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(54) Title: TRANSCRIPTIONAL CONTROL ELEMENTS AND USES THEREFOR - II

(57) Abstract: This invention discloses transcriptional control elements for use in plant genetic engineering. More particularly, the present invention relates discloses tissue-specific promoters for expression of heterologous nucleic acids in plants including monocotyledonous plants. The invention also discloses chimeric nucleic acid constructs comprising a promoter of the invention operably linked to a foreign or endogenous polynucleotide that codes for a protein of interest or a transcript that is capable of modulating expression of a target gene. The invention is further concerned with transformed plant cells, as well as differentiated plants and plant parts, containing the construct of the invention.



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“TRANSCRIPTIONAL CONTROL ELEMENTS AND USES THEREFOR - II”

FIELD OF THE INVENTION

[0001] This invention relates generally to transcriptional control elements for use in
5 plant genetic engineering. More particularly, the present invention relates to tissue-specific
promoters for expression of heterologous nucleic acids in plants including monocotyledonous
plants. The invention also relates to chimeric nucleic acid constructs comprising a promoter of
the invention operably linked to a foreign or endogenous polynucleotide that codes for a
protein of interest or a transcript that is capable of modulating expression of a target gene. The
10 invention is further concerned with transformed plant cells, as well as differentiated plants
and plant parts, containing the construct of the invention.

BACKGROUND OF THE INVENTION

[0002] A primary goal of genetic engineering is to obtain plants having improved
characteristics or traits. Many different types of characteristics or traits are considered
15 advantageous, but those of particular importance include enhanced yield or quality of
harvestable plant products, production of additional compounds in plants, enhanced stability
or shelf life of the ultimate consumer product obtained from a plant, improvement in the
nutritional value of edible portions of a plant, and plant resistance to diseases, insects,
herbicides, cold stress, water stress or soil salinity.

20 [0003] Recent advances in genetic engineering have enabled the incorporation of a
selected gene (or genes) into plant cells to impart a desired quality (or qualities) to a plant of
interest. The selected gene (or genes) may be derived from a source different from the plant of
interest or may be native to the desired plant, but engineered to have different or improved
qualities. This new gene (or genes) may then be expressed in cells of the regenerated plant to
25 exhibit the new trait or characteristic.

[0004] In order for the newly incorporated gene to express the transcript and/or
protein for which it codes in a plant cell, the proper regulatory signals must be present and in
the proper location with respect to the gene. These regulatory signals include at least a
promoter, which is a region involved in the regulation of transcription. Other regulatory
30 signals that common in protein-coding genes for example include a 5' non-translated leader
sequence and a 3' polyadenylation signal.

[0005] Generally, the efficiency of gene expression is governed largely by the promoter used to express the gene. A promoter is a DNA sequence that directs the cellular machinery of a plant to produce (transcribe) RNA (transcript) from a contiguous transcribable region downstream (3') of the promoter. By corollary, the promoter region lies upstream (5') of the transcribable region. DNA is typically comprised of two polynucleotide strands in anti-parallel orientation with complementary base pairing. The terminology for the location and orientation of DNA sequence elements, including non-transcribed regulatory signals, uses as a reference the sequence of the corresponding transcript, which is a single-stranded polynucleotide with unambiguous 5' and 3' ends. Nucleotides are commonly counted from the point at which transcription commences, with positive (+) numbering in the downstream direction and negative (-) numbering in the upstream direction.

[0006] Promoters typically comprise sequence modules that function in concert to determine the overall promoter activity. Conserved sequence motifs near the transcription start site are believed to function in the binding and orientation of RNA polymerase, whereas sequence motifs more distant from the transcription start site substantially modulate the level and developmental pattern of promoter activity. For example, many plant promoters include a motif with a TATA consensus in the vicinity of -30 nt, and a motif with a CAAT or AGGA consensus at about -70 nt upstream of the transcription start site (for reviews, see Messing, Geraghty, Heidecker, Hu, Kridl and Rubenstein, 1983, pp 211-21 in *Genetic Engineering of Plants* ed Kosuge, Meredith and Hollaender, Plenum; Waugh and Brown, 1991, pp 1-37 in *Plant Genetic Engineering*, ed Grierson, Blackie; Ferl and Paul, 2000, pp 312-57 in *Biochemistry and Molecular Biology of Plants*, ed Buchanan, Gruissem and Jones, ASPP.). Isolated regions of about 40-90 nt upstream of the transcription start site and containing such conserved sequence motifs commonly have little or no promoter activity alone. However, when combined with upstream regulatory sequences from the same promoter or from other promoters, they can show promoter activity in a pattern determined substantially by the upstream regulatory sequences (for example, see Puente, Wei and Deng, 1996, *EMBO J.* **15**: 3732).

[0007] The promoter influences the rate at which the transcript of the gene is made. Assuming the transcript includes a coding region with appropriate translational signals, the promoter also influences the rate at which the resultant protein product of the gene is produced. Promoter activity also can depend on the presence of several other *cis*-acting regulatory elements which, in conjunction with cellular factors, determine strength,

specificity, and transcription initiation site (for a review, see Zawel and Reinberg, 1992, *Curr. Opin. Cell Biol.* 4:488).

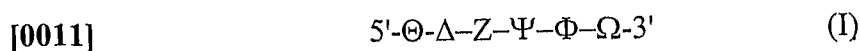
5 [0008] It has been shown that certain promoters are able to direct RNA synthesis at a higher rate relative to other promoters. These are called “strong promoters.” Certain other promoters have been shown to direct RNA production at higher levels only in particular types of cells or tissues and are often referred to as “tissue-specific promoters”. Promoters that are capable of directing RNA production in many or all tissues of a plant are called “constitutive promoters”. Thus, expression of a chimeric gene (or genes) introduced into a plant may potentially be controlled by identifying and using a promoter with the desired characteristics.

10 The desired expression pattern depends on the nature of the gene product and the trait associated with expression of any particular gene. For example, in the case of an enzyme for conversion of an endogenous storage compound into an alternative industrial product, it may be useful to drive expression preferentially in mature storage tissues, to avoid interference with other plant functions in non-storage tissues.

15 [0009] There is currently a dearth of promoters that can be used for effective expression of foreign or endogenous coding sequences in desired developmental patterns in plants in general, and monocotyledonous plants in particular.

SUMMARY OF THE INVENTION

[0010] In work leading up to the present invention, studies were carried out to understand the molecular basis for developmental control of plant gene expression, and in particular to understand the basis for preferential expression of certain plant genes in sink tissues, sucrose-storage tissues and mature stem tissues. During the course of these studies, the present inventors surprisingly discovered a class of plant genes with developmental patterns of interest, which enabled the isolation of a structurally related class of promoter sequences that can be used to express heterologous genes in plants, including preferential expression in plant sink (*e.g.*, stem) tissue. Sequence analysis has revealed that these promoter sequences share a common segmental architecture, which can be represented by the following formula:



[0012] in which segment Ω , which comprises core promoter elements, is more conserved across the class of promoter sequences than segment Φ , segment Φ is more conserved than segment Z , segment Z is more conserved than segment Δ , segment Δ is more conserved than segment Θ , and Ψ is an optional spacer or intervening segment. Based on studies investigating another class of promoter sequences with a similar segmental architecture, as disclosed in co-pending United States Provisional Application No 61/106,922 filed October 20, 2008, which is hereby incorporated by reference herein in its entirety, the present inventors propose that segment Ω is transcriptionally active in plant cells.

[0013] Accordingly, in one aspect of the present invention, isolated nucleic acid molecules are provided, which comprise a promoter sequence that is operable in plant cells, including monocotyledonous plant cells. The promoter sequence generally comprises a nucleotide sequence (also referred to herein as segment Ω sequence) selected from the group consisting of:

[0014] (a) YGASMTTKAYWAKTSGCTARRRARN_aKTYRRAKRMRWARWT
GKYRSRRN_bHTTYN_cDMYTYKTN_dTTCCAASRGYTWKMAAAYYAHRMTMCN_eRA
RGTATAKRWMAHAN_fWRMATKWGSRRTYYTRRRHKAWTTHYWRMTYAMYMYA
WTCAKYTYWAYWCWWTTYYYN_gMKKWYWTN_hAWMYTTSYMTTWTRRGWACM
YKATTWRWRWKCYAAYCN_iYRRTTTRN_jAARYSCYARTYN_kTYGRATTTASWAACA
AAAAYGKCAAATTGCATACCCAACCTCAN_lCYCATATAAAAMGKYACCAACCCAA
CATN_mTN_nTTYC [SEQ ID NO: 1];

[0015] (b) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 1 or a complement thereof; and

[0016] (c) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 1 or a complement thereof, under at least medium or high stringency conditions,

[0017] wherein:

[0018] A, C, G and T represent the nucleic acid bases adenine, cytosine, guanine and thymine, respectively;

[0019] M is A or C;

10 [0020] R is A or G;

[0021] W is A or T;

[0022] S is C or G;

[0023] Y is C or T

[0024] K is G or T;

15 [0025] V is A or C or G;

[0026] H is A or C or T;

[0027] D is A or G or T;

[0028] B is C or G or T;

[0029] N is G or A or C or T; and

20 [0030] each of a , b , c , d , e , g , i , k and m is an integer from 0-1;

[0031] each of h , j and n is an integer from 0-2;

[0032] l is an integer from 0-3; and

[0033] f is an integer from 0-5.

[0034] In some embodiments, N_a comprises 0 bases or is A; N_b comprises 0 bases or is K; N_c comprises 0 bases or is K; N_d comprises 0 bases or is A; N_e comprises 0 bases or is T; N_f comprises 0 bases or a sequence selected from ATTT or ACTC; N_g comprises 0 bases or is T; N_h comprises 0 bases or is K; N_i comprises 0 bases or is A; N_j comprises 0 bases or is the sequence TC; N_k comprises 0 bases or is W; N_l comprises 0 bases or is a sequence

selected from GA or CT; N_l comprises 0 bases or is the sequence TGC; N_m comprises 0 bases or is A; and N_n comprises 0 bases or is the sequence AT.

[0035] Illustrative examples of sequences according to SEQ ID NO: 1 are suitably selected from the group consisting of:

5 [0036] TGAGATTTACAAGTCGCTAAAAAAGTTGGAGGCGTAATTGTTG
GGGCTTTTGACTTTTTTCCAACAGTTTATAAAATCACGCTCCTAAAGTATAGAAAA
TAATTTTGCATTAGGAATCTTAAACTATTTTCAAATTACCCTAATCATTTTTTATACT
TTTTTTTCTTTCTTGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGG
TTAATCAAACCCAGTTTTTCGGATTTAGAAACAAAAACGGCAAATTGCATACCCA
10 ACTCATCGCCCATATAAAACGTTACCAACCCAACATATATTTC [SEQ ID NO: 2];

[0037] TGAGATTTACAAGTCGCTAAAAAGGTTGGAGGCATAATTGTTGG
GGCTTTGACTTTTTTCCAACAGTTTATAAAATCACGCTCCTAAAGTATAGAAAAC
ATGCATTAGGAATCTTAAACTATTTTCAAATTACTCTAATCATTTTTTATACTTTTTT
TCTTTCTTGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAAT
15 CAAACCCAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACCTCA
TCGCCCATATAAAACGGCACCAACCCAACATATATTTC [SEQ ID NO: 3];

[0038] TGAGATTTACAAGTCGCTAAAAAAGTTGGAGGCGTATTTGTTG
GGGGTTTTTGACTTTTTTCCAACAGTTTATAAAATCATGCTCCTAAAGTATAGAAA
ACAATTTTGCATTAGGAATCCTAAACTATTTCTAAATTACCCTAATCATTTTTTATA
20 CTTTTTTTTCTTTCTTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGG
TTAATCAAACCCAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCA
ACTCATCGCCCATATAAAACGGCACCAACCCAACATATATTTC [SEQ ID NO: 4];

[0039] CGACCTTGATTATTGGCTAGGGAAATTCAAATAAAAAGATGGCA
CAATATTCTTACTCGTATTCCAAGGGCTAGGCAAATAAAATACGAGGTATATGT
25 CAAAACTCAAATGTGCGGTTTTGGGTGAATTATTGCTCAACACATTCAGCTCAA
CTCAATTCCCTAGGATATTTAACCCTCTCTTATAAGTACATTATTTATGTGCCAAC
CCAATTAGAAGTGCTAATCTTGAATTTACTAACAAAAATGGCAAATTGCATACCC
AACTCACCCATATAAAAAGGCACCAACCCAACATTATTTTC [SEQ ID NO: 5];

[0040] CGACCTTGATTATTGGCTAGGGAAATTCAAATAAAAAGATGGCA
30 CAATATTCTTACTCGTATTCCAAGGGCTAGGCAAATAAAATACGAGGTATATGT
CAAACTCAAATGTGCGGTTTTGGGTGAATTATTGCTCAACACATTCAGCTCAA
CTCAATTCCCTAGGATATTTAACCCTCTCTTATAAGTACATTATTTATGTGCCAAC

