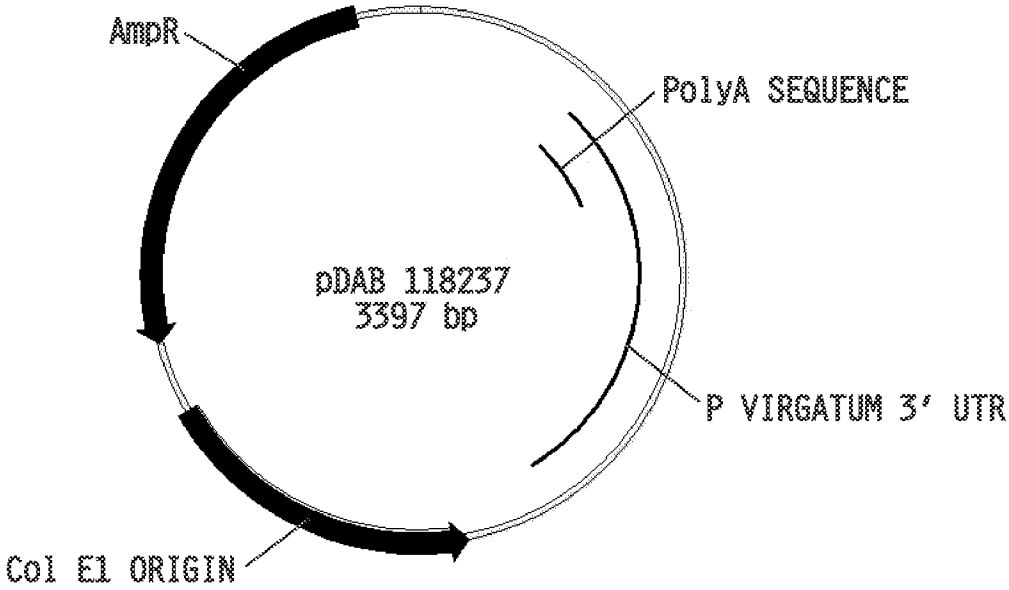
AmpR
PolyA SEQUENCE
pDAB 118237
3397 bp
P VIRGATUM 3' UTR
Col E1 ORIGIN

FIG. 23

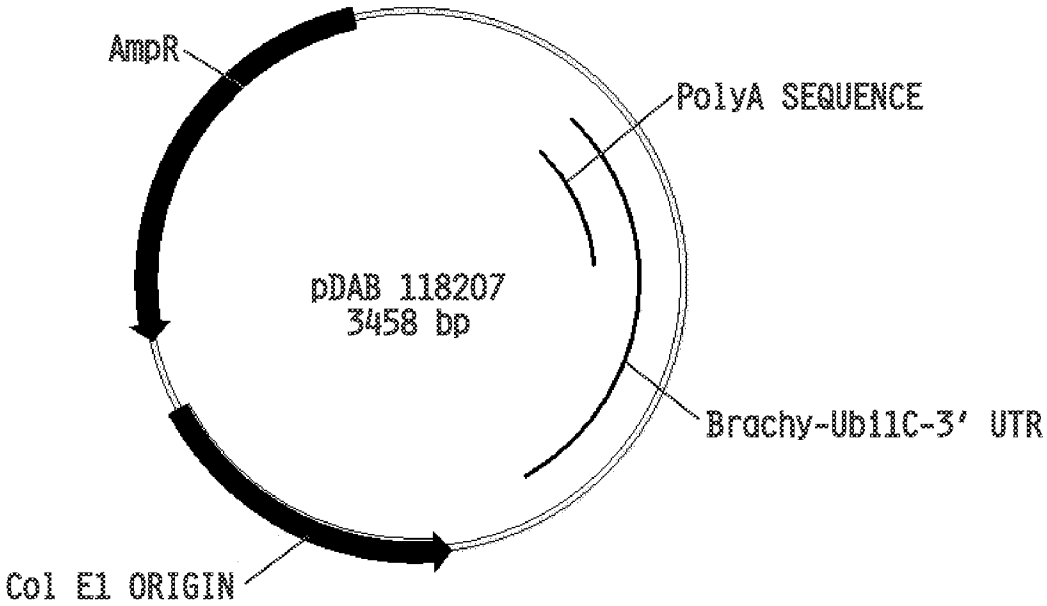
AmpR
PolyA SEQUENCE
pDAB 118207
3458 bp
Brachy-Ub11C-3' UTR
Col E1 ORIGIN

FIG. 24

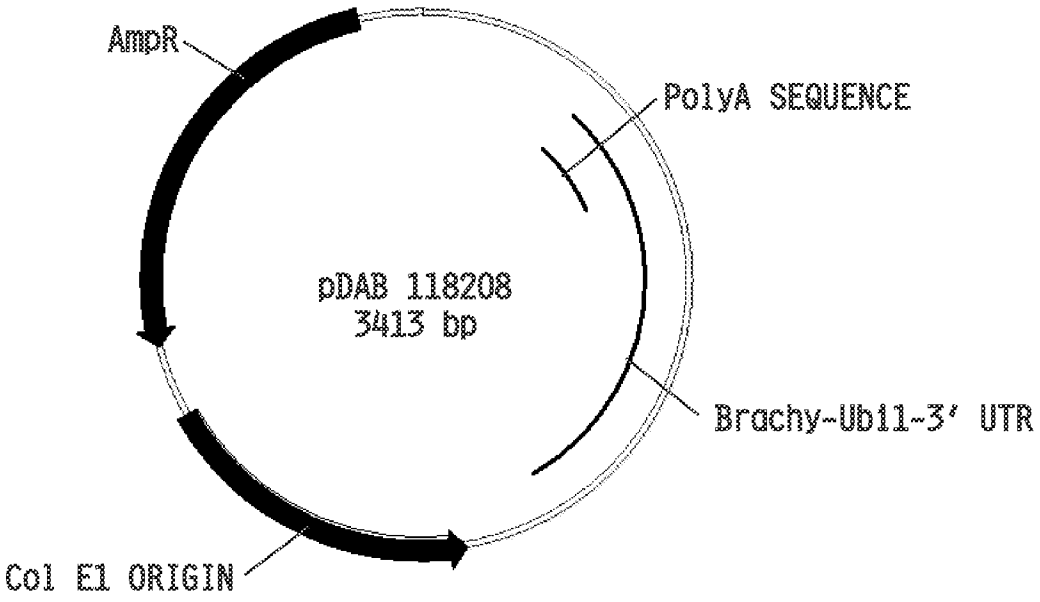
AmpR
PolyA SEQUENCE
pDAB 118208
3413 bp
Brachy-Ub11-3' UTR
Col E1 ORIGIN

FIG. 25

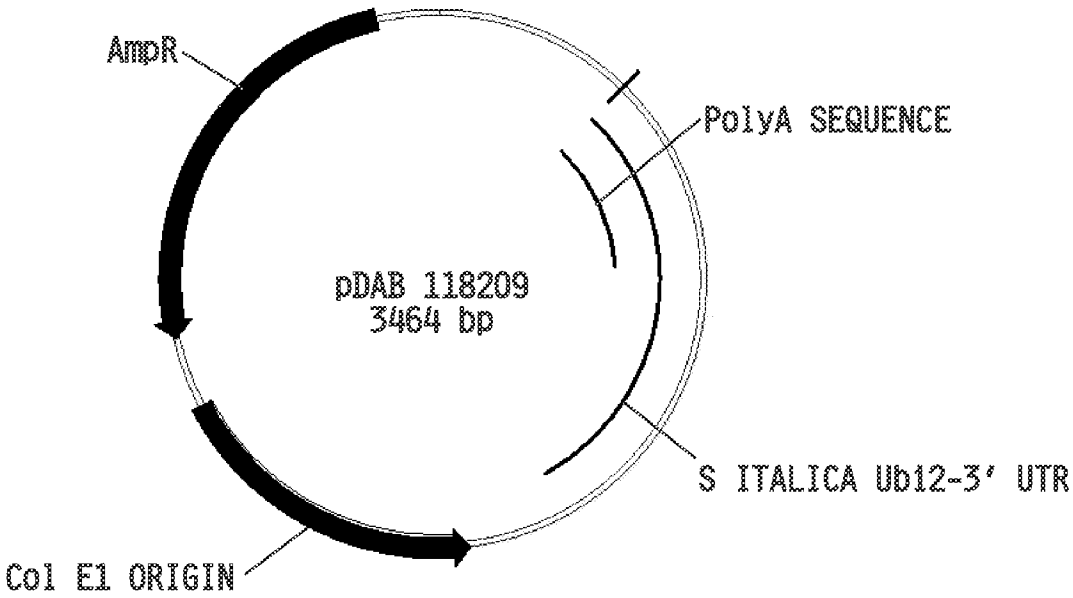
AmpR
PolyA SEQUENCE
pDAB 118209
3464 bp
S ITALICA Ub12-3' UTR
Col E1 ORIGIN