CCAATTGGAAGTGCTAATCTTGAATTTACTAACAAAAATGTCAAATTGCATACCC
AACTCACCCATATAAAAAGGCACCAACCCAACATTATTTTC [SEQ ID NO: 6]; and

[0041] TGAGATTTACAAGTCGCTAAAAAAGTTGGAGGCGTAATTGTTGG
GGCTTTGACTTTTTTCCCGTTTATAAAATCACGCTCCTAAAGTATAGAAAACAAT
5 TTTGCATTAGGAATCTTAAAATATTTTCAAATTACCCTAATCATTTTTATACTTTTT
TTCTTTCTTGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAA
TCAAACCCAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACCTC
ATCGCTCATATAAAACGGCACCAACCCAACATATTTCC [SEQ ID NO: 7];

[0042] In some embodiments, the promoter sequence further comprises upstream of
10 (a), (b) or (c) a nucleotide sequence (also referred to herein as segment Φ sequence) selected
from the group consisting of:

[0043] (d) YR WYWRWTAYS YCTWDGAWMRTYKTYRAWARAYMAN_oRVC
ARAKMTSRARWTTGTTGASKYN_pYRYWRYAASCRTKGN_qCACTYRAAYDVR YRKR
YHSCWTAYTRWWGAAWMRMGAASMWRTSWWKMATWWRYYYAADMAMAN_rW
15 MYKAGCWAGMRMRYYWWTCSARKACTTKMCMRAYKRRRTRN_sRAYATRYTCM
AYN_tCAYAAKWGKWGMDSN_uAYCMAMMYYYMCYKARTAWMTTATWMYTTWG
AGCN_vYAATWGTTTN_wAWAMAAN_xWYAYMRCWMAMAARN_yGYTWTCAAARMW
N_zYARTTGGYWN_{aa} [SEQ ID NO: 8];

[0044] (e) a nucleotide sequence that shares at least 80% (and at least 81% to at
20 least 99% and all integer percentages in between) sequence identity with the sequence set
forth in SEQ ID NO: 8 or a complement thereof; and

[0045] (f) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID
NO: 8 or a complement thereof, under at least medium or high stringency conditions,

[0046] wherein:

25 [0047] A, C, G, T, M, R, W, S, Y, K, V, H, D, B and N are as defined above;

[0048] each of p , s , t , x , y and z is an integer from 0-1;

[0049] each of q and r is an integer from 0-2;

[0050] o is an integer from 0-4;

[0051] w is an integer from 0-5;

30 [0052] each of v and aa is an integer from 0-6; and

[0053] u is an integer from 0-23.

[0054] In some embodiments, N_o is the sequence AGAC; N_p is G; N_q is the sequence GC; N_r is a sequence selected from AA or TG; N_s is T; N_t is A; N_u is a sequence selected from AATTTCTAATCATGGTCAATATA [SEQ ID NO: 9],

5 AATTTATAATCATGGCTAATATA [SEQ ID NO: 10], TATATATATATTAATCTATC [SEQ ID NO: 11], TATATATATTAATCTATC [SEQ ID NO: 12] or

AATTTCTAATCATGGCTAATATA [SEQ ID NO: 13]; N_v is the sequence ATCTC; N_w is A or a sequence selected from TCAA, GGCAC or TTTT; N_x is D; N_y is A; N_z is A; or N_{aa} is the sequence AATCAA. In other embodiments, any one or more of N_o , N_p , N_q , N_r , N_s , N_t , N_u ,

10 N_v , N_w , N_x , N_y , N_z or N_{aa} comprises 0 bases.

[0055] Representative examples of sequences according to SEQ ID NO: 8 are suitably selected from the group consisting of:

[0056] CAACTAATATCTCTATGAGCATCTTCGAAAGACAAAGACGGCAG
ATCTCAAAATTGTTGAGGTCATAATAAGCATTGCACTCAAACAGGCATGTTGCTT
15 ATTAAAGAATAGCGAAGCAATGAAGAATAAATCCAAGAAAATATGAGCAAGCAC
GTCAATCGAGCACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGTGCTCA
ATTTCTAATCATGGTCAATATAATCAAACCCACCGAGTATATTACTTAGAGC
ATCTCCAATAGTTTTCAAAAAAAAAAATCATCGCAAAAAAAGTTTTCAAAAAAATA
ATTGGTAAATCAA [SEQ ID NO: 14];

20 [0057] CAACTAATATCTCTAGGAGCATCTTCAAAGACAAAGACGACAG
ATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTGCTT
ATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAAAAAAAAAATATGAGCAAGC
ACATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGC
TCAATTTATAATCATGGCTAATATAATCAAACCCACCGAGTATATTACTTAG
25 AGCATCTCCAATAGTTTTCAAAAAAAAAAATTCATCGCAAAAAAAGTTTTCAAAAA
ATAATTGGTAAATCAA [SEQ ID NO: 15];

[0058] CAACTAATATCTCTATGAGCATCTTTAAAGACAAAGACAACAG
ATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGAGCATGTGCTT
ATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAGAAAATATGAGCAAGCAC
30 ATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGCTC
ATCAAACCCACCGAGTATATTACTTAGAGCATCTCCAATAGTTTAAAAAA
ATCATCGCAAAAAAAGGTTTTCAAAAAAATAATTGGTAAATCAA [SEQ ID NO: 16];

[0059] TGTTAGTTACCCCTTAGAAAGTTGTTAATAAATCAGCCAAAGATG
 GAATTTGTTGACTCGTGCTACAACCATGGGCCACTTGAATTCATGGACACCATAC
 TGTTGAAACAAGAACATGTGTTTCATTTGCTTAATCACATGACCTAGCTAGAGAG
 CTTTTCCAATACTTTCCAGATGAAATGTAATATACTCCACACATAATTGTTGAAGT
 5 ATATATATATTAATCTATCACCCACATTTCCCTTAATAACTTATACTTTTGAGCTAA
 TTGTTTGGCACATACAAGATACAACCTCACAATGCTATCAAAGCTCAGTTGGCT
 [SEQ ID NO: 17];

[0060] TGTTAGTTACCCCTTAGAAAGTTGTTAATAAATCAGCCAAAGATG
 GAATTTGTTGACTCGTGCTACAACCATGGGCCACTTGAATTCATGGACACCATAC
 10 TGTTGAAACAAGAACATGTGTTTCATTTGCTTAATCACATGACCTAGCTAGAGAG
 CTTTTCCAATACTTTCCAGATGAAATGTAATATACTCCACACATAATTGTTGAGGT
 ATATATATTAATCTATCACCCACATTTCCCTTAATAACTTATACTTTTGAGCTAATT
 GTTTGGCACATACAAGATACAACCTCACAATGCTATCAAAGCTCAGTTGGCT [SEQ
 ID NO: 18]; and

[0061] CAACTAATATGTCTATGAGCATCTTCAAAGACAAAGACGACAG
 ATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTCGCTT
 ATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAAAAAATATGAGCAAGCAC
 ATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGCTC
 AATTTCTAATCATGGCTAATATAATCAAACCCACCCAGTATATTACTTAGAG
 20 CATCTCCAATAGTTTTTTTTTAAAAAATCATCGCAAAAAAAGCTTTCAAAAAAAT
 AATTGGTAAATCAA [SEQ ID NO: 19].

[0062] Suitably, the promoter sequence further comprises upstream of (d), (e) or (f)
 a nucleotide sequence (also referred to herein as segment Z sequence) selected from the
 group consisting of:

[0063] (g) WYHAWCRKTAYWWRAN_{ab}AGRAGYRWSN_{ac}YATGASAGCATR
 25 WGTRTMMCWMATGTRN_{ad}YWTTCTWRAAWATARYWWYYTWYRRARWT
 RKTATAKYAATAAATSARWCWAWRAKKRAWTWWRWTKWTGAMTMRHKHTWY
 AAAYATCKRYCAMTYRN_{ae}AKWMATRRASWMCMTAMWRKTTWAAAAAYRRAMW
 MAWGTRTTTGAWWN_{af}RHKTTTTMYWTYMATRAATN_{ag}AYAWYWWRWWTAYRS
 30 WHAWTCAWRWKMRAYYWYWYMCMTYATGRW [SEQ ID NO: 20];

[0064] (h) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 20 or a complement thereof; and

[0065] (i) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 20 or a complement thereof, under at least medium or high stringency conditions,

[0066] wherein:

[0067] A, C, G, T, M, R, W, S, Y, K, V, H, D, B and N are as defined above;

[0068] each of *ae*, *af* and *ag* is an integer from 0-1;

[0069] *ab* is an integer from 0-3;

10 [0070] *ac* is an integer from 0-8; and

[0071] *ad* is an integer from 0-9.

[0072] In some embodiments, N_{ab} is the sequence CAT; N_{ac} is the sequence AATAAAA; N_{ad} is the sequence GATAAAGAT; N_{ae} is G; N_{af} is C; or N_{ag} is C. In other embodiments any one or more of N_{ab} , N_{ac} , N_{ad} , N_{ae} , N_{af} or N_{ag} comprises 0 nucleotides.

15 [0073] Representative examples of sequences according to SEQ ID NO: 20 are suitably selected from the group consisting of:

[0074] TCTACCGGTACTCGAAGGAGCGTCCATGAGAGCATGTGTGTCCCT
AATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCA
AACAAAGATGGAATTTGTTGATGACTCGCTTTACAAACATCGGCCACTTGGATTC
20 ATGGACACCATACTGTTATAAAACAGAAACATGTGTTTGATTCGTGTTTTCTTTTC
ATGAATCACAATATAAATATAGATATTCATGTGCGCACTCACCCATTATGAA
[SEQ ID NO: 21];

[0075] TCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCT
AATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCA
25 AACAAAGATGGAATTTGTTGATGACTCGTGCTACAAACATCGGCCACTTGGAGTC
ATGGACACCCTACTGTTATAAAACGGAAACATGTGTTTGATTCGCTTTTTCTTTTC
ATGAATCACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGA
[SEQ ID NO: 22];

[0076] TCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCT
30 AATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCA

AACAAAGATGGAATTTGTTGATGACTCGCGCTACAAACATCGGCCACTTGGATTC
 ATGGACACCATACTGTTATAAAACGGAAACATGTGTTTGATTGCTTTTTCTTTTC
 ATGAATCACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGA
 [SEQ ID NO: 23];

5 [0077] ATAATCATTATATAACATAGAAGTAAGGAATAAAATATGACAGC
 ATAAGTATAACACATGTAGATAAAGATCATTCATAGAAAATAACATATTTACAAA
 TAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAAATATTTGAATAAATATT
 TAAATATCTATCAATCAATAAATAAAGTACATAAAAGTTAAAAATGAACTAAAGT
 ATTTGAAAAAGTTTTACATCAATAAATATATCTAGTTTACGCTAAATCAAAATAA
 10 TATATTTACATCATGGT [SEQ ID NO: 24];

 [0078] ATAATCATTATATAACATAGAAGTAAGGAATAAAATATGACAGC
 ATAAGTATAACACATGTAGATAAAGATCATTCATAGAAAATAACATATTTACAAA
 TAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAAATATTTGAATAAATATT
 TAAATATCTATCAATCAATAAATAAAGTACATAAAAGTTAAAAATGAACTAAAGT
 15 ATTTGAAAAAGTTTTACATCAATAAATATATCTAGTTTACGCTAAATCAAAATAA
 TATATTTACATCATGGT [SEQ ID NO: 25]; and

 [0079] TCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCT
 AATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCA
 AACAAAGATGGAATTTGTTGATGACTCGCGCTACAAACATCGGCCACTTGGATTC
 20 ATGGACACCATACTGTTATAAAACGGAAACATGTGTTTGATTGCTTTTTCTTTTC
 ATGAATCACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGA
 [SEQ ID NO: 26].

 [0080] In some embodiments, the promoter sequence further comprises upstream of
 (g), (h) or (i) a nucleotide sequence (also referred to herein as segment Δ sequence) selected
 25 from the group consisting of:

 [0081] (j) RRSKWMWAKYTYWRKAMWTRGTAYAKSRRTRMWYRWRTYT
 WTMARTAWTTAWWTWWRTAARWAWCTAYN_{ah}YDATWYAWWTARWDRN_{ai}RAG
 KWSMYWSRWGWYAMMRAKGWRTTWATAKGWMTTYGWMAAYSTYWASATYR
 RTMARYWTAMTMTYTYAN_{aj}GGYWRRRKCMAAAKMAN_{ak}SATYATGGTYWRMW
 30 WWGKWKYWKATWSTAKWMWWWTMCRKATWWTAWTKMAWATMCWWMW
 TWMRWTWMTYTCAKATRYRRATMYAAATYMAGN_{al}TARTKWRAAWTSAAWTM

WGMRWTAWSMATTTTAKWTWCATACYYGRWWTGGRTGKKAGMAAKWWMKG
AWTMRYWTWWWTYRRWSRKYTTSAWRWMCWKW [SEQ ID NO: 27];

[0082] (k) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 27 or a complement thereof; and

[0083] (l) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 27 or a complement thereof, under at least medium or high stringency conditions,

[0084] wherein:

[0085] A, C, G, T, M, R, W, S, Y, K, V, H, D, B and N are as defined above;

10 [0086] each of ah and al is an integer from 0-1;

[0087] aj is an integer from 0-2;

[0088] ai is an integer from 0-5; and

[0089] ak is an integer from 0-8.

15 [0090] In some embodiments, N_{ah} is G; N_{ai} is a sequence selected from AATAA, AATA or TAGGT; N_{aj} is the sequence AA; N_{ak} is a sequence selected from TATGTTTA or AATTAAAC; or N_{al} is C. In some embodiments, any one or more of N_{ah} , N_{ai} , N_{aj} , N_{ak} or N_{al} comprises 0 bases.

[0091] Representative sequences according to SEQ ID NO: 27 may be selected from the group consisting of:

20 [0092] AACTAATATCTCAGTAAATAGTATAGCAATAAATGAGTCTATAA
GTATTTAAATATATAAATATCTACTGATATAAATAAATAAAAGTACATACA
AGTTACAAATGAATTAATAAGGTATTTGAAAACGTTTACATCAATAAATATACTA
TTTAGGCTAAATCAAAATAATATGTTTACATCATGGTTTACATAGTTGCTTTATT
CTATACAAATACGGATAATATTGAATATCCATCGTACGTTTATTTTCATATACGGAT
25 CCAAATTCAGCTAATGAAAAATGAATTCAGAAATATCTATTTTATTTTCATACCTG
AAATGGATGTGAGAAATATATGAATAATATAATTCAGCCAGCTTCATATCCATT
[SEQ ID NO: 28];

30 [0093] AACGAATATATCAATAAATAGTATAGCAATAAATGAGTCTATAA
GTAATTAAATATATAAATATCTATCAATATAAATAAATAAAGTACATACAAGTTA
CAAATGAATTAATAAGGTATTTGAAAACGTTTAAATCAATAAATATACTATTTTA

GGCTAAATCAAATAACATCATGGTTTACATAGTTGCTTTATTCTATACAAATAC
 GGATAATATTGAATATCCTTCATACGTTTATTTTCATATATGAATCCAAATTCAGCT
 AATGAAAAATGAATTCAGAGATATCCATTTTATTTTCATACCTGAAATGGATGTG
 AGAAATATATGAATAATATAATTCAGACAGCTTCATATCCATT [SEQ ID NO: 29];

5 [0094] AACGAATATCTCAATAAATAGTATAGCAATAAATGAGTCTATAA
 GTAATTAAATATATAAATATCTATCGATATAAATAAATAAATAAAGTACATACAA
 GTTACAAATGAATTAATAAGGTATTTGAAAACGTTTACATTAATAAATATACTAT
 TTTAGGCTAAATCAAATAACATCATGGTTTACATAGTTGCTTTATTCTATACAAA
 TACGGATAATATTGAATATCCTTCATACGTTTATTTTCATATATGAATCCAAATTCA
 10 GCTAATGAAAAATGAATTCAGAGATATCCATTTTATTTTCATACCTGAAATGGAT
 GTGAGAAATATATGAATAATATAATTCAGACAGCTTCATATCCATT [SEQ ID NO:
 30];

 [0095] GGGTTCAAGTTTTAGACTTGGTACATGGGTGCTCATATTTTTCAA
 TATTTATTTTAGTAAGAACTATGTTATTCATTTAGTAGTAGGTGAGGTGCCTGGT
 15 GACAACGAGGTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCT
 CTCAAAGGTAGGGGCCAAAGCAAATTAAACGATTATGGTCAGATATGGATTCAG
 ATAGTAGTATTTTCCATATTTTAATTCAAATACTAATTTAAATACTCTCAGATGCA
 AATATAAATCAAGTAGTTTGAATTCAAATATGCATTAAGAATTTTAGATACATAC
 TCGGTTTGGGTGGTAGCAAGTACGGATTCGCTTTTATTGATGGTTTTGAAGAACG
 20 GA [SEQ ID NO: 31]; and

 [0096] GGGTTCAAGTTTTAGACTTGGTACATGGGTGCTCATATTTTTCAA
 TATTTATTTTAGTAAGAACTATGTTATTCATTTAGTGGTAGGTGAGGTGCCTGGT
 GACAACGAGGTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCT
 CTCAAAGGTAGGGGCCAAAGCAAATTAAACGATTATGGTCAGATATGGATTCAG
 25 ATAGTAGTATTTTCCATATTTTAATTCAAATACTAATTTAAATACTCTCAGATGCA
 AATATAAATCAAGTAGTTTGAATTCAAATATGCATTAAGAATTTTAGATACATAC
 TCGGTTTGGGTGGTAGCAAGTACGGATTCGCTTTTATTGATGGTTTTGAAGAACG
 GA [SEQ ID NO: 32].

 [0097] In some embodiments, the promoter sequence further comprises upstream of
 30 (j), (k) or (l) a nucleotide sequence (also referred to herein as segment Θ sequence) selected
 from the group consisting of:

[0098] (m) YWYTYWRWKTGKSYKWWYWDMAAGWRWSYATWN_{am}RMKWT
RWRRTBTWGAAGMWTBKAATWRYSMCATRTATAN_{an}TAAAGAAWARN_{ao}AN_{ap}TK
ACAYTAYWWGWAYAWYAYATSTARRYAMWKGTCATWYATRRAMMMTWYMW
YAWWKAKWN_{aq} [SEQ ID NO: 33];

5 [0099] (n) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 33 or a complement thereof; and

[0100] (o) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 33 or a complement thereof, under at least medium or high stringency conditions,

10 [0101] wherein:

[0102] A, C, G, T, M, R, W, S, Y, K, V, H, D, B and N are as defined above;

[0103] each of *am* and *ao* is an integer from 0-1;

[0104] *ap* is an integer from 0-2;

[0105] *an* is an integer from 0-11; and

15 [0106] *aq* is an integer from 0-26.

[0107] In some embodiments, N_{am} is W; N_{an} is a sequence selected from AAACGCAAAAT [SEQ ID NO: 34], AAACGCAAAAC [SEQ ID NO: 35] or TAAAGAGTATA [SEQ ID NO: 36]; N_{ao} is T; N_{ap} is a sequence selected from TA or AA; or N_{aq} is a sequence selected from GCAATAAATGAGTCTATAATATGCAC [SEQ ID NO: 37] or GGGGGTGGAAACCTTCCTTCAC [SEQ ID NO: 38]. In other embodiments, any one or more of N_{am}, N_{an}, N_{ao}, N_{ap} or N_{aq} comprises 0 nucleotides.

[0108] Representative examples of sequences according to SEQ ID NO: 33 include:

25 [0109] TATTCAATTTGGGTGATAGCAAGTATGCATTGCTTTATGGTTTTG
AAGAATGGAATAATCACATATATAAAACGCAAAATTAAAGAATAAATATGACAC
TATATGTATAATACATGTAGACAATGGTCATTCATAGAAAATAACATATTTATAG
CAATAAATGAGTCTATAATATGCAC [SEQ ID NO: 39];

[0110] TATTCAATTTGGGCGATATCAAGTATGCATTTGCTTTATGGTTTTG
AAGAATGGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACTA

TAAGTATAATACATGTAGACAATGGTCATTCATAGAACATAACATATTTAGA
[SEQ ID NO: 40];

[0111] TATTCAATTTGGGCGATAGCAAGTATGCATTTGCTTTATGGTTTT
GAAGAATTGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACT
5 ATAAGTATAATACATGTAGGCAATGGTCATTCATAGAACATAACATATTTAGA
[SEQ ID NO: 41];

[0112] CTCTTTGAGTGTCTTTCTAAAAGAGACTATAAAAGATGAAATGTA
GAAGCTTCTAATTGCGCCATGTATATAAAGAGTATATAAAGAAAAGTAAATTACA
TTACTTGAATATCATATCTAAATACATGTCATATATGAACCTTTTCCAAAGAGTG
10 GGGGTGGAACCTTCCTTCAC [SEQ ID NO: 42]; and

[0113] CTCTTTGAGTGTCTTTCTAAAAGAGACTATAAAAGATGAAATCTA
GAAGCTTCTAATTGCGCCATGTATATAAAGAAAAGTAAATTACATTACTTGAACA
TCATATCTAAATACATGTCATATATGAACCTTTTCCAAAGAGTGGGGGTGGAAC
CTTCCTTCAC [SEQ ID NO: 43].

15 [0114] In some embodiments, an intervening sequence (also referred to herein as
“segment Ψ sequence”) is interposed between the nucleotide sequence according to any one
of (d), (e) or (f) and the nucleotide sequence according to the nucleotide sequence according
to any one of (g), (h) or (i). Generally, the intervening sequence comprises from about 150 to
about 250 nucleotides, typically from about 180 to about 220 nucleotides and suitably from
20 about 190 to about 210 nucleotides. In illustrative examples of this type, the intervening
sequence is selected from the group consisting of:

[0115] TCTATACCTAATAATAAAGAGGCAAAATTTCTCTTCACCTGTTTT
TTTTGGGTCCGCCATCCCTTAACCTTTGCGAATGTGAAAACTGCTTATAGC
CCTTCTCTTTATATAATTAGGAATCATAATCCAATTAGATCTTTCTGATTTCTGGGT
25 GAATAGGAATATTAATCCAAATAGAAAAAATATAATAATATGCA [SEQ ID NO:
44], or a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all
integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO:
44 or a complement thereof, or a nucleotide sequence that hybridizes to the sequence set forth
in SEQ ID NO: 44 or a complement thereof, under at least medium or high stringency
30 conditions;

[0116] TTCATTAGGTCCTTTATTCTATACAAATACGAATAATATTGAATA
TCCATCCCATATTGATTCATATATAGATCCAAATTTAGCTAATAAAAATGAATTC

AGATATATCCAGCTTCAGATCCATTTCCACCGTTACTTGAAGATGCTAATAGGGG
 TGTTTATAGGGGTGAGTATGCGTGAGAGCGTGTATGTCTGTACTT [SEQ ID NO:
 45] , or a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and
 all integer percentages in between) sequence identity with the sequence set forth in SEQ ID
 NO: 45 or a complement thereof, or a nucleotide sequence that hybridizes to the sequence set
 forth in SEQ ID NO: 45 or a complement thereof, under at least medium or high stringency
 conditions; and

[0117] TTCATTAGGTCCTTTATTCTATACAAATACGAATAATATTGAATA
 TCCATCCCATATTGATTTTCATATATAGATCCAAATTTAGCTAATAAAAATGAATTC
 10 AGATATATCCAGCTTCAGATCCATTTCCACCGTTACTTGAAGATGCTAATAGGGG
 TGTTTATAGGGGTGAGTATGCGTGAGAGCGTGTATGTCTGTACTT [SEQ ID NO:
 46] , or a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and
 all integer percentages in between) sequence identity with the sequence set forth in SEQ ID
 NO: 46 or a complement thereof, or a nucleotide sequence that hybridizes to the sequence set
 15 forth in SEQ ID NO: 46 or a complement thereof, under at least medium or high stringency
 conditions.

[0118] In specific embodiments, the promoter sequence has a structure represented
 by formula (I):

[0119] $5' - \Theta - \Delta - Z - \Psi - \Phi - \Omega - 3'$ (I)

20 [0120] wherein:

[0121] Θ may be present or absent and is selected from any one of (m), (n) or (o);

[0122] Δ may be present or absent and is selected from any one of (j), (k) or (l);

[0123] Z is selected from any one of (g), (h) or (i);

[0124] Ψ is an optional spacer or intervening sequence;

25 [0125] Φ is selected from any one of (d), (e) or (f); and

[0126] Ω is selected from any one of (a), (b) or (c).