FIG. 26

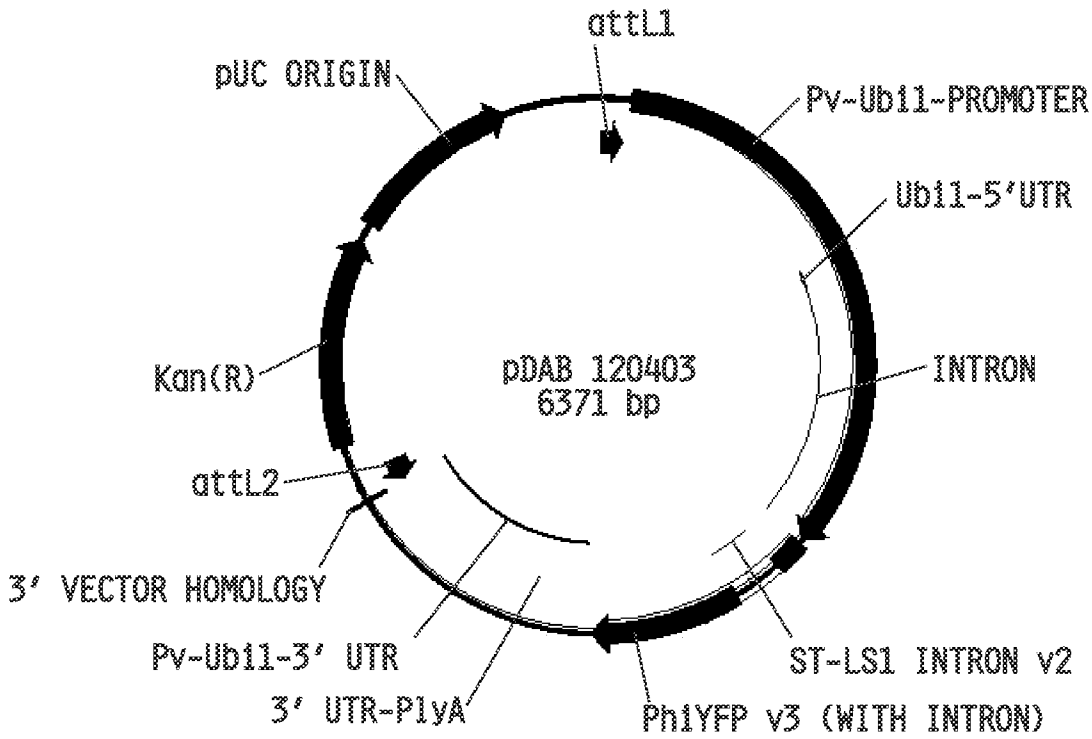
attL1
pUC ORIGIN
Pv-Ub11-PROMOTER
Ub11-5'UTR
Kan(R)
pDAB 120403
6371 bp
INTRON
attL2
3' VECTOR HOMOLOGY
Pv-Ub11-3' UTR
ST-LS1 INTRON v2
3' UTR-PlyA
PhlYFP v3 (WITH INTRON)

FIG. 27

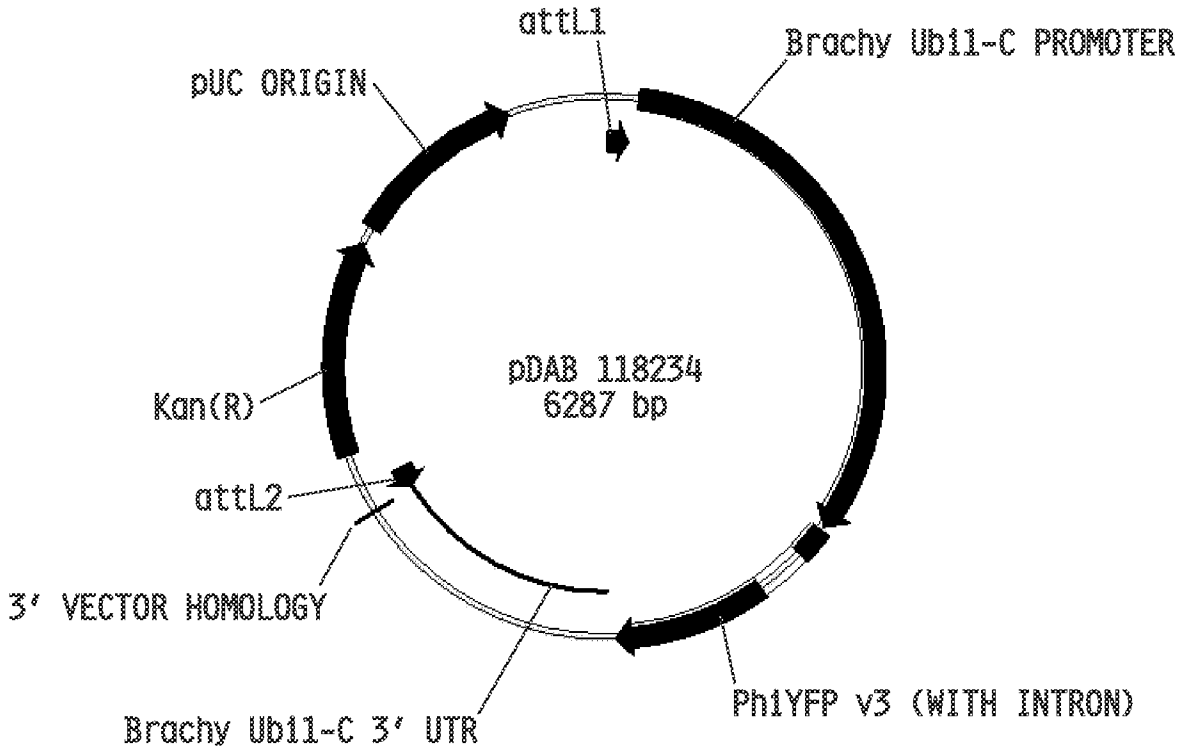
attL1
Brachy Ubi1-C PROMOTER
pUC ORIGIN
pDAB 118234
6287 bp
Kan(R)
attL2
3' VECTOR HOMOLOGY
Brachy Ubi1-C 3' UTR
PhiYFP v3 (WITH INTRON)