[0127] In illustrative examples of this type, the promoter sequence comprises a
 nucleotide sequence selected from the group consisting of:

[0128] TATTCAATTTGGGTGATAGCAAGTATGCATTGCTTTATGGTTTTG
 30 AAGAATGGAATAATCACATATATAAAACGCAAAATTAAAGAATAAATATGACAC

TATATGTATAATACATGTAGACAATGGTCATTCATAGAAAATAACATATTTATAG
 CAATAAATGAGTCTATAATATGCACAATAATATCTCAGTAAATAGTATAGCAAT
 AAATGAGTCTATAAGTATTTAAATATATAAATATCTACTGATATAAATAAATAAA
 TAAAAGTACATACAAGTTACAAATGAATTAATAAGGTATTTGAAAACGTTTACAT
 5 CAATAAATATACTATTTTAGGCTAAATCAAATAATATGTTTACATCATGGTTTAC
 ATAGTTGCTTTATTCTATACAAATACGGATAATATTGAATATCCATCGTACGTTTA
 TTTCATATACGGATCCAAATTCAGCTAATGAAAAATGAATTCAGAAATATCTATT
 TTATTTTCATACCTGAAATGGATGTGAGAAATATATGAATAATATAATTCAGCCA
 GCTTCATATCCATTTCTACCGGTACTCGAAGGAGCGTCCATGAGAGCATGTGTGT
 10 CCCTAATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAA
 ATCAAACAAAGATGGAATTTGTTGATGACTCGCTTTACAAACATCGGCCACTTGG
 ATTCATGGACACCATACTGTTATAAAACAGAAACATGTGTTTGATTTCGTGTTTTCT
 TTTCATGAATCACAATATAAATATAGATATTCATGTGCGCACTCACCCATTATGA
 ATCTATACCTAATAATAAAGAGGCCAAAATTTCTCTTCACCTGTTTTTTTTGGGTCC
 15 GGCCATCCCTTAACCTTTGCGAATGTGAAAAACTGCTTATAGCCCTTCTCTTT
 ATATAATTAGGAATCATAATCCAATTAGATCTTTCTGATTTTCGGGTGAATAGGAA
 TATTAATCCAAATAGAAAAAATATAATAATATGCACAATAATATCTCTATGAGC
 ATCTTCGAAAGACAAAGACGGCAGATCTCAAAATTGTTGAGGTCATAATAAGCAT
 TGCACCTCAAACAGGCATGTTGCTTATTAAAGAATAGCGAAGCAATGAAGAATAA
 20 ATCCAAGAAAATATGAGCAAGCACGTCAATCGAGCACTTGACCAACTGGGTAGA
 CATGTTCAATCACAAGAGGTGCTCAATTTCTAATCATGGTCAATATAATCAAACC
 CCACCGAGTATATTACTTAGAGCATCTCCAATAGTTTTCAAAAAAAAAAATCA
 TCGCAAAAAAAGTTTTCAAAAAAATAATTGGTAAATCAATGAGATTTACAAGTCG
 CTAAAAAAAGTTGGAGGCGTAATTGTTGGGGCTTTTGACTTTTTTCCAACAGTTTA
 25 TAAAATCACGCTCCTAAAGTATAGAAAATAATTTTGCATTAGGAATCTTAAACTA
 TTTTCAAATTACCCTAATCATTTTTTATACTTTTTTTCTTTCTTGTATATTTGCATTT
 TGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCCAGTTTTTCGGATTT
 AGAAACAAAAACGGCAAATTGCATACCCAACCTCATCGCCCATATAAAACGTTAC
 CAACCCAACATATATTTCC [SEQ ID NO: 47], or a nucleotide sequence that shares at
 30 least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence
 identity with the sequence set forth in SEQ ID NO: 47 or a complement thereof, or a
 nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 47 or a
 complement thereof, under at least medium or high stringency conditions;

[0129] TATTCAATTTGGGCGATATCAAGTATGCATTTGCTTTATGGTTTTG
 AAGAATGGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACTA
 TAAGTATAATACATGTAGACAATGGTCATTCATAGAACATAACATATTTAGAAAC
 GAATATATCAATAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAATATAT
 5 AAATATCTATCAATATAAATAAATAAAGTACATACAAGTTACAAATGAATTAATA
 AGGTATTTGAAAACGTTTAAATCAATAAATATACTATTTTAGGCTAAATCAAAAT
 AACATCATGGTTTACATAGTTGCTTTATTCTATACAAATACGGATAATATTGAATA
 TCCTTCATACGTTTATTTTCATATATGAATCCAAATTCAGCTAATGAAAAATGAATT
 CAGAGATATCCATTTTATTTTCATACCTGAAATGGATGTGAGAAATATATGAATA
 10 ATATAATTCAGACAGCTTCATATCCATTTCCACCGTTACTTGAAGGAGTGTCCATG
 AGAGCATGTGTGTCCCTAATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTT
 GTTATATTAATAAATCAAACAAAGATGGAATTTGTTGATGACTCGTGCTACAAAC
 ATCGGCCACTTGGAGTCATGGACACCCTACTGTTATAAAACGGAAACATGTGTTT
 GATTCGCTTTTTCTTTTCATGAATCACAATATAAATATAGACATTCATGTGCGCAC
 15 TCACACCTTATGGACAACTAATATCTCTAGGAGCATCTTCAAAAGACAAAGACGA
 CAGATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTGCG
 CTTATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAAAAAAAAATATGAGCA
 AGCACATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGG
 AGCTCAATTTATAATCATGGCTAATATAATCAAACCCACCGAGTATATTACT
 20 TAGAGCATCTCCAATAGTTTTCAAAAAAAAAATTCATCGCAAAAAAAAAAGTTTTCAA
 AAAAATAATTGGTAAATCAATGAGATTTACAAGTCGCTAAAAGGTTGGAGGCA
 TAATTGTTGGGGCTTTGACTTTTTTTCCAACAGTTTATAAAATCACGCTCCTAAAG
 TATAGAAAACATGCATTAGGAATCTTAAACTATTTTCAAATTACTCTAATCATTTT
 TATACTTTTTTTCTTTCTTGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAAT
 25 CATGGTTAATCAAACCCAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCAT
 ACCCAACTCATCGCCCATATAAAACGGCACCAACCAACATATTTCC [SEQ ID
 NO: 48], or a nucleotide sequence that shares at least 80% (and at least 81% to at least 99%
 and all integer percentages in between) sequence identity with the sequence set forth in SEQ
 ID NO: 48 or a complement thereof, or a nucleotide sequence that hybridizes to the sequence
 30 set forth in SEQ ID NO: 48 or a complement thereof, under at least medium or high
 stringency conditions;

[0130] TATTCAATTTGGGCGATAGCAAGTATGCATTTGCTTTATGGTTTTT
 GAAGAATTGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACT
 ATAAGTATAATACATGTAGGCAATGGTCATTCATAGAACATAACATATTTAGAAA

CGAATATCTCAATAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAAATATA
 TAAATATCTATCGATATAAATAAATAAATAAAGTACATACAAGTTACAAATGAAT
 TAATAAGGTATTTGAAAACGTTTACATTAATAAATATACTATTTTAGGCTAAATC
 AAAATAACATCATGGTTTACATAGTTGCTTTATTCTATACAAATACGGATAATATT
 5 GAATATCCTTCATACGTTTATTTTCATATATGAATCCAAATTCAGCTAATGAAAAAT
 GAATTCAGAGATATCCATTTTATTTTCATACCTGAAATGGATGTGAGAAATATAT
 GAATAATATAATTCAGACAGCTTCATATCCATTTCCACCGTTACTTGAAGGAGTG
 TCCATGAGAGCATGTGTGTCCCTAATGTGTTTTCTTTAAATATAGTTATCCTTTGG
 AAAGTTGTTATATTAATAAATCAAACAAAGATGGAATTTGTTGATGACTCGCGCT
 10 ACAAACATCGGCCACTTGGATTCATGGACACCATACTGTTATAAAACGGAAACAT
 GTGTTTGATTGCTTTTTCTTTTCATGAATCACAATATAAATATAGACATTCATGT
 GCGCACTCACACCTTATGGACAATAATATCTCTATGAGCATCTTTAAAAGACAA
 AGACAACAGATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGAG
 CATGTCGCTTATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAGAAAATAT
 15 GAGCAAGCACATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACA
 AGAGGAGCTCATCAAACCCACCGAGTATATTACTTAGAGCATCTCCAATAG
 TTAAAAAAAATCATCGCAAAAAAGGTTTTCAAAAAAATAATTGGTAAATCAATG
 AGATTTACAAGTCGCTAAAAAAAGTTGGAGGCGTATTTGTTGGGGGTTTTTGACT
 TTTTCCAACAGTTTATAAAATCATGCTCCTAAAGTATAGAAAACAATTTTGCATT
 20 AGGAATCCTAAACTATTTCTAAATTACCCTAATCATTTTTTATACTTTTTTTCTTTC
 TTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCC
 AGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACCTCATCGCCCAT
 ATAAACGGCACCAACCCAACATATATTTCC [SEQ ID NO: 49], or a nucleotide
 sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages
 25 in between) sequence identity with the sequence set forth in SEQ ID NO: 49 or a complement
 thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 49
 or a complement thereof, under at least medium or high stringency conditions;

[0131] CTCTTTGAGTGTCTTTCTAAAAGAGACTATAAAAGATGAAATGTA
 GAAGCTTCTAATTGCGCCATGTATATAAAGAGTATATAAAGAAAAGTAAATTACA
 30 TTAATTGAATATCATATCTAAATACATGTCATATATGAACCCTTTTCCAAAGAGTG
 GGGGTGGAACCTTCCTTCACGGGTTCAAGTTTTAGACTTGGTACATGGGTGCTCA
 TATTTTTCAATATTTATTTTAGTAAGAACTATGTTATTCATTTAGTAGTAGGTGA
 GGTGCCTGGTGACAACGAGGTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTC
 AGCTTAATCTCTCAAAGGTAGGGGCCAAAGCAAATTAACGATTATGGTCAGATA

TGGATTCAGATAGTAGTATTTTCCATATTTTAATTCAAATACTAATTTAAATACTC
 TCAGATGCAAATATAAATCAAGTAGTTTGAATTCAAATATGCATTAAGAATTTTA
 GATACATACTCGGTTTGGGTGGTAGCAAGTACGGATTCGCTTTTATTGATGGTTTT
 GAAGAACGGAATAATCATTATATAACATAGAAGTAAGGAATAAAATATGACAGC
 5 ATAAGTATAACACATGTAGATAAAGATCATTCATAGAAAATAACATATTTACAAA
 TAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAAATATTTGAATAAATATT
 TAAATATCTATCAATCAATAAATAAAGTACATAAAAGTTAAAAATGAACTAAAGT
 ATTTGAAAAAGTTTTACATCAATAAATATATCTAGTTTACGCTAAATCAAAATAA
 TATATTTACATCATGGTTTCATTAGGTCCTTTATTCTATACAAATACGAATAATAT
 10 TGAATATCCATCCCATATTGATTTTCATATATAGATCCAAATTTAGCTAATAAAAAAT
 GAATTCAGATATATCCAGCTTCAGATCCATTTCCACCGTTACTTGAAGATGCTAAT
 AGGGGTGTTTATAGGGGTGAGTATGCGTGAGAGCGTGTATGTCTGTACTTTGTTA
 GTTACCCCTTAGAAAAGTTGTTAATAAATCAGCCAAAGATGGAATTTGTTGACTCG
 TGCTACAACCATGGGCCACTTGAATTCATGGACACCATACTGTTGAAACAAGAAC
 15 ATGTGTTTCATTTGCTTAATCACATGACCTAGCTAGAGAGCTTTTCCAATACTTTC
 CAGATGAAATGTAATATACTCCACACATAATTGTTGAAGTATATATATATTAATC
 TATCACCCACATTTCTTAATAACTTATACTTTTGAGCTAATTGTTTGGCACATAC
 AAGATACAACCTCACAATGCTATCAAAGCTCAGTTGGCTCGACCTTGATTATTGGC
 TAGGGAAATTCAAATAAAAAGATGGCACAATATTCTTACTCGTATTCCAAGGGCT
 20 AGGCCAACTAAAATACGAGGTATATGTCAAACTCAAATGTGCGGTTTTGGGTG
 AATTATTGCTCAACACATTCAGCTCAACTCAATTCCCTAGGATATTTAACCCTCTC
 TTATAAGTACATTATTTATGTGCCAACCCAATTAGAAGTGCTAATCTTGAATTTAC
 TAACAAAAATGGCAAATTGCATACCCAACCTCACCCATATAAAAAGGCACCAACC
 CAACATTATTTTC [SEQ ID NO: 50], or a nucleotide sequence that shares at least 80%
 25 (and at least 81% to at least 99% and all integer percentages in between) sequence identity
 with the sequence set forth in SEQ ID NO: 50 or a complement thereof, or a nucleotide
 sequence that hybridizes to the sequence set forth in SEQ ID NO: 50 or a complement thereof,
 under at least medium or high stringency conditions;

[0132] CTCTTTGAGTGTCTTTCTAAAAGAGACTATAAAAGATGAAATCTA
 30 GAAGCTTCTAATTGCGCCATGTATATAAAGAAAAGTAAATTACATTACTTGAACA
 TCATATCTAAATACATGTCATATATGAACCCTTTTCCAAAGAGTGGGGGTGGAAC
 CTTCTTCACGGGTTCAGTTTTAGACTTGGTACATGGGTGCTCATATTTTCAAT
 ATTTATTTTAGTAAGAACTATGTTATTCATTTAGTGGTAGGTGAGGTGCCTGGTG
 ACAACGAGGTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCTCT

CAAAGGTAGGGGCCAAAGCAAATTAACGATTATGGTCAGATATGGATTCAGAT
 AGTAGTATTTTCCATATTTTAATTCAAATACTAATTTAAATACTCTCAGATGCAAA
 TATAAATCAAGTAGTTTGAATTCAAATATGCATTAAGAATTTTAGATACATACTC
 GGTTTGGGTGGTAGCAAGTACGGATTTCGCTTTTATTGATGGTTTTGAAGAACGGA
 5 ATAATCATTATATAACATAGAAGTAAGGAATAAAATATGACAGCATAAGTATAA
 CACATGTAGATAAAGATCATTCATAGAAAATAACATATTTACAAATAAATAGTAT
 AGCAATAAATGAGTCTATAAGTAATTAAATATTTGAATAAATATTTAAATATCTA
 TCAATCAATAAATAAAGTACATAAAAGTTAAAAATGAACTAAAGTATTTGAAAA
 AGTTTTACATCAATAAATATATCTAGTTTACGCTAAATCAAAATAATATATTTACA
 10 TCATGGTTTCATTAGGTCCTTTATTCTATACAAATACGAATAATATTGAATATCCA
 TCCCATATTGATTTTCATATATAGATCCAAATTTAGCTAATAAAAAATGAATTCAGAT
 ATATCCAGCTTCAGATCCATTTCCACCGTTACTTGAAGATGCTAATAGGGGTGTTT
 ATAGGGGTGAGTATGCGTGAGAGCGTGTATGTCTGTACTTTGTTAGTTACCCCTTA
 GAAAGTTGTTAATAAATCAGCCAAAGATGGAATTTGTTGACTCGTGCTACAACCA
 15 TGGGCCACTTGAATTCATGGACACCATACTGTTGAAACAAGAACATGTGTTTCAT
 TTGCTTAATCACATGACCTAGCTAGAGAGCTTTTCCAATACTTTCCAGATGAAATG
 TAATATACTCCACACATAATTGTTGAGGTATATATATTAATCTATCACCCACATTT
 CCTTAATAACTTATACTTTTGAGCTAATTGTTTGGCACATACAAGATACAACCTCAC
 AATGCTATCAAAGCTCAGTTGGCTCGACCTTGATTATTGGCTAGGGAAATTCAAA
 20 TAAAAAGATGGCACAATATTCTTACTCGTATTCCAAGGGCTAGGCAAACTAAAAT
 ACGAGGTATATGTCAAACTCAAAATGTGCGGTTTTGGGTGAATTATTGCTCAAC
 ACATTCAGCTCAACTCAATTCCCTAGGATATTTAACCCTCTCTTATAAGTACATTA
 TTTATGTGCCAACCCAATTGGAAGTGCTAATCTTGAATTTACTAACAAAAATGTC
 AAATTGCATACCCAACCTCACCCATATAAAAAGGCACCAACCCAACATTATTTTC

25 [SEQ ID NO: 51], or a nucleotide sequence that shares at least 80% (and at least 81% to at
 least 99% and all integer percentages in between) sequence identity with the sequence set
 forth in SEQ ID NO: 51 or a complement thereof, or a nucleotide sequence that hybridizes to
 the sequence set forth in SEQ ID NO: 51 or a complement thereof, under at least medium or
 high stringency conditions; and

30 [0133] TCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCT
 AATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCA
 AACAAAGATGGAATTTGTTGATGACTCGCGCTACAAACATCGGCCACTTGGATTC
 ATGGACACCATACTGTTATAAAACGGAAACATGTGTTTGATTTCGCTTTTTCTTTTC
 ATGAATCACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGACAA

CTAATATGTCTATGAGCATCTTCAAAAGACAAAGACGACAGATCTCAAGATTGTT
 GAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTCGCTTATTAAAGAATAGCG
 AAGCAATCAAGAATAAATTCAAAAAAATATGAGCAAGCACATCAATCGAGGACT
 TGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGCTCAATTTCTAATCATGG
 5 CTAATATAATCAAACCCACCCAGTATATTACTTAGAGCATCTCCAATAGTTT
 TTTTAAAAAATCATCGCAAAAAAAGCTTTCAAAAAAATAATTGGTAAATCAAT
 GAGATTTACAAGTCGCTAAAAAAGTTGGAGGCGTAATTGTTGGGGCTTTGACTTT
 TTTCCCGTTTATAAAATCACGCTCCTAAAGTATAGAAAACAATTTTGCATTAGG
 AATCTTAAATATTTTCAAATTACCCTAATCATTTTTTATACTTTTTTCTTTCTTGT
 10 ATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCCA
 GTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCATCGCTCATA
 TAAACGGCACCAACCAACATATTTCC [SEQ ID NO: 52], or a nucleotide sequence
 that shares at least 80% (and at least 81% to at least 99% and all integer percentages in
 between) sequence identity with the sequence set forth in SEQ ID NO: 52 or a complement
 15 thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 52
 or a complement thereof, under at least medium or high stringency conditions.

[0134] In other illustrative examples, the promoter sequence comprises a nucleotide
 sequence selected from the group consisting of:

[0135] TATTCAATTTGGGTGATAGCAAGTATGCATTGCTTTATGGTTTTG
 20 AAGAATGGAATAATCACATATATAAAACGCAAAATTAAAGAATAAATATGACAC
 TATATGTATAATACATGTAGACAATGGTCATTCATAGAAAATAACATATTTATAG
 CAATAAATGAGTCTATAATATGCACAATAATATCTCAGTAAATAGTATAGCAAT
 AAATGAGTCTATAAGTATTTAAATATATAAATATCTACTGATATAAATAAATAAA
 TAAAAGTACATACAAGTTACAAATGAATTAATAAGGTATTTGAAAACGTTTACAT
 25 CAATAAATATACTATTTTAGGCTAAATCAAAATAATATGTTTACATCATGGTTTAC
 ATAGTTGCTTTATTCTATACAAATACGGATAATATTGAATATCCATCGTACGTTTA
 TTTCATATACGGATCCAAATTCAGCTAATGAAAAATGAATTCAGAAATATCTATT
 TTATTTTCATACCTGAAATGGATGTGAGAAATATATGAATAATATAATTCAGCCA
 GCTTCATATCCATTTCTACCGGTACTCGAAGGAGCGTCCATGAGAGCATGTGTGT
 30 CCCTAATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAA
 ATCAAACAAAGATGGAATTTGTTGATGACTCGCTTTACAAACATCGGCCACTTGG
 ATTCATGGACACCATACTGTTATAAAACAGAAACATGTGTTTGATTCGTGTTTTCT
 TTTCATGAATCACAATATAAATATAGATATTCATGTGCGCACTCACCCATTATGA

ATCTATACCTAATAATAAAGAGGGCAAAATTTCTCTTCACCTGTTTTTTTTTGGGTCC
 GGCCATCCCTTAACCTAATTTGCGAATGTGAAAACTGCTTATAGCCCTTCTCTTT
 ATATAATTAGGAATCATAATCCAATTAGATCTTTCTGATTTTCGGGTGAATAGGAA
 TATTAATCCAAATAGAAAAAATATAATAATATGCACAATAATATCTCTATGAGC
 5 ATCTTCGAAAGACAAAGACGGCAGATCTCAAATTGTTGAGGTCATAATAAGCAT
 TGCACCTCAAACAGGCATGTTGCTTATTAAAGAATAGCGAAGCAATGAAGAATAA
 ATCCAAGAAAATATGAGCAAGCACGTCAATCGAGCACTTGACCAACTGGGTAGA
 CATGTTCAATCACAAGAGGTGCTCAATTTCTAATCATGGTCAATATAATCAAACC
 CCACCGAGTATATTACTTAGAGCATCTCCAATAGTTTTCAAAAAAAAAAATCA
 10 TCGCAAAAAAAGTTTTCAAAAAAATAATTGGTAAATCAATGAGATTTACAAGTCG
 CTAAAAAAGTTGGAGGCGTAATTGTTGGGGCTTTTGACTTTTTTCCAACAGTTTA
 TAAATCACGCTCCTAAAGTATAGAAAATAATTTTGCATTAGGAATCTTAAACTA
 TTTTCAAATTACCCTAATCATTTTTTATACTTTTTTTCTTTCTTGTATATTTGCATTT
 TGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCCAAGTTTTTCGGATTT
 15 AGAAACAAAAACGGCAAATTGCATACCCAATCATCGCCCATATAAAACGTTAC
 CAACCCAACATATATTTCCACCTAATGCCGTCGCCCACTAATTGCCATAACACT
 AAGACGAGCGCCAACCTTCGGTGCTCTGCTTGCCTCGCTGTTGCTGTTGCCCATGG
 [SEQ ID NO: 53], or a nucleotide sequence that shares at least 80% (and at least 81% to at
 least 99% and all integer percentages in between) sequence identity with the sequence set
 20 forth in SEQ ID NO: 53 or a complement thereof, or a nucleotide sequence that hybridizes to
 the sequence set forth in SEQ ID NO: 53 or a complement thereof, under at least medium or
 high stringency conditions;

[0136] TCAAATAAATATAAATCAGGTAGTTCAAATTCAAATATACATTTA
 GATATGTATTCAATTTGGGCGATATCAAGTATGCATTTGCTTTATGGTTTTGAAGA
 25 ATGGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACTATAAG
 TATAATACATGTAGACAATGGTCATTCATAGAACATAACATATTTAGAAACGAAT
 ATATCAATAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAATATATAAAT
 ATCTATCAATATAAATAAATAAAGTACATACAAGTTACAAATGAATTAATAAGGT
 ATTTGAAAACGTTTAAATCAATAAATATACTATTTTAGGCTAAATCAAATAACA
 30 TCATGGTTTACATAGTTGCTTTATTCTATACAAATACGGATAATATTGAATATCCT
 TCATACGTTTATTTTCATATATGAATCCAAATTCAGCTAATGAAAAATGAATTCAG
 AGATATCCATTTTATTTTCATACCTGAAATGGATGTGAGAAATATATGAATAATA
 TAATTCAGACAGCTTCATATCCATTTCCACCGTTACTTGAAGGAGTGTCCATGAG
 AGCATGTGTGTCCCTAATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGT

TATATTAATAAATCAAACAAAGATGGAATTTGTTGATGACTCGTGCTACAAACAT
 CGGCCACTTGGAGTCATGGACACCCTACTGTTATAAAACGGAAACATGTGTTTGA
 TTCGCTTTTTCTTTTCATGAATCACAATATAAATATAGACATTCATGTGCGCACTC
 ACACCTTATGGACAATAATATCTCTAGGAGCATCTTCAAAAGACAAAGACGACA
 5 GATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTGCGCT
 TATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAAAAAAAAATATGAGCAAG
 CACATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAG
 CTCAATTTATAATCATGGCTAATATAATCAAACCCCAACCGAGTATATTATTACTTA
 GAGCATCTCCAATAGTTTTCAAAAAAAAAATTCATCGCAAAAAAAAAAGTTTTCAAAA
 10 AAATAATTGGTAAATCAATGAGATTTACAAGTCGCTAAAAAGGTTGGAGGCATA
 ATTGTTGGGGCTTTGACTTTTTTTTCCAACAGTTTATAAAATCACGCTCCTAAAGTA
 TAGAAAACATGCATTAGGAATCTTAAACTATTTTCAAATTACTCTAATCATTTTTTA
 TACTTTTTTTCTTTCTTGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCA
 TGGTTAATCAAACCCCAAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATAC
 15 CCAACTCATCGCCCATATAAAACGGCACCAACCCAACATATTTCCACCTAATGCC
 GTCGCCCAACTAATTGCCATAACACTAAGACGAGCGCCAACCTTGCGGTGCTCTG
 CTTGCCTCGCTGTTGCTGTTGCCCATGG [SEQ ID NO: 54], or a nucleotide sequence
 that shares at least 80% (and at least 81% to at least 99% and all integer percentages in
 between) sequence identity with the sequence set forth in SEQ ID NO: 54 or a complement
 20 thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 54
 or a complement thereof, under at least medium or high stringency conditions;

[0137] TTGGATCAAATAAATATAAATCAAGTAGTTCAAATTCAAATATAC
 ATTTAGATATGTATTCAATTTGGGCGATAGCAAGTATGCATTTGCTTTATGGTTTT
 GAAGAATTGAATAATCATATATAAAACGCAAACTAAAGAATAAATGACACT
 25 ATAAGTATAATACATGTAGGCAATGGTCATTCATAGAACATAACATATTTAGAAA
 CGAATATCTCAATAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAAATATA
 TAAATATCTATCGATATAAATAAATAAATAAAGTACATACAAGTTACAAATGAAT
 TAATAAGGTATTTGAAAACGTTTACATTAATAAATATACTATTTTAGGCTAAATC
 AAAATAACATCATGGTTTACATAGTTGCTTTATTCTATACAAATACGGATAATATT
 30 GAATATCCTTCATACGTTTATTTTCATATATGAATCCAAATTCAGCTAATGAAAAAT
 GAATTCAGAGATATCCATTTTATTTTCATACCTGAAATGGATGTGAGAAATATAT
 GAATAATATAATTGAGACAGCTTCATATCCATTTCCACCGTTACTTGAAGGAGTG
 TCCATGAGAGCATGTGTGTCCCTAATGTGTTTTCTTTAAATATAGTTATCCTTTGG
 AAAGTTGTTATATTAATAAATCAAACAAAGATGGAATTTGTTGATGACTCGCGCT