FIG. 28

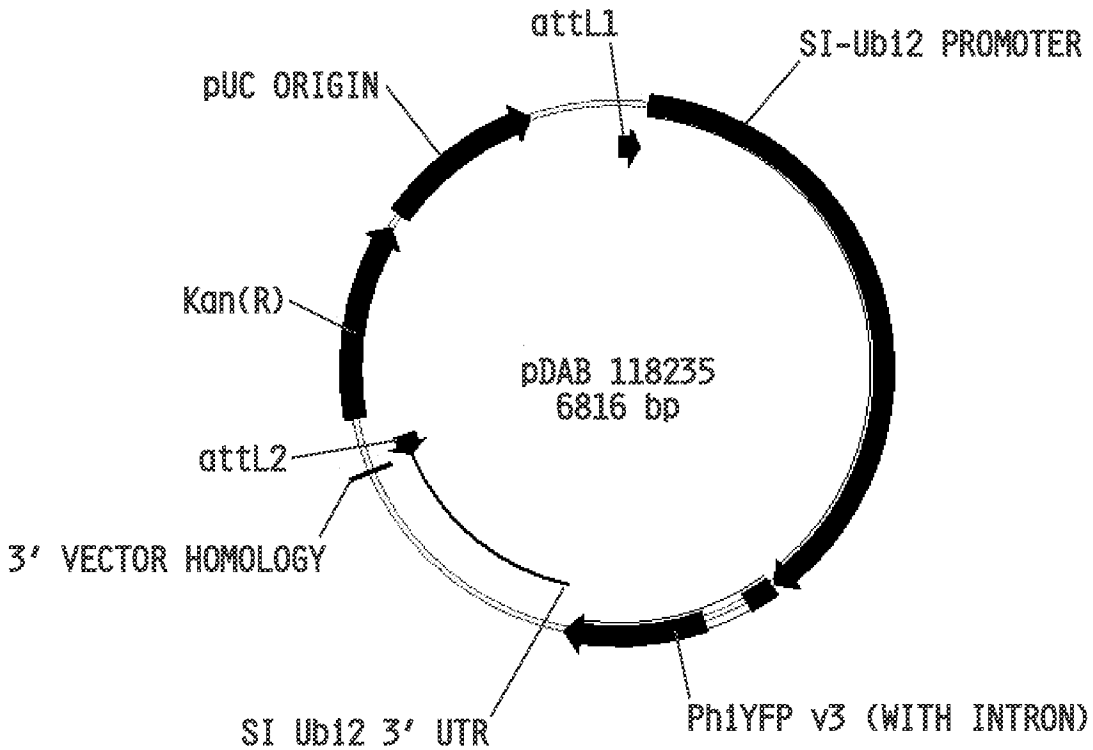
attL1
SI-Ubi2 PROMOTER
pUC ORIGIN
pDAB 118235
6816 bp
Kan(R)
attL2
3' VECTOR HOMOLOGY
SI Ubi2 3' UTR
PhiYFP v3 (WITH INTRON)

FIG. 29

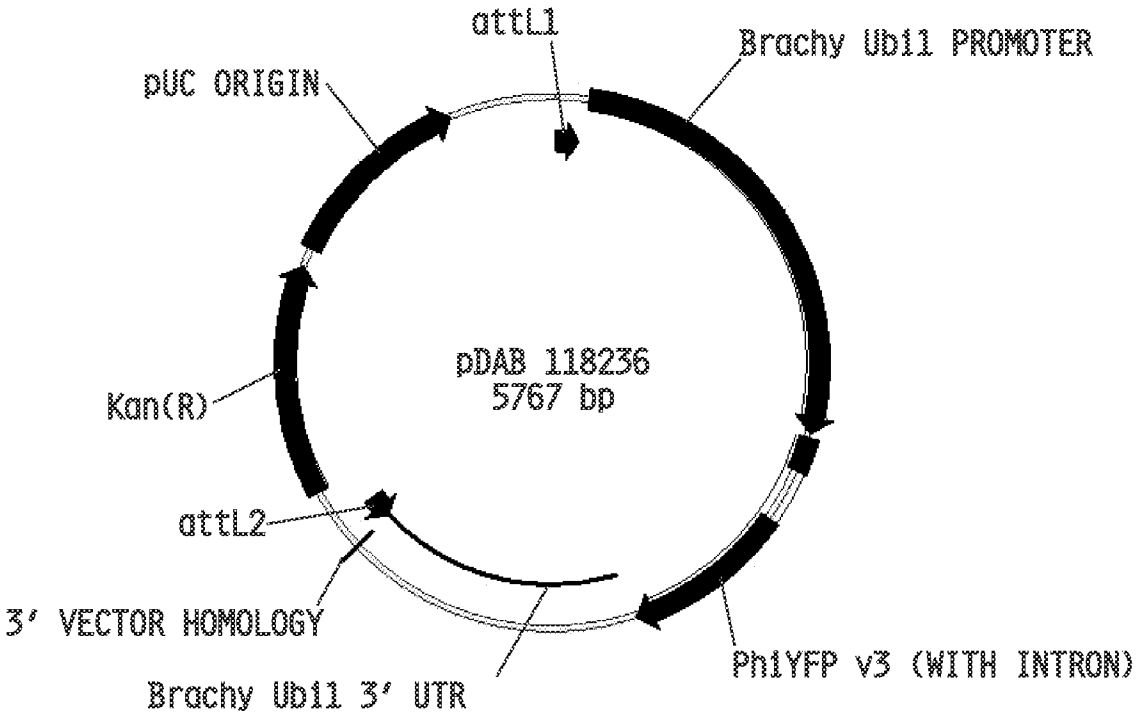
attL1
Brachy Ub11 PROMOTER
pUC ORIGIN
pDAB 118236
5767 bp
Kan(R)
attL2
3' VECTOR HOMOLOGY
Brachy Ub11 3' UTR
Ph1YFP v3 (WITH INTRON)

FIG. 30

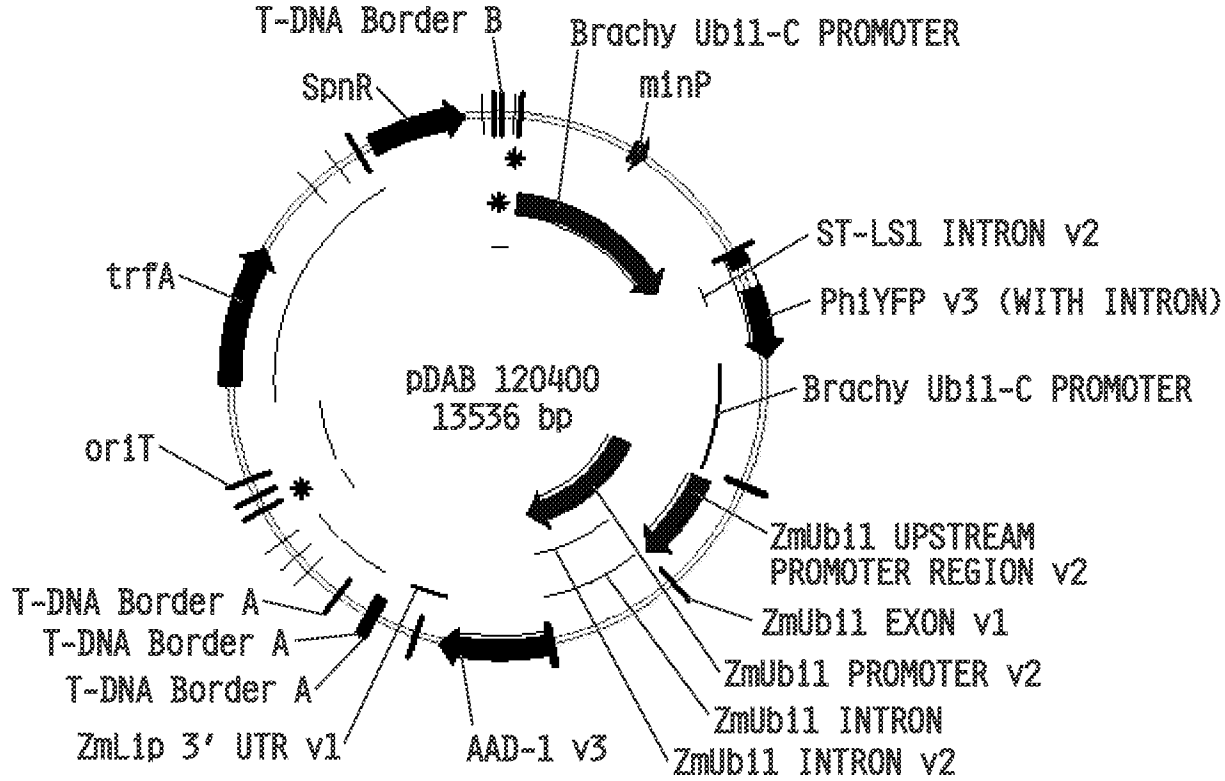
T-DNA Border B
Brachy Ub11-C PROMOTER
SpnR
minP
ST-LS1 INTRON v2
Ph1YFP v3 (WITH INTRON)
trfA
pDAB 120400
13536 bp
Brachy Ub11-C PROMOTER
oriT
ZmUb11 UPSTREAM
PROMOTER REGION v2
T-DNA Border A
T-DNA Border A
T-DNA Border A
ZmUb11 EXON v1
ZmUb11 PROMOTER v2
ZmUb11 INTRON
ZmL1p 3' UTR v1
AAD-1 v3
ZmUb11 INTRON v2