ACAAACATCGGCCACTTGGATTCATGGACACCATACTGTTATAAAACGGAAACAT
 GTGTTTGATTCGCTTTTTCTTTTCATGAATCACAATATAAATATAGACATTCATGT
 GCGCACTCACACCTTATGGACAATAATCTCTATGAGCATCTTTAAAAGACAA
 AGACAACAGATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGAG
 5 CATGTCGCTTATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAGAAAATAT
 GAGCAAGCACATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACA
 AGAGGAGCTCATCAAACCCACCGAGTATATTACTTAGAGCATCTCCAATAG
 TTAAAAAAAATCATCGCAAAAAAGGTTTTCAAAAAATAATTGGTAAATCAATG
 AGATTTACAAGTCGCTAAAAAAGTTGGAGGCGTATTTGTTGGGGGTTTTTGACT
 10 TTTTCCAACAGTTTATAAAATCATGCTCCTAAAGTATAGAAAACAATTTTGCATT
 AGGAATCCTAAACTATTTCTAAATTACCCTAATCATTTTTTATACTTTTTTTCTTTC
 TTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCC
 AGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCATCGCCCAT
 ATAAACGGCACCAACCCAACATATATTTCCACCTAATGCCGTCGCCCGACTAAT
 15 TGCCATAACTCTTAAAGACGAGCGCCAACCTTGCGGTGCTCTGCTTGCCCTCGCTGT
 TGCTGTTGCCCATGG [SEQ ID NO: 55], or a nucleotide sequence that shares at least 80%
 (and at least 81% to at least 99% and all integer percentages in between) sequence identity
 with the sequence set forth in SEQ ID NO: 55 or a complement thereof, or a nucleotide
 sequence that hybridizes to the sequence set forth in SEQ ID NO: 55 or a complement thereof,
 20 under at least medium or high stringency conditions;

[0138] CTGCAGGCGGCCGCGAATTCAGTAGTGATTACTATAGGGCACGC
 GTGGTCGACGGCCCGGGCTGGTATCGGGGCGTAGATTCCTCTCAACATATCAGCG
 TTGGAATATGTTTGCCTTTTGAGTGGCCAGCACCAAGTACCATAGCCCCAGCTAA
 CTAGCTCGAGTGGCCCTCGAGAATATCAGGGGTAGTTGCTATATACCAACATCAT
 25 GGTGAATTAACCTCAATGTCTTTCCTCACCAAATATTTAAAGGTGTTTACAGGTG
 TCCCTTTCCTGCTTTCATGTTGTAATCTTCAAGCTCTAGCAGCCAGGCGAACAAC
 CGAAGGAATTAAGAAGACGACTAGAAAGGCCTTGGTTGTATTTTTATTTTGGAT
 GTTATTTTGTGTCAAGTTTCTCTCTTTCGTACTGGCTATTTACATATGAGACACTCT
 TTGAGTGTCTTCTAAAAGAGACTATAAAAGATGAAATGTAGAAGCTTCTAATTG
 30 CGCCATGTATATAAAGAGTATATAAAGAAAAGTAAATTACATTACTTGAATATCA
 TATCTAAATACATGTCATATATGAACCCTTTTCCAAAGAGTGGGGGTGGAACCTT
 CCTTCACGGGTTCAGTTTTAGACTTGGTACATGGGTGCTCATATTTTCAATATT
 TATTTTAGTAAGAACTATGTTATTCATTTAGTAGTAGGTGAGGTGCCTGGTGAC
 AACGAGGTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCTCTCA

AAGGTAGGGGCCAAAGCAAATTAAACGATTATGGTCAGATATGGATTGAGATAG
 TAGTATTTTCCATATTTTAATTCAAATACTAATTTAAATACTCTCAGATGCAAATA
 TAAATCAAGTAGTTTGAATTCAAATATGCATTAAGAATTTTAGATACATACTCGG
 TTTGGGTGGTAGCAAGTACGGATTCGCTTTTATTGATGGTTTTGAAGAACGGAAT
 5 AATCATTATATAACATAGAAGTAAGGAATAAAATATGACAGCATAAGTATAACA
 CATGTAGATAAAGATCATTATAGAAAATAACATATTTACAAATAAATAGTATAG
 CAATAAATGAGTCTATAAGTAATTAAATATTTGAATAAATATTTAAATATCTATC
 AATCAATAAATAAAGTACATAAAAGTTAAAAATGAACTAAAGTATTTGAAAAAG
 TTTTACATCAATAAATATATCTAGTTTACGCTAAATCAAATAATATATTTACATC
 10 ATGGTTTCATTAGGTCCTTTATTCTATACAAATACGAATAATATTGAATATCCATC
 CCATATTGATTTTCATATATAGATCCAAATTTAGCTAATAAAAAATGAATTCAGATA
 TATCCAGCTTCAGATCCATTTCCACCGTTACTTGAAGATGCTAATAGGGGTGTTTA
 TAGGGGTGAGTATGCGTGAGAGCGTGTATGTCTGTACTTTGTTAGTTACCCCTTAG
 AAAGTTGTTAATAAATCAGCCAAAGATGGAATTTGTTGACTCGTGCTACAACCAT
 15 GGGCCACTTGAATTCATGGACACCATACTGTTGAAACAAGAACATGTGTTTCATT
 TGCTTAATCACATGACCTAGCTAGAGAGCTTTTCCAATACTTTCCAGATGAAATGT
 AATATACTCCACACATAATTGTTGAAGTATATATATATTAATCTATCACCCACATT
 TCCTTAATAACTTATACTTTTGAGCTAATTGTTTGGCACATACAAGATACAACCTCA
 CAATGCTATCAAAGCTCAGTTGGCTCGACCTTGATTATTGGCTAGGGAAATTCAA
 20 ATAAAAAGATGGCACAATATTCTTACTCGTATTCCAAGGGCTAGGCAAACCTAAAA
 TACGAGGTATATGTCAAACTCAAAATGTGCGGTTTTGGGTGAATTATTGCTCAA
 CACATTCAGCTCAACTCAATTCCCTAGGATATTTAACCCTCTCTTATAAGTACATT
 ATTTATGTGCCAACCCAATTAGAAGTGCTAATCTTGAATTTACTAACAAAAATGG
 CAAATTGCATACCCAACCTCACCCATATAAAAAGGCACCAACCCAACATTATTTTC
 25 ACCTAGTGTGTCGTCGCCGAACCTCATTGCCATAACACTAAGACGAGCGCCAACCTTG
 TGGTGCTCTTGCCCTCGCTGTTGCTGTCGCCCATGG [SEQ ID NO: 56], or a nucleotide
 sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages
 in between) sequence identity with the sequence set forth in SEQ ID NO: 56 or a complement
 thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 56
 30 or a complement thereof, under at least medium or high stringency conditions;

[0139] CTGCAGGCGGCCGCGAATTCAGTAGTACTATAGGGCACGC
 GTGGTCGACGGCCCGGGCTGGTATCGGGGCGTAGATTCCTCTCAACATATCAGCG
 TTGGAATATGTTTGCCTATTGAGTGGCCAGCACCAAGTACCATAGCCCCAGCTAA
 CTAGCTCGAGTGGCCCTCGAGAATATCAGAGGTAGTTGCTATATACCAACATCAT

GGTGAATTAACCTCAATGTCTTTCCTCACCAAAATATTTAAAGGTGTTTACAGGTG
TCCCTTTCCTGCTTTCATGGTGTAATCTTCAAGCTCTAGCAGGCAGGCGAACAAC
CGAAGGAATTAAGAAGACGACTAGAAAGGCCTTGGTTGTATTTTTATTTTTGGAT
GTTATTTTTGTGTCAAGTTTCTCTCTTTCGTAAGTGGCTATTTAGATATGAGACACTCT
5 TTGAGTGTCTTCTCTAAAAGAGACTATAAAAAGATGAAATCTAGAAGCTTCTAATTG
CGCCATGTATATAAAGAAAAGTAAATTACATTACTTGAACATCATATCTAAATAC
ATGTCATATATGAACCCCTTTTCCAAAGAGTGGGGGTGGAACCTTCCTTCACGGGT
TCAAGTTTTAGACTTGGTACATGGGTGCTCATATTTTTCAATATTTATTTTAGTAA
GAAACTATGTTATTCATTTAGTGGTAGGTGAGGTGCCTGGTGACAACGAGGTGTT
10 TATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCTCTCAAAGGTAGGGG
CCAAAGCAAATTAACGATTATGGTCAGATATGGATTCAGATAGTAGTATTTTCC
ATATTTTAATTCAAATACTAATTTAAATACTCTCAGATGCAAATATAAATCAAGT
AGTTTGAATTCAAATATGCATTAAGAATTTTAGATACATACTCGGTTTGGGTGGT
AGCAAGTACGGATTCGCTTTTATTGATGGTTTTGAAGAACGGAATAATCATTATA
15 TAACATAGAAGTAAGGAATAAAATATGACAGCATAAGTATAACACATGTAGATA
AAGATCATTCATAGAAAATAACATATTTACAAATAAATAGTATAGCAATAAATGA
GTCTATAAGTAATTAATATTTGAATAAATATTTAAATATCTATCAATCAATAAAT
AAAGTACATAAAAGTTAAAAATGAACTAAAGTATTTGAAAAAGTTTACATCAAT
AAATATATCTAGTTTACGCTAAATCAAATAATATATTTACATCATGGTTTCATTA
20 GGTCTTTTATTCTATACAAATACGAATAATATTGAATATCCATCCCATATTGATTT
CATATATAGATCCAAATTTAGCTAATAAAAATGAATTCAGATATATCCAGCTTCA
GATCCATTTCCACCGTTACTTGAAGATGCTAATAGGGGTGTTTATAGGGGTGAGT
ATGCGTGAGAGCGTGTATGTCTGTACTTTGTTAGTTACCCCTTAGAAAGTTGTAA
TAAATCAGCCAAAGATGGAATTTGTTGACTCGTGCTACAACCATGGGCCACTTGA
25 ATTCATGGACACCATACTGTTGAAACAAGAACATGTGTTTCATTTGCTTAATCAC
ATGACCTAGCTAGAGAGCTTTTCCAATACTTTCCAGATGAAATGTAATATACTCC
ACACATAATTGTTGAGGTATATATATTAATCTATCACCCACATTTCTTAATAACT
TATACTTTTGAGCTAATTGTTTGGCACATACAAGATACAACCTCACAATGCTATCA
AAGCTCAGTTGGCTCGACCTTGATTATTGGCTAGGGAAATTCAAATAAAAAGATG
30 GCACAATATTCTTACTCGTATTCCAAGGGCTAGGCAAACCTAAAATACGAGGTATA
TGTCAAAACTCAAATGTGCGGTTTTGGGTGAATTATTGCTCAACACATTCAGCT
CAACTCAATTCCTAGGATATTTAACCCTCTCTTATAAGTACATTATTTATGTGCC
AACCCAATTGGAAGTGCTAATCTTGAATTTACTAACAAAAATGTCAAATTGCATA
CCCAACTCACCCATATAAAAAGGCACCAACCCAACATTATTTTCGCCTAGTGTCG

TCGCCGAACTCATTGCCATAACACTAAGACGAGCGCCAACCTTGTGGTGCTCTTG
 CCTCGCTGTTGCTGTCGCCCATGG [SEQ ID NO: 57], or a nucleotide sequence that
 shares at least 80% (and at least 81% to at least 99% and all integer percentages in between)
 sequence identity with the sequence set forth in SEQ ID NO: 57 or a complement thereof, or a
 5 nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 57 or a
 complement thereof, under at least medium or high stringency conditions; and

[0140] TCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCT
 AATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCA
 AACAAAGATGGAATTTGTTGATGACTCGCGCTACAAACATCGGCCACTTGGATTC
 10 ATGGACACCATACTGTTATAAAACGGAAACATGTGTTTGATTCTGCTTTTTCTTTTC
 ATGAATCACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGACAA
 CTAATATGTCTATGAGCATCTTCAAAAGACAAAGACGACAGATCTCAAGATTGTT
 GAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTCGCTTATTAAAGAATAGCG
 AAGCAATCAAGAATAAATTCAAAAAAATATGAGCAAGCACATCAATCGAGGACT
 15 TGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGCTCAATTTCTAATCATGG
 CTAATATAATCAAACCCACCCAGTATATTACTTAGAGCATCTCCAATAGTTT
 TTTTAAAAAATCATCGCAAAAAAAGCTTTCAAAAAAATAATTGGTAAATCAAT
 GAGATTTACAAGTCGCTAAAAAAGTTGGAGGCGTAATTGTTGGGGCTTTGACTTT
 TTTCCCGTTTATAAAATCACGCTCCTAAAGTATAGAAAACAATTTTGCATTAGG
 20 AATCTTAAAATATTTTCAAATTACCCTAATCATTTTTATACTTTTTTCTTTCTTGT
 ATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCCA
 GTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCATCGCTCATA
 TAAAACGGCACCAACCCAACATATTTCCACCTAATGCCGTCGCCCAACTAATTGC
 CATAACACTAAGTCGAGCGCCAACCTTGCGGTGCTCTGCTTGCCTCGCTGTTGCTG
 25 TTGCCCATGG [SEQ ID NO: 58], or a nucleotide sequence that shares at least 80% (and at
 least 81% to at least 99% and all integer percentages in between) sequence identity with the
 sequence set forth in SEQ ID NO: 58 or a complement thereof, or a nucleotide sequence that
 hybridizes to the sequence set forth in SEQ ID NO: 58 or a complement thereof, under at least
 medium or high stringency conditions.

30 [0141] Non-limiting examples of nucleotide sequences that share at least 80%
 sequence identity with the sequence set forth in SEQ ID NO: 47, or that hybridize to the
 sequence set forth in SEQ ID NO: 47 or a complement thereof, under at least medium or high
 stringency conditions are suitably selected from any one of SEQ ID NO: 48, 49, 52, 53, 54, 55

and 58. Representative examples of nucleotide sequences that share at least 80% sequence identity with the sequence set forth in SEQ ID NO: 48, or that hybridize to the sequence set forth in SEQ ID NO: 48 or a complement thereof, under at least medium or high stringency conditions are suitably selected from any one of SEQ ID NO: 47, 49, 52, 53, 54, 55 and 58.

5 Non-limiting examples of nucleotide sequences that share at least 80% sequence identity with the sequence set forth in SEQ ID NO: 49, or that hybridize to the sequence set forth in SEQ ID NO: 49 or a complement thereof, under at least medium or high stringency conditions are suitably selected from any one of SEQ ID NO: 47, 48, 52, 53, 54, 55 and 58. Illustrative
10 examples of nucleotide sequences that share at least 80% sequence identity with the sequence set forth in SEQ ID NO: 52, or that hybridize to the sequence set forth in SEQ ID NO: 52 or a complement thereof, under at least medium or high stringency conditions are suitably selected from any one of SEQ ID NO: 47, 48, 49, 53, 54, 55 and 58.

[0142] Non-limiting examples of nucleotide sequences that share at least 80% sequence identity with the sequence set forth in SEQ ID NO: 50, or that hybridize to the
15 sequence set forth in SEQ ID NO: 50 or a complement thereof, under at least medium or high stringency conditions are suitably selected from any one of SEQ ID NO: 51, 56 and 57. Representative examples of nucleotide sequences that share at least 80% sequence identity with the sequence set forth in SEQ ID NO: 51, or that hybridize to the sequence set forth in
SEQ ID NO: 51 or a complement thereof, under at least medium or high stringency conditions
20 are suitably selected from any one of SEQ ID NO: 50, 56 and 57.

[0143] In a related aspect, the present invention provides isolated nucleic acid molecules, which comprise a promoter sequence that is operable in plant cells, including monocotyledonous plant cells. The promoter sequence generally comprises a nucleotide sequence selected from the group consisting of:

25 [0144] (i) a nucleotide sequence set forth in any one of SEQ ID NO: 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 and 58 or a complement thereof;

[0145] (ii) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in any one of SEQ ID NO: 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 and 58 or a
30 complement thereof; and

[0146] (iii) a nucleotide sequence that hybridizes to the sequence set forth in any one of SEQ ID NO: 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 and 58 or a complement thereof, under at least medium or high stringency conditions.

[0147] In accordance with the present invention, it is predicted that promoter elements positioned upstream or 5' to the TATA box in segment Ω of the exemplified promoter sequences set forth in SEQ ID NO: 47-58 (*e.g.*, promoter elements in any one or more of segments Φ , Ψ , Z , Δ , Θ or segment Λ that corresponds to the portion of segment Ω , which is upstream of the TATA box) influence the transcription initiation rate and/or tissue (*e.g.*, sink tissue) expression of operably connected nucleic acid sequences, and could be fused, therefore, to heterologous core promoter sequences to produce chimeric promoters with similar expression patterns to the exemplified promoter sequences. Accordingly, in another aspect, the present invention provides isolated nucleic acid molecules comprising chimeric promoter sequences that generally comprise at least one segment selected from the group consisting of a heterologous core promoter sequence in operable connection with a nucleotide sequence selected from the group consisting of:

[0148] (1) a nucleotide sequence corresponding to at least one segment selected from segments Λ , Φ , Ψ , Z , Δ and Θ of SEQ ID NO: 47-58, or

[0149] (2) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence of the at least one segment or a complement thereof, or

[0150] (3) a nucleotide sequence that hybridizes to the sequence s of the at least one segment or a complement thereof, under at least medium or high stringency conditions .

[0151] In some embodiments, the nucleotide sequence represented by (1) comprises segment Λ . In other embodiments, the nucleotide sequence represented by (1) comprises segments Λ and Φ , and optionally segment Ψ . In still other embodiments, the nucleotide sequence represented by (1) comprises segments Λ , Φ and Z , and optionally segment Ψ . In yet other embodiments, the nucleotide sequence represented by (1) comprises segments Λ , Φ , Z and Δ , and optionally segment Ψ . In still other embodiments, the nucleotide sequence represented by (1) comprises segments Λ , Φ , Z , Δ and Θ , and optionally segment Ψ . In other embodiments, the nucleotide sequence represented by (1) comprises segment Φ , and optionally segment Ψ . In still other embodiments, the nucleotide sequence represented by (1) comprises segments Φ and Z , and optionally segment Ψ . In yet other embodiments, the

nucleotide sequence represented by (1) comprises segments Φ , Z and Δ , and optionally segment Ψ . In still other embodiments, the nucleotide sequence represented by (1) comprises segments Φ , Z, Δ and Θ , and optionally segments Ψ .

5 [0152] Generally, a promoter of the invention can be fused to a transcribable sequence to create a chimeric construct. This construct can then be introduced into a host cell, typically a plant cell or plant or plant part, by any method of choice. Accordingly, in another aspect of the present invention, there is provided chimeric nucleic acid constructs comprising an isolated nucleic acid comprising a promoter sequence as broadly described above, in operable connection with a heterologous (*e.g.*, foreign or endogenous) nucleic acid sequence
10 to be transcribed.

[0153] In some embodiments, the chimeric construct further comprises a 3' non-translated sequence that is operably linked to the heterologous nucleic acid sequence and that functions in plant cells to terminate transcription and/or to cause addition of a polyadenylated nucleotide sequence to the 3' end of a transcribed RNA sequence.

15 [0154] The heterologous nucleic acid sequence is heterologous with respect to the plant cell in which it is or will be introduced. In some embodiments, the heterologous nucleic acid sequence encodes a structural or regulatory protein, or alternatively, a transcript capable of modulating expression of a corresponding target gene. In illustrative examples of this type, the transcript comprises an antisense RNA or a ribozyme or other transcribed region aimed at
20 downregulation of expression of the corresponding target gene. For example, the other transcribed region may comprise a sense transcript aimed at sense suppression (co-suppression) of the corresponding target gene or a hairpin transcript aimed at RNAi-mediated downregulation of the target gene.

[0155] Depending upon the heterologous nucleic acid sequence selected, the
25 chimeric construct in some embodiments may be further characterized in that the promoter sequence is capable of conferring transcription of the heterologous nucleic acid sequence in a specific tissue (*e.g.*, stem tissue) of the plant. In specific embodiments, the promoter sequence is capable of regulating transcription of the heterologous nucleic acid preferentially in stem tissue of a plant. In illustrative examples of this type, the chimeric construct may be further
30 characterized in that the promoter sequence is capable of regulating transcription of the heterologous nucleic acid sequence in mature stem tissue of a plant.

[0156] Plants falling within the scope of the present invention encompass any taxonomic grouping, including angiosperms, gymnosperms, monocotyledons and dicotyledons. In some embodiments, the plant is selected from monocotyledonous plants such as cereals, sugarcane, bananas and pineapples. In specific embodiments, the plant is a sucrose-
5 accumulating plant such as sugarcane, sugarbeet or sweet sorghum.

[0157] In yet another aspect, the present invention provides methods for expression of a heterologous nucleic acid sequence. These methods generally comprise introducing into a plant cell a chimeric construct as broadly described above.

[0158] In still another aspect, the present invention contemplates methods for
10 producing a transformed plant cell, wherein the methods generally comprise introducing into a plant cell a chimeric construct as broadly described above.

[0159] In a further aspect, the present invention provides methods for producing transformed plant cells. These methods generally comprise introducing into regenerable plant cells a chimeric construct as broadly described above so as to yield transformed plant cells
15 and identifying or selecting the transformed plant cells.

[0160] In still a further aspect, the present invention provides methods for selecting stable genetic transformants from transformed plant cells, wherein the methods generally comprise introducing into regenerable plant cells a chimeric construct as broadly described above so as to yield transformed plant cells and identifying or selecting a transformed plant
20 cell line from the transformed plant cells.

[0161] In some embodiments, the regenerable cells are regenerable dicotyledonous plant cells. In other embodiments, the regenerable cells are monocotyledonous plant cells such as regenerable graminaceous or non-graminaceous monocotyledonous plant cells. In some embodiments, the expression of the chimeric construct in the transformed cells imparts
25 a phenotypic characteristic to the transformed cells. Suitably, the imparted phenotype results from expression of the heterologous nucleic acid sequence.

[0162] According to another aspect of the present invention, transformed plant cells are provided, containing a chimeric construct as broadly described above.

[0163] In still another aspect, the present invention contemplates methods for
30 producing a differentiated transgenic plant. These methods generally comprise introducing a chimeric construct as broadly described above into regenerable plant cells so as to yield

regenerable transformed cells, identifying or selecting a population of transformed cells, and regenerating a differentiated transgenic plant from the population.

[0164] In some embodiments, the expression of the chimeric construct renders the differentiated transgenic plant identifiable over the corresponding non-transgenic plant.

5 [0165] In still a further aspect, the invention provides differentiated transgenic plants comprising plant cells containing a chimeric construct as broadly described above. In some embodiments, the chimeric construct is transmitted through a complete cycle of the differentiated transgenic plant to its progeny so that it is expressed by the progeny plants. Accordingly, the present invention also provides cells, tissues, leaves, fruit, flowers, seeds and
10 other reproductive material, material used for vegetative propagation, progeny plants including F1 hybrids, male-sterile plants and all other plants and plant products derivable from the differentiated transgenic plant.

[0166] The 1248-nt sequence set forth in SEQ ID NO: 59 is a transcribable nucleic acid sequence comprising an ORF, which codes for a 415-amino acid sequence, as set forth in
15 SEQ ID NO: 60. This ORF is transcribed at high levels in stem tissues, including mature stem tissues, of sugarcane (*Saccharum* sp.). Accordingly, nucleotide sequences that correspond or are complementary to at least a portion of the sequence set forth in SEQ ID NO: 59 may be useful as probes for isolating homologous transcribable sequences from other plants, especially from other sugarcane plants and more broadly other monocotyledonous plants such
20 as cereals, bananas and pineapples to, in turn, permit the isolation of promoter sequences with analogous qualities to those described herein. Thus, in another aspect, the present invention contemplates isolated nucleic acid molecules comprising a promoter sequence or biologically active fragment thereof or variant of these, wherein the promoter sequence is located upstream of a transcribable nucleic acid sequence that hybridizes to a nucleic acid probe
25 derived from the polynucleotide sequence set forth in SEQ ID NO: 59. Advantageously, the isolated promoter sequence is of sufficient length such that it is capable of initiating and/or regulating transcription of a DNA sequence to which it is coupled. The promoter sequence may be between about 150 nts and 2500 nts in length and usually greater than 250 nts in length. Suitably, analogous promoter sequences may be obtained from plants, especially from
30 monocotyledonous plants such as cereals, sugarcane, bananas and pineapples, which contain a nucleotide sequence that is capable of hybridizing to a nucleic acid probe derived from the sequence set forth in SEQ ID NO: 59 under at least medium stringency conditions, and especially under high stringency conditions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0167] Figure 1 is a photographic representation of various Northern analyses showing expression of *ScCIPK1* in different sugarcane tissues. (a) Northern analysis using total RNA isolated from meristems (M), internodes 1 to 3 (1-3), internode 5 (5), internode 12 (12), internode 20 (20), expanding leaf (EL) and mature leaf (ML) from field-grown Q117 plants, plus root (R) from glasshouse-grown Q117 plants, hybridized to a probe comprising nt 960-1248 of SEQ ID NO: 59. (b) Northern analysis using total RNA from internode 20 (IN20), mature roots (MR) and root tips (RT) from glasshouse-grown Q117 plants, hybridized to a probe comprising nt 960-1248 of SEQ ID NO: 59. (c) Northern analysis using total RNA from dissected rind (R), storage parenchyma (P) and vascular strands (V) from internode 8 of field-grown Q117 plants, hybridized to a probe comprising nt 960-1248 of SEQ ID NO: 59.

[0168] Figure 2 is a diagrammatic representation of a sequence alignment of the promoter sequences corresponding to promoter alleles A, B, 5, 49, 51 and 77. The nucleotide sequences delineating segments Θ , Δ , Z, Ψ , Φ , Λ and Ω are shown.