FIG. 31

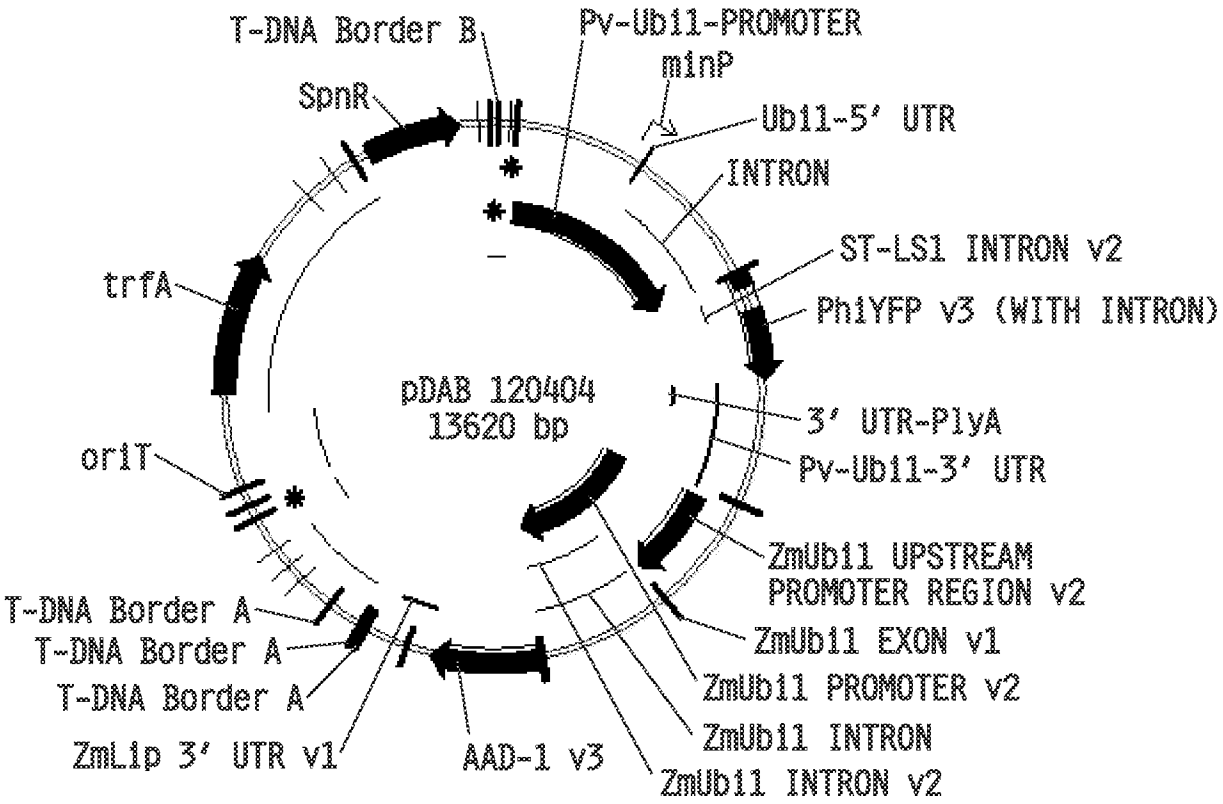
T-DNA Border B
Pv-Ub11-PROMOTER
minP
SpnR
Ub11-5' UTR
INTRON
ST-LS1 INTRON v2
trfA
Ph1YFP v3 (WITH INTRON)
pDAB 120404
13620 bp
3' UTR-P1yA
Pv-Ub11-3' UTR
oriT
ZmUb11 UPSTREAM
PROMOTER REGION v2
T-DNA Border A
ZmUb11 EXON v1
T-DNA Border A
ZmUb11 PROMOTER v2
T-DNA Border A
ZmUb11 INTRON
ZmL1p 3' UTR v1
AAD-1 v3
ZmUb11 INTRON v2

FIG. 32

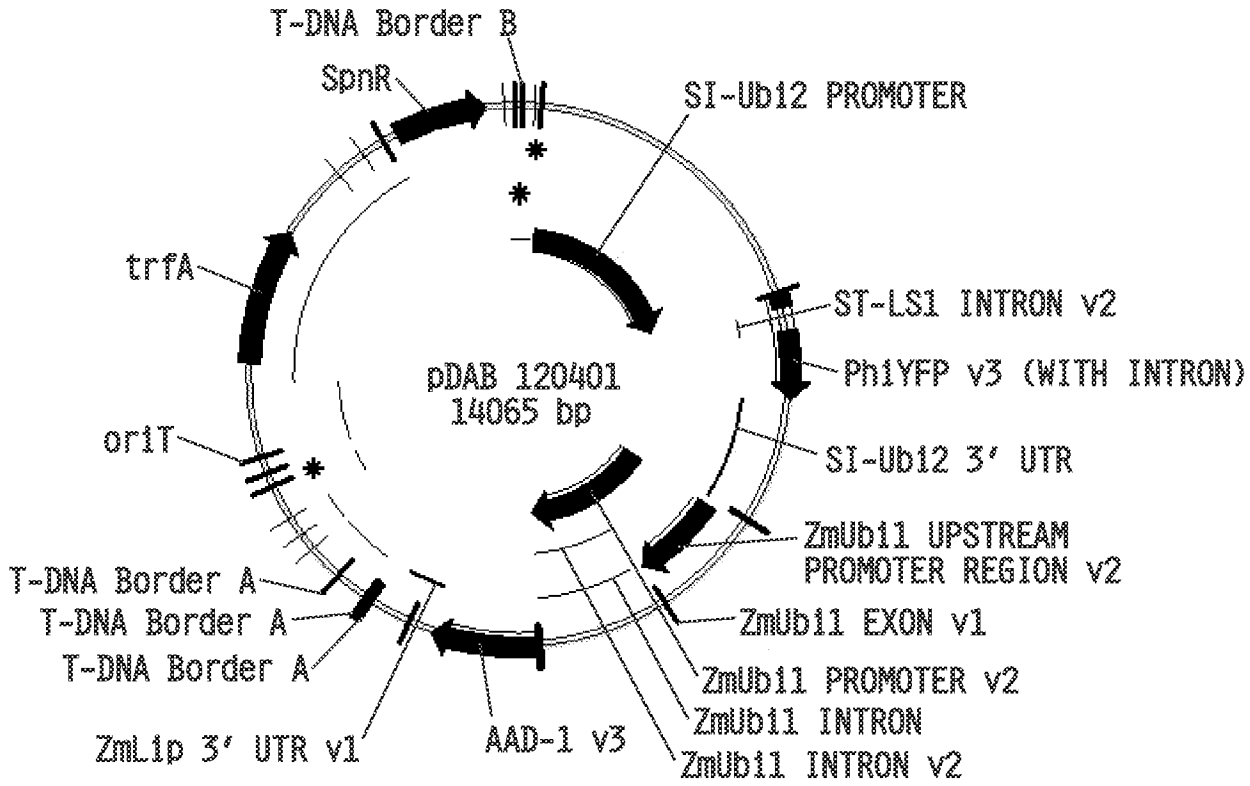
T-DNA Border B
SpnR
SI-Ub12 PROMOTER
trfA
ST-LS1 INTRON v2
pDAB 120401
14065 bp
Ph1YFP v3 (WITH INTRON)
oriT
SI-Ub12 3' UTR
ZmUb11 UPSTREAM
PROMOTER REGION v2
T-DNA Border A
T-DNA Border A
ZmUb11 EXON v1
T-DNA Border A
ZmUb11 PROMOTER v2
ZmUb11 INTRON
ZmL1p 3' UTR v1
AAD-1 v3
ZmUb11 INTRON v2

FIG. 33

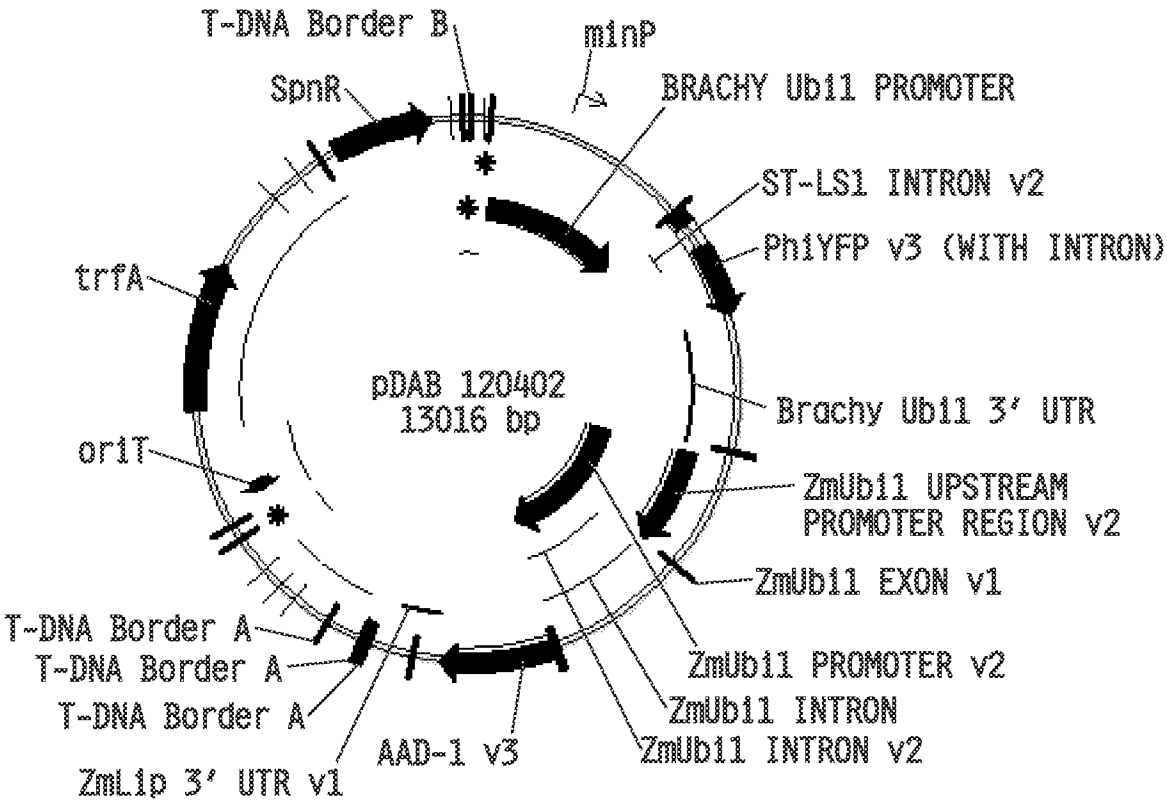
T-DNA Border B
mInP
SpnR
BRACHY Ub11 PROMOTER
ST-LS1 INTRON v2
Ph1YFP v3 (WITH INTRON)
trfA
pDAB 120402
13016 bp
Brachy Ub11 3' UTR
oriT
ZmUb11 UPSTREAM
PROMOTER REGION v2
ZmUb11 EXON v1
T-DNA Border A
T-DNA Border A
T-DNA Border A
ZmUb11 PROMOTER v2
ZmUb11 INTRON
ZmUb11 INTRON v2
AAD-1 v3
ZmLip 3' UTR v1

FIG. 34

ctgctcgttcagcccacagtaacacgccgtgcgacatgcagatgccctccaccacgccgaccaaccccaagt
ccgccgcgctcgtccacggcgccatccgcatccgcgcgtcaacgtcatccggaggaggcgagcgcgatgtcg
acggccacggcggcggcggacacgacggcgacgccccgactccgcgcgcgcgtcaaggctgcagtggcgtcg
tggtggccgtccgcctgcacgagatccccgcgtggacgagcgccgcctccacccagcccctatatcgagaaa
tcaacggtgggctcgagctcctcagcaacctccccacccccccttccgaccacgctcccttcccccgtgccc
ctcttctccgtaaacccgagccgccgagaacaacaccaacgaaagggcgaagagaatcgccatagagaggag
atgggcggaggcggatagtttcagccattcacggagaaatggggaggagagaacacgacatcatacggacgc
gaccctctagctggctggctgtcctaaagaatcgaacggaatcgctgcgccaggagaaaacgaacggtcctg
aagcatgtgcgcccggttcttccaaaacacttatctttaagattgaagtagtatatatgactgaaattttta
caaggtttttccccataaaacaggtgagcttatctcatccttttgtttaggatgtacgtattatatatgact
gaatatttttattttcattgaatgaagattttcgacccccaaaaataaaaaacggagggagtacctttgt
gccgtgtatatggactagagccatcgggacgtttccggagactgcgtggtgggggcgatggacgcacaacga
ccgcattttcggttgccgactcgccgttcgcatctggtaggcacgactcgtcgggttcggctcttgcgtgag
ccgtgacgtaacagacccgttctcttcccccgtctggccatccataaatcccccctccatcggcttcccttt
cctcaatccagcaccctgattCCGATCGAAAAGTCCCCGCAAGAGCAAGCGACCGATCTCGTGAATCTCCGT
CAAGgtatgcagcctcgcttcctcctcgctaccgtttcaattctggagtaggtcgtagaggataccatgttg
atttgacagagggagtagattagatacttgtagatcgaagtgcggatgttccatggtagatgataccatgtt
gatttcgattagatcggattaaatctttgtagatcgaagtgcgcatgttccatgaattgcctgttaccagta
gattcaagtttttctgtgttatagaggtgggatctactcgttgagatgattagctcctagaggacaccatgc
cgttttggaaaatagatcagaaccgtgtagatcgatgtgagcatgtgttcctgtagatccaagttctttcgc
atgttactagttgtgatctattgtttgtgtaatacgctctcgatctatccgtgtagatttcactcgattact
gttactgtggcttgatcgttcatagttgttcgttaggtttgatcgaacagtgtctgaacctaattggatatg
tattcttgatctatcaacgtgtaggtttcagtcatgtatttatgtactccctccgtcccaaattaactgacg
tggattttgtataagaatctatacaaatccatgtcagttaattcgggatggagtaccatattcaataatttg
tttattgctgtccacttatgtaccatatgtttgttgttcctcatgtggattctactaattatcattgattgg
tgatcttctattttgctagtttcctagctcaatctggttattcatgtagatgtgttgttgaaatcggagacc
atgcttgttattagatagtttattgcttatcagtttcatgttctggttgatgcaacacatattcatgttcgc
tatctggttgctgcttgatattctctgatttacattcattataagaatatattctgctctggttgttgcttc
tcatgactttacctactcggtaggtgacttacctttggtttacaattgtcaactatgcag***ATG***cagatctt
tgtgaagaccctcaccggcaagaccatcacccttgaggtcgagtcttctgatacgatcgacaatgtcaaggc
aaagatccaggacaaggagggcatccccccggaccagcagcgtctcatcttcgccgggaagcagctggagga
tggccgcaccctggcagattacaacatccagaaggagtccaccctccatctggtgctcaggctcaggggtgg
catgcaaatctttgtgaagacccttactggcaagaccatcacactcgaggtcgagtcgtctgacacgatcga
caatgtgaaggcaaagatccaggacaaggagggcatccccccagaccagcagcgcctcatctttgctggcaa
gcaactggaagacggtcgcaccctggcagattacaatatccagaaggagtccaccttgcaccttgtgcttcg
cctccgtggtggcatgcaaatctttgtcaagaccttgacaggcaagaccattactctggaggtcgagtcgtc
tgacacaatcgataatgtgaaggcgaagatccaggacaaggagggaattccaccggaccagcagcgcctaat
ctttgctggcaaacagcttgaggatggccgcaccctggcagattacaacatccagaaagaatccactctgca
cctggtgcttcgcctccgtggtggcatgcagatctttgtcaagaccttgacagggaagacaatcacactgga
ggtcgagtcgtctgacacaatcgataatgtgaaggcgaagatccaggacaaggagggcattccaccggacca
gcagcgccttatcttcgccggcaagcagcttgaggatggccgcaccttgctgattacaatatccagaagga

FIG. 35

atccaccctgcacctggtgcttcgcctccgtggtggcatgcagatctttgtcaagactttgaccgggaagac
cattacactggaggttgaatcttcagacaccatcgacaacgtgaaggcgaagatccaggacaaggagggcat
ccccccagaccagcagcgcctgatctttgctggtaagcagcttgaggatggacgcactctggcggattataa
*catccagaaggagtctaccctacacctggtgctccgcctccgtggtggccag**TAA**gtttgtcaaaaactggc*
ctacagtctgctgcccctgttggtctgccccttggaagtagtcgtgtctatggttatgtgagaagtcgttgt
gttctttctaatcccgtactgtttgtgtgaacatctgctgctgtcgtattgcatcgtgaagaatcctgttat
gaataagtgaacatgaaccttgttctgtgattacggcttcgtggttatgcgaacgttcttacaaacgcaatt
gcacctgatgtaaaatcgttttgctagctgtatggaacaagtgctcatgatgttcatgcaagatgcaattc
cagcttttgttggtttgtcatctttgtactgtgcttaccgcacataaagattgcatcttgcttattgctttg
ttgctttggtgctcgtccgcttctccttgcaccttatcaaacctttgtttagattctcttcttatagcactt
ggtaactctcagctttacaacgccagtactgtttctgaaatttcatgactgataaagctgatagatggagta
ctaatatatgacatctttccataaatgttcgggtgcagagatatggaggccccaggatcctatttacaggat
gaacctacctgggccgctgtacgcatgacatccgcgagcaagtctgaggttctcaatgtacacatgaaattg
atttttgctgcgtttggcttggctgatcgttgcatttgttctgattcatcagagttaaataacggatatatc
agcaaatatccgcagcatccacaccgaccacacgtccggttaacagagtccccctgccttgctttaattatt
acggagtactccgctattaatccttagatatgtttcgaaggaactcaaaccttcctccatctgcaaatctca
gtgcttcaaaactggaattagataattgaaaccttcattcggttgcaattcacaactgcaaattgaacagca
ctgtcaatttcaatttcgggttcacgattccaccgataggttgacatgatccatgatccacccattgtacaa
c (SEQ ID NO:19)

FIG. 35 cont.

ggcgtcaggactggcgaagtctggactctgcagggccgaactgctgaagacgaagcagaggaagagaaaggg
aagtgttcgacttgtaatttgtaggggttttttttagaggaacttgtaatttgtaggtgggctggcctcgtt
ggaaaaacgatgctggctggttgggctgggccgatgtacgcttgcaaacaacttgtggcggcccgttctgga
cgagcaggagtttcttttttgttctcacttttctggtcttctttagttacggagtacctttgtttttttaaa
ggagttacctttttttttaggaattctttagttacctttcgcttgctctcaaaaaatatttaactttcgcttt
ttttcattttaatttttgcaactatttacgagtttcatgaatgcttattttccagcatatcattatttgcaa
gtatttttatgccgtatgtattggacgagagccatcgggactgttccagagactgcgtggtggggacggctc
ccaaccgccttttctatctctgttcgcatccggtggccgacttggctcgcgcgtgagccgtgacgtaacaga
cttggtctcttccccatctggccatctataaattcccccatcgatcgaccctccctttccCCAATCCAGCAC
CCCCGATCCCGATCGAAAATTCTCCGCAACAGCAAGCGATCGATCTAGCGAATCCCCGTCAAGgtatgtagc
ctctcgattcctcctcagccctgccctcgatttggtgtacgcgttgagatgatgatctcgtagatgtctaga
tgacaccatgtcgatttgaaatagatcagatccgtgtagatcgatgagctcctgtgtacctgtggattcaag
ttattttcgcatgctattgttgtgatctactagatctagtgtgtgtattctatgctatcgatttctccgtgt
agatttcactcgattactgttactgtggcttgatcggccatagatgttggttaaggtttgatcggttagtgt
ttgaacctgcgtggatatctagcatccatctattatcgtgtaggtttcgaacaaacaagcactattattgta
ctgatggttcgtctatggttggttttgaccgttttagtgttgaacgagccttctgtatttgtttattgctgt
ccagtgatgtaccatgttcgttgagtgtcggattatactaattattgttgattgataatcttgtagtttgct
ttcctaatttatttatcgtagtcctgatttgcctcagctgtgcctcacccgtgcgatggtcaatcaacttg
ttagcccaatctgcttaatcatgtacatttgttgttagaatcagagatcaagccaattagctatcttattgc
ttatctgttccatgttctgatcgatgtaacagtctacacttttgctctgtgctacttgattaaaacattctg
acttaaattcatgattggaagtttcagatctgattgttgccttacttgactaatatctattcatgtgacacc
tctctgtcttggtaacttaccgctgtttgtttgtaatttctgactatgcag***ATG***cagatctttgtgaagacc
ctcactggcaaaaccatcacccttgaggtcgagtcgtccgacacgatcgacaacgtcaaggcaaagatccag
gacaaggagggcattcctccagaccagcagcgcctcatctttgctggaaagcagcttgaggacggccgcacc
ctcgccgactacaacatccagaaggagtccaccctccacctggtcctgaggctccgtggtggcatgcagatc
ttcgtcaagacccttaccggcaagaccatcacgctggaggtcgagtcctctgacacgatcgacaatgtgaag
gcgaagatccaggataaggagggcatccccccggaccagcagcgcctcatctttgccggcaagcagcttgag
gacggccgtaccctcgccgactacaacatccagaaggaatccacactccatctggtgctcaggctgcgtggt
ggcatgcagatcttcgtcaagaccctaaccggcaagaccatcactctggaggttgagtcctctgacacgatc
gacaatgtgaaggcaaagatccaggataaggagggcattcccccggaccagcagcgcctcatcttcgctggc
aagcagcttgaggatggccgcaccctggcagattacaatatccagaaggaatccaccttgcacctggtgctt
cgcctccgtggtggcatgcagatctttgtaaagaccttgactggcaagacaattaccctggaggttgagtcg
tccgacacaattgacaatgtcaaggcgaagatccaggacaaggagggcatcccaccggaccagcagcgcctc
atcttcgccggcaagcagcttgaggatggtcgcacccttgcagattacaatatccagaaggaatccactctg
catctggtgcttcgtctccgcggtggaatgcagatcttcgttaagacgttgacagggaagaccatcacactg
gaggttgaatcttcggacaccattgacaacgtgaaggcaaagatccaggacaaggagggcatccccccagac
cagcagcgcctcatctttgctggtaagcagcttgaggatggccgcacccttgcagattacaacattcagaag
gagtccaccctgcacctggtgctccgtctccgtggtgggcag***TAA***gcttctgccgaactggttcacagtctg
ctgcccttggtggtctgcccccttagtggtcatgccttttgttatgtgtcttgcgtcccaatcctgtatcgtt
tgtgtgaacatctctgctgctgtatagcagcttgaatcctgttatgaatttgtgaacctgaaccttgttccg
tgaatcatgttatgaataagtgaacctgaaccttgttccgtgattattgttacaatctgttgtgccgtatgg

FIG. 36

ttggtcgtgtgtgatttatgttgaactggagaaccaagttcgttccaggacatattgcaacctaagctaaac
catgtagaactacttgttctgggagacataaaacgtcatttttatgcattcgtaacatttaagcatactaca
ataattgtattgtccttttcctactcatccttgaaaccatatgcctcttctcagcgcctctacatgcagtgt
gctcagaacaaacaggccctgccagctgcttttcaattttccaattaataaccacaatagtcggactatggc
atctgtgggtgactatgcaagatgttgctgtcaggtctctgaaacttttcccatgtatctgttgaaattacc
cagtaaattcatgcctctatttaatctggcatggttgattttcaaacagaatgtgtttttttttgttctgga
agctatattggtaaataaatacaaagctggagtgtgattatatttccaacagatattcaagaaaatctcagt
tgatttatttactactgtagtatatatatatatatcttacagttgacttctcatatttcaaacgacatgtgagc
acattgttcagtttcttaggatgtgttgtgtgctcaaaggtgtaattttgcattctgccctccgagtaaaca
ctacacgtatttttttgagtggcagtgcatttgattacaaggcaacaacaacaaaaacctatggcaagatat
ccttcttagaggctgccaggatcattttgactgaactatgtaaggctgaagaaaagg (SEQ ID NO:20)

FIG. 36 cont.

tgcgtctggacgcacaagtcatagcattatcggctaaaatttcttaatttctaaattagtcatatcggctaa
gaaagtggggagcactatcatttcgtagaacaagaacaaggtatcatatatatatatatatataatatttaa
actttgttaagtggaatcaaagtgctagtattaatggagtttcatgtgcattaaattttatgtcacatcagc
aattttgttgacttggcaaggtcatttagggtgtgtttggaagacaggggctattaggagtattaaacatag
tctaattacaaaactaattgcacaaccgctaagctgaatcgcgagatggatctattaagcttaattagtcca
tgatttgacaatgtggtgctacaataaccatttgctaatgatggattacttaggtttaatagattcgtctcg
tgatttagcctatgggttctgctattaattttgtaattagctcatatttagttcttataattagtatccgaa
catccaatgtgacatgctaaagtttaaccctggtatccaaatgaagtcttatgagagtttcatcactccggt
ggtatatgtacttaggctccgttttcttccaccgacttatttttagcacccgtcacattgaatgtttagata
ctaattagaagtattaaacgtagactatttacaaaatccattacataagacgaatctaaacggcgagacgaa
tctattaaacctaattagtccatgatttgacaatgtgttgctacagtaaacatttgctaatgatggattaat
taggcttaatagattcgtctcgccgtttagcctccacttatgtaatgggttttctaaacaatctacgtttaa
tactcctaattagtatctaaatattcaatgtgacacgtgctaaaaataagtcagtggaaggaagagaacgtc
cccttagttttccatcttattaattgtacgatgaaactgtgcagccagatgattgacaatcgcaatacttca
actagtgggccatgcacatcagcgacgtgtaacgtcgtgagttgctgttcccgtagAGAAATATCAACTGGT
GGGCCACGCACATCAGCGTCGTGTAACGTGGACGGAGGAGCCCCGTGACGGCGTCGACATCGAACGGCCACC
AACCACGGAACCACCCGTCCCCACCTCTCGGAAGCTCCGCTCCACGGCGTCGACATCTAACGGCTACCAGCA
GGCGTACGGGTTGGAGTGGACTCCTTGCCTCTTTGCGCTGGCGGCTTCCGGAAATTGCGTGGCGGAGACGAG
GCGGGCTCGTCTCACACGGCACGGAAGACgtcacgggttccttccccacctctcctcttccccaccgccata
aatagCCGACCCCCTCGCCTTTCTCCCCAATCTCATCTCGTCTCGTGTTGTTCGGAGCACACCACCCGCCCC
AAATCGTTCTTCCCGCAAGCCTCGGCGATCCTTCACCCGCTTCAAGgtacggcgatcgtcttcctcctctag
atcggcgtgatctgcaagtagttgatttggtagatggttaggatctgtgcactgaagaaatcatgttagatc
cgcgatgtttctgttcgtagatggctgggaggtggaatttttgtgtagatctgatatgttctcctgtttatc
ttgtcacgctcctgcgatttgtggggattttaggtcgttgatctgggaatcgtggggttgcttctaggctgt
tcgtagatgaggtcgttctcacggtttactggatcattgcctagtagatcagctcgggctttcgtctttgta
tatggtgcccatacttgcatctatgatctggttccgtggtgttacctaggtttctgcgcctgattcgtccga
tcgattttgttagcatgtggtaaacgtttggtcatggtctgatttagattagagtcgaataggatgatctcg
atctagctcttgggattaatatgcatgtgtcaccaatctgttccgtggttaagatgatgaatctatgcttag
ttaatgggtgtagatatatatgctgctgttcctcaatgatgccgtagcttttacctgagcagcatggatcct
cctgttacttaggtagatgcacatgcttatagatcaagatatgtactgctactgttggaattctttagtata
cctgatgatcatccatgctcttgttacttgttttggtatacttggatgatggcatgctgctgcttttgttg
atttgagcccatccatatctgcatatgtcacatgattaagatgattacgctgtttctgtatgatgccatagc
ttttatgtgagcaacatgcatcctcctggttatatgcattaatagatggaagatatctattgctacaatttg
atgattattttgtacatacgatgatcaagcatgctcttcatactttgttgatatacttggataatgaaatgc
tgctgcacgttcattctatagcactaatgatgtgatgaacacgcacgacctgtttgtggcatctgtttgaat
gtgttgttgctgttcactagagactgttttattaacctactgctagatacttacccttctgtctgtttattc
ttttgcag***ATG***cagatctttgtcaagaccctcaccggcaagaccatcaccctcgaggtggagtcttctgaca
ccattgacaacgtcaaggccaagatccaggacaaggaaggcattcccccggatcagcagcggctcatctttg
ccggcaagcagcttgaggatgggcgcaccctggctgactacaacatccagaaggagagcaccctccacctgg
tgctccgtctcaggggaggcatgcagatctttgtgaagaccttgactggcaagaccatcacccttgaggtgg

FIG. 37

agtcttccgacaccatcgacaacgtcaaggccaagatccaggacaaggagggcatccccccggaccagcaga
ggctcatctttgcgggcaagcagcttgaggatggacgcaccctggctgactataacatccagaaggagagca
ccctccatctggtgctccgtctcaggggaggcatgcagatcttcgtgaagactctcactggcaagaccatca
ccctcgaggtggagtcttccgacaccatcgacaacgtcaaggccaagatccaggacaaggagggcatccccc
cagaccagcagaggctcatctttgctggcaagcagcttgaggacggacgcaccctggctgactataacatcc
agaaggagagcaccctccacctggtgctccgcctgaggggtgggatgcagatctttgtgaagactttgactg
gcaagaccattactttggaggttgagagctccgacaccatcgacaacgtgaaggccaagatccaggacaagg
aaggcatccccccggaccagcagaggctcatcttcgccggcaagcagcttgaggacggacgcaccctggctg
actataacatccagaaggagagcaccctccacctggtgctccgtctcaggggaggcatgcagatcttcgtga
agaccctcactggcaagaccatcacccttgaggtggagtcttccgacaccatcgacaatgtcaaggccaaga
tccaggacaaggagggcatccccccagaccagcagagactcatctttgcaggcaagcagcttgaggacggac
gcaccctggctgactacaacatccagaaggagagcaccctccacctggtgctccgtctcaggggaggcatgc
agatcttcgtgaagaccctcactggcaagaccatcaccctcgaggtggagtcttctgacaccatcgacaacg
tcaaggccaagatccaggacaaggagggcatccccccggaccagcagcgtcttatctttgccggcaagcagc
tggaggatggccgcaccttgcggattacaatatccagaaggagagcaccctccatctggtgctccgtctga
ggggtgggatgcagatattcgtgaagactttgaccggcaagaccatcactttggaggttgagagctccgaca
ccattgacaatgtgaaggccaagatccaggacaaggagggtatccccccggaccagcagcgtctgatcttcg
ccggcaagcaactggaggatggccgcaccctggcggactacaatatccagaaggagtccaccctccacctgg
*tgctccgcctccgtggtggtcag**TAA**gcccatcggtcatggatgcttctactgtacctgggtcgtctggtct*
ctgcctgtgtcacctttgaagtacctgtgtcgggattgtgtttggtcatgaactgcagtttgtctttgatgt
tcttttgtctggtcttatgaactggttgtatctgtatgtttactgtaaactgttgttgcggtgcagcagtat
ggcatccgaatgaataaaatgatgtttggacttaaatctgtactctgtttgttttcggttatgccagttctat
attgcctgagatcagaatgtttagcttttgagttctgtttggcttgtggtcgactcctgtttcttacttgag
gcgtaactctgttctggcaaactcaaatgtctaactgaatgttttaggacttaattgttggacagattaacg
tgtttggtttgtttctagattgtgattcggaaggcttgttagttgtggaatcaaggagagcagctaggtctg
tgcagaacgttattttggatttaagccttctcagattatgccattactctaaacctaatgatatcatatttc
actcggggatgttggagtagtcttttctttctcctgcagacaaaatgattttgctttcgtgtgtgtacatga
ttttgtgcaactgttgcaacaactgaagtagacaagttttgacctcaccagaagaatgaaaaagattttgga
atttgttacatcgacaaaccattgtaacttggcccatcagaatgcacagaagagcggctacaaattgacatg
cgttgcaaactttgcaatagttgatgcacatgtttgccattgcctgccagtcttaggaaaagtgtgtggttc
gagaaatctaagcatatgtgctctgctcacattgcgtggaacccacacagctttgtcacactcttgtccact
ccagaagtcattcctggcgctgtttacccctggtaaaaggtaaccgaaaacttctcaaggctgtacccaaaa
ctggaaggaaatttggaggaaatctttgcttttgatcggctcactctttc (SEQ ID NO:21)

FIG. 37 cont.

ttgaattttaatttcaaattttgcagggtagtagtggacatcacaatacatatttagaaaaagttttataat
tttcctccgttagttttcatataatttttgaactccaacgattaatctattattaaatatcccgatctatcaa
aataatgataaaaatttatgattaatttttctaacatgtgttatggtgtgtactatcgtcttataaaatttc
aacttaaaactccacctatacatggagaaatgaaaaagacgaattacagtagggagtaatttgaaccaaatg
gaatagtttgagggtaaaatgaactaaacaatagtttaggaggttattcagattttagttatagttgagagg
agtaatttagacttttttcctatcttgaattgttgacggctctcctatcggatatcggatggagtctttcagc
ccaacataacttcattcgggcccaaacgttcgtccatccagcctagggagaacattttgcccatgatatctg
ttttttcttttttttctattttcactggtattataggagggaaatatacaacgtgttcacctttggtttcattc
ttgttccatctgaatttatctaaaactgtgtttgaacttcgtaagaattttgttcgatctgtccggtacatc
gtgttgataggtggcctccgagattcttctttttaaccggcaaagtaaaataatctcagctccagcctaacg
tcaattatcagagagagaaaaaaatatttttttatgattgatcggaaaccaaccgccttacgtgtcgatcct
ggttcctggccggcacggcggaggaaagcgaccgacctcgcaacgccggcgcacggcgccgccgtgttggac
ttggtctcccgcgactccgtgggcctcggcttatcgccgccgctccatctcaaccgtccgcttggacacgtg
gaagttgatccgtcgcgcaccagcctcggaggtaacctaactgcccgtactataaatccgggatccggcctc
tccaatccccatcgccaCAAGTTCGCGATCTCTCGATTTCACAAATCGCCGAGAAGACCCGAGCAGAGAAGT
TCCCTCCGATCGCCTTGCCAAGgtactcctacctaatcctccttaactgatctctcctctatcacgttggta
atcttcgaatgatctgctgcctggctcgctgttccccctcgttatgcactgtttccatcacgagttttttt
ttcatcatctaatctatgcggttgcggaagaattgtggctagtggagtagttttctgtgcttgatcggtaga
ttcgatgtgtgggtgtatggatgttttctgaaaagttgctggattagtttacgctttcaggccgcaggtctg
ttcgaaattgattatgaagtctatatgctttggatctatcgatttccagttttattcagatgtaggccaaaa
aattgtcggcatttgtgtggaattagttggcctttaggtctgcacattcatggtgacggcacagttgctgct
ggctgttgcgtgggacgagttattatagttgtttttgtttttccctgattgattcacattttcaatgataac
tagcctttgtcacctaaccaagtccaggttgatcctatctgtgttcttcagctaccagtttgcatagatgat
ggtgtattcgattgctttagtaggccttctgatttcacatctaattctgtcatgaatatagataactttaca
tgcttttgatatactttatatttgaactgttcactgtccagcctattttggataattgagtgcattggcttt
tgatgcctgaattattcacatgttcctggataattgacctgtgtcacctagttgactgtttttgaggtgcg
acccgtctgttcagctgatttgtgtattcgattgctctagttaatcttttgattatgcagctagtgctttgt
catatgtagctttataggcttctgatgtccttggatatagttcagtctacttgtcaagttgctttacaagta
gtagctctgattctatttggcttcctgagtcagagctttgcaaattgcttgttgttacattacatcatatta
cttgaattgcagttatttaatggttggattgttgctgtttacttctacatttttgctgttttatattatac
taaaatgtttgtgttgctgcttttcag***ATG***cagatctttgtgaagacactcactggcaagactatcaccctt
gaggtggagtcttctgacacaattgacaatgtcaaggcaaagatccaggacaaggaagggattcctccagac
cagcagcgccttatcttcgctggcaagcagcttgaggatggccgtacacttgcagattacaacattcagaag
gagtccacactgcaccttgtcctcaggctgcgtggaggcatgcagattttcgtgaagaccctcactggcaag
acgatcaccctagaggtggagtcatctgacaccatcgacaatgtgaaggcaaagatccaggacaaggagggc
atccccccctgaccagcagcgcctcatctttgcaggcaagcagttggaggatgggcgaactctggctgactat
aatatccagaaagagtccacccttcacctcgtcctccgcctgcgaggtggcatgcagatctttgtgaagacg
ctgacaggcaagaccatcacattggaagttgagtcctccgatacaatcgacaatgtgaaggccaagatccag
gataaggagggtatccccccggaccagcagcgcctcatcttcgccggcaagcagctcgaggatggccgcact
ctcgctgactacaacatccagaaggaatcaaccctgcacctggtgctccgcctgcgtggtggaatgcagatc

FIG. 38

tttgtgaagacgcttaccggcaagaccatcaccttggaggtggagtcttcggacaccatcgacaatgtgaag
gcgaagattcaggacaaggagggcattcctccggaccagcagcgcctcatctttgctggcaagcagctagag
gacgggcgtaccctggcggattacaacatccagaaggagtccaccctccaccttgtcctgcgcctccgtggt
*ggtttc**TGA***gcctagtgctcctgagttgccttttgtcgttatggtcaacctctggtttaagtcgtgtgaact
ctctgcattgcgttgctagtgtctggttgtggttgtaataagaacatgaagaacatgttgctgtggatcaca
tgacttttttttttgaaccggaagatcacatgactttcatggctttaagttcctgaactctgaaatctggac
ccctttttaagctctgaactcatcattcttgcatttacatctggtgttgatcttattgatgtgatgcagtcc
tgctgaaatagtcaatgtagattcatgactgactgattgcgtttatggtgtgtatgttgttaacaagctgaa
ggtcgtgtggtgtctttccagttagacgaagtgtgctttattgtagcgtgtagtgctgctggatgattgatg
aactgaaacattctgcatttagcaactagcgagccaaaggtgatgactgagtttctgtagacctgttttttt
atgcccatggtcgttcttcaattgcacttgattttcacattagctggatcataatctgagcagactactcaa
aagtacaaagttcatcttcgctatgacgctttgccactaggattttctttgtatgatttgtttacaaatcct
gtaatctagtcaaaagaaaagccaaaatttttctttgtatgatttgtttacaaatcctctaatctagtcaaa
gaaaagccaaatttatccctcctggtcccctacatcacgtagctatgtggcccgcaagcagatgaaagcagc
cccgtcagccgacgccgacgccgacgccaacacatcctgctcctccctcgccggcgccggcgccggcgaggc
cgcaccgccgctgccccgtggccgcaggcacacggtgccgcactgccgccgccccgtggccgcaggcacacg
gtgccgcactgccgccgcctccccttccggcattgccggacggctgggctactgtccccgccgccttcccaa
t (SEQ ID NO:34)

FIG. 38 cont.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/053364

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/82 (2014.01)
CPC - C12N 15/8216(2014.11)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12N 5/10, 15/29, 15/63, 15/82; C12Q 1/68 (2014.01)
USPC - 435/320.1, 419, 468; 536/24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12N 15/62, 15/63, 15/67, 15/79, 15/82, 15/113, 15/8216, 15/8222, 15/8286 (2014.11) (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, PubMead

Search terms used: ubiquitin promoter terminator plant Setaria

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/101343 A1 (KUMAR et al) 04 July 2013 (04.07.2013) entire document	1-6, 12-30
A	US 2010/0199377 A1 (SEKAR et al) 05 August 2010 (05.08.2010) entire document	1-6, 12-30
A	US 2009/0007301 A1 (WINTZ et al) 01 January 2009 (01.01.2009) entire document	1-6, 12-30
A	US 2012/0180158 A1 (ABBITT) 12 July 2012 (12.07.2012) entire document	1-6, 12-30
A	US 2003/0066108 A1 (JILKA et al) 03 April 2003 (03.04.2003) entire document	1-6, 12-30

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
30 November 2014	18 DEC 2014

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/053364

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 3, 6, 13, 14, 17, 40, 41, and 42 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/053364

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-11
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.