TABLE A
BRIEF DESCRIPTION OF THE SEQUENCES

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 1	CIPK1 promoter consensus sequence – segment Ω	307 nts
SEQ ID NO: 2	Allele 77 promoter sequence – segment Ω	313 nts
SEQ ID NO: 3	Allele 5 promoter sequence – segment Ω	305 nts
SEQ ID NO: 4	Allele 51 promoter sequence – segment Ω	312 nts
SEQ ID NO: 5	Allele A promoter sequence – segment Ω	306 nts
SEQ ID NO: 6	Allele B promoter sequence – segment Ω	306 nts
SEQ ID NO: 7	Allele 49 promoter sequence – segment Ω	306 nts
SEQ ID NO: 8	CIPK1 promoter consensus sequence – segment Φ	300 nts
SEQ ID NO: 9	N_u sequence 1	23 nts
SEQ ID NO: 10	N_u sequence 2	23 nts
SEQ ID NO: 11	N_u sequence 3	20 nts
SEQ ID NO: 12	N_u sequence 4	18 nts
SEQ ID NO: 13	N_u sequence 5	23 nts
SEQ ID NO: 14	Allele 77 promoter sequence – segment Φ	332 nts
SEQ ID NO: 15	Allele 5 promoter sequence – segment Φ	335 nts
SEQ ID NO: 16	Allele 51 promoter sequence – segment Φ	305 nts
SEQ ID NO: 17	Allele A promoter sequence – segment Φ	320 nts
SEQ ID NO: 18	Allele B promoter sequence – segment Φ	318 nts
SEQ ID NO: 19	Allele 49 promoter sequence – segment Φ	332 nts
SEQ ID NO: 20	CIPK1 promoter consensus sequence – segment Z	267 nts
SEQ ID NO: 21	Allele 77 promoter sequence – segment Z	264 nts
SEQ ID NO: 22	Allele 5 promoter sequence – segment Z	264 nts

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 23	Allele 51 promoter sequence – segment Z	264 nts
SEQ ID NO: 24	Allele A promoter sequence – segment Z	281 nts
SEQ ID NO: 25	Allele B promoter sequence – segment Z	281 nts
SEQ ID NO: 26	Allele 49 promoter sequence – segment Z	264 nts
SEQ ID NO: 27	CIPK1 promoter consensus sequence – segment Δ	366 nts
SEQ ID NO: 28	Allele 77 promoter sequence – segment Δ	376 nts
SEQ ID NO: 29	Allele 5 promoter sequence – segment Δ	363 nts
SEQ ID NO: 30	Allele 51 promoter sequence – segment Δ	367 nts
SEQ ID NO: 31	Allele A promoter sequence – segment Δ	378 nts
SEQ ID NO: 32	Allele B promoter sequence – segment Δ	378 nts
SEQ ID NO: 33	CIPK1 promoter consensus sequence – segment Θ	145 nts
SEQ ID NO: 34	N _{an} sequence 1	11 nts
SEQ ID NO: 35	N _{an} sequence 2	11 nts
SEQ ID NO: 36	N _{an} sequence 3	11 nts
SEQ ID NO: 37	N _{aq} sequence 1	26 nts
SEQ ID NO: 38	N _{aq} sequence 2	21 nts
SEQ ID NO: 39	Allele 77 promoter sequence – segment Θ	179 nts
SEQ ID NO: 40	Allele 5 promoter sequence – segment Θ	152 nts
SEQ ID NO: 41	Allele 51 promoter sequence – segment Θ	152 nts
SEQ ID NO: 42	Allele A promoter sequence – segment Θ	176 nts
SEQ ID NO: 43	Allele B promoter sequence – segment Θ	165 nts
SEQ ID NO: 44	Allele 77 intervening sequence Ψ	201 nts
SEQ ID NO: 45	Allele A intervening sequence Ψ	201 nts
SEQ ID NO: 46	Allele B intervening sequence Ψ	201 nts

SEQUENCE ID NUMBER	SEQUENCE	LENGTH
SEQ ID NO: 47	Allele 77 promoter sequence – segments Θ – Ω	1665 nts
SEQ ID NO: 48	Allele 5 promoter sequence – segments Θ – Ω	1419 nts
SEQ ID NO: 49	Allele 51 promoter sequence – segments Θ – Ω	1400 nts
SEQ ID NO: 50	Allele A promoter sequence – segments Θ – Ω	1662 nts
SEQ ID NO: 51	Allele B promoter sequence – segments Θ – Ω	1649 nts
SEQ ID NO: 52	Allele 49 promoter sequence – segments Θ – Ω	902 nts
SEQ ID NO: 53	Allele 77 promoter sequence – full-length	1757 nts
SEQ ID NO: 54	Allele 5 promoter sequence – full-length	1563 nts
SEQ ID NO: 55	Allele 51 promoter sequence – full-length	1551 nts
SEQ ID NO: 56	Allele A promoter sequence – full-length	2181 nts
SEQ ID NO: 57	Allele B promoter sequence – full-length	2168 nts
SEQ ID NO: 58	Allele 49 promoter sequence – full-length	995 nts
SEQ ID NO: 59	<i>ScCIPK1</i> CDS	1248 nts
SEQ ID NO: 60	Amino acid sequence encoded by <i>ScCIPK1</i> CDS	415 aa
SEQ ID NO: 61	<i>Nos</i> core promoter region	101 nts
SEQ ID NO: 62	CAMV core promoter region	65 nts
SEQ ID NO: 63	CAMV core promoter region with as-1 enhancer	90 nts
SEQ ID NO: 64	Allele 77 promoter sequence – segment Λ	281 nts
SEQ ID NO: 65	Allele 5 promoter sequence – segment Λ	275 nts
SEQ ID NO: 66	Allele 51 promoter sequence – segment Λ	280 nts
SEQ ID NO: 67	Allele A promoter sequence – segment Λ	275 nts
SEQ ID NO: 68	Allele B promoter sequence – segment Λ	275 nts
SEQ ID NO: 69	Allele 49 promoter sequence – segment Λ	276 nts

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0169] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0170] The articles “a” and “an” are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0171] By “about” is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 % to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[0172] The term “amplicon” refers to a target sequence for amplification, and/or the amplification products of a target sequence for amplification. In certain other embodiments an “amplicon” may include the sequence of probes or primers used in amplification.

[0173] The term “biologically active fragment”, as applied to promoter sequences, refers to a fragment that has at least about 0.1, 0.5, 1, 2, 5, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30% of the activity of a reference promoter sequence. It will also be understood that the phrase “biologically active fragment” refers to a part of an indicated DNA sequence that modulates RNA transcription or that, when fused to a particular gene and introduced into a plant cell, causes expression of the gene at a level higher than is possible in the absence of such part of the indicated DNA sequence. In specific embodiments, “biologically active fragments” encompass a portion of a promoter sequence that when added to a sequence including one or more ‘core’ promoter elements or motifs such as a TATA motif, promotes transcription in at least one tissue type to a greater extent than the ‘core’ sequence without the addition of the portion. Included within the scope of the present invention are biologically active fragments of at least about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 550, 600, or 650

nucleotides in length, or almost up to the number of nucleotides present in a full-length promoter sequence.

[0174] The terms “chimeric construct,” “chimeric nucleic acid” or “chimeric DNA” and the like are used herein to refer to a gene or nucleic acid sequence, either single- or double-stranded, comprising at least two nucleic acid sequences from species which do not combine nucleic acids such as DNA under natural conditions, or which nucleic acid sequences are positioned or linked in a manner which does not normally occur in the native genome of the untransformed plant.

[0175] Throughout this specification, unless the context requires otherwise, the words “comprise,” “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

[0176] By “corresponds to” or “corresponding to” is meant a polynucleotide (a) having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or (b) encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein. This phrase also includes within its scope a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein.

[0177] The terms “growing” or “regeneration” as used herein mean growing a whole, differentiated plant from a plant cell, a group of plant cells, a plant part (including seeds), or a plant piece (e.g., from a protoplast, callus, or tissue part).

[0178] As used herein, the term “heterologous” refers to a nucleic acid sequence linked to a nucleic acid sequence to which it is not naturally linked.

[0179] “Hybridization” is used herein to denote the pairing of complementary nucleotide sequences to produce a DNA-DNA hybrid or a DNA-RNA hybrid. Complementary base sequences are those sequences that are related by the base-pairing rules. In DNA, A pairs with T and C pairs with G. In RNA, U pairs with A and C pairs with G. In this regard, the terms “match” and “mismatch” as used herein refer to the hybridization potential of paired nucleotides in complementary nucleic acid strands. Matched nucleotides hybridize efficiently, such as the classical A-T and G-C base pair mentioned above. Mismatches are other combinations of nucleotides that do not hybridize efficiently.

[0180] By “isolated” is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an “isolated polynucleotide”, as used herein, refers to a polynucleotide, which has been purified from the sequences which flank it in a naturally-occurring state, *e.g.*, a DNA fragment which has been removed from the sequences that are normally adjacent to the fragment. Suitably, an “isolated” polynucleotide is free of sequences (*e.g.*, protein encoding sequences) that naturally flank the polynucleotide (*i.e.*, sequences located at the 5' and 3' ends of the polynucleotide) in the genomic DNA of the organism from which the polynucleotide was derived. For example, in various embodiments, an isolated promoter polynucleotide can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the polynucleotide in genomic DNA of the cell from which the polynucleotide was derived.

[0181] As used herein, “maturation” refers to the process of accumulating stored carbohydrate in sink tissue. Accordingly, the term “mature tissue” refers to tissue with more than 80%, 85%, 90% or 95% of the maximum stored carbohydrate concentration achieved during plant development. For example, in sugarcane, maturation increases initially with internode age, so that if internodes are counted from the internode attached to the youngest emerged leaf as number 1, maturation typically occurs up to about internode number 11 and older internodes are mature, as reviewed by Moore, 1995, *Aust. J. Plant Physiol.* 22, 661.

[0182] By “marker gene” is meant a gene that imparts a distinct phenotype to cells expressing the marker gene and thus allows such transformed cells to be distinguished from cells that do not have the marker. A selectable marker gene confers a trait for which one can ‘select’ based on resistance to a selective agent (*e.g.*, a herbicide, antibiotic, radiation, heat, or other treatment damaging to untransformed cells). A screenable marker gene (or reporter gene) confers a trait that one can identify through observation or testing, *i.e.*, by ‘screening’ (*e.g.*, β -glucuronidase, neomycin phosphotransferase II, luciferase, or other enzyme activity not present in untransformed cells).

[0183] As used herein, a “naturally-occurring” nucleic acid molecule refers to a RNA or DNA molecule having a nucleotide sequence that occurs in nature. For example a naturally-occurring nucleic acid molecule can encode a protein that occurs in nature.

[0184] By “obtained from” is meant that a sample such as, for example, a nucleic acid extract is isolated from, or derived from, a particular source. For instance, the nucleic acid extract may be obtained from tissue isolated directly from a host plant.

[0185] The term “oligonucleotide” as used herein refers to a polymer composed of a multiplicity of nucleotide residues (deoxyribonucleotides or ribonucleotides, or related structural variants or synthetic analogues thereof, including nucleotides with modified or substituted sugar groups and the like) linked via phosphodiester bonds (or related structural variants or synthetic analogues thereof). Thus, while the term “oligonucleotide” typically refers to a nucleotide polymer in which the nucleotide residues and linkages between them are naturally-occurring, it will be understood that the term also includes within its scope various analogues including, but not restricted to, peptide nucleic acids (PNAs), phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, methyl phosphonates, 2-O-methyl ribonucleic acids, and the like. The exact size of the molecule can vary depending on the particular application. Oligonucleotides are a polynucleotide subset with 200 bases or fewer in length. Preferably, oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g., for probes; although oligonucleotides may be double stranded, e.g., for use in the construction of a variant nucleic acid sequence. Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

[0186] The term “operably connected” or “operably linked” as used herein means placing a transcribable nucleic acid sequence as defined herein under the regulatory control of a promoter sequence, which then controls the transcription and optionally translation of the gene. In the construction of heterologous promoter/ transcribable nucleic acid combinations, it is generally desirable to position the promoter sequence at a distance from the transcription start site that is approximately the same as the distance between the promoter sequence and the gene it controls in its natural setting; *i.e.*, the gene from which the promoter sequence was derived. As is known in the art, some variation in this distance can be accommodated without loss of function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous nucleic acid sequence to be placed under its control is defined by the positioning of the element in its natural setting; *i.e.*, the genes from which it is derived.

[0187] As used herein, “plant” and “differentiated plant” refer to a whole plant or plant part containing differentiated plant cell types, tissues and/or organ systems. Plantlets and seeds are also included within the meaning of the foregoing terms. Plants included in the invention are any plants amenable to transformation techniques, including angiosperms, gymnosperms, monocotyledons and dicotyledons. In specific embodiments, the plant is a

monocotyledonous plant, illustrative examples of which include turf, turf grass, cereal, maize, rice, oat, wheat, barley, orchid, iris, lily, onion, banana, pineapple, sugarcane, sorghum, and palm.

[0188] The term “plant cell” as used herein refers to any plant cell or cell line including protoplasts, gamete-producing cells, and cells which regenerate into whole plants. Plant cells also include cells in plants as well as protoplasts in culture.

[0189] By “plant tissue” is meant differentiated and undifferentiated tissue derived from roots, shoots, pollen, seeds, tumour tissue, such as crown galls, and various forms of aggregations of plant cells in culture, such as embryos and calluses.

[0190] The term “polynucleotide” or “nucleic acid” as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[0191] The terms “polynucleotide variant” and “variant” refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridize with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompass polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains a biological function or activity of the reference polynucleotide. The terms “polynucleotide variant” and “variant” also include naturally-occurring allelic variants.

[0192] “Polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally-occurring amino acid, such as a chemical analogue of a corresponding naturally-occurring amino acid, as well as to naturally-occurring amino acid polymers.

[0193] By “primer” is meant an oligonucleotide which, when paired with a strand of DNA, is capable of initiating the synthesis of a primer extension product in the presence of a suitable polymerizing agent. The primer is usually single-stranded for maximum efficiency in amplification but can alternatively be double-stranded. A primer must be sufficiently long

to prime the synthesis of extension products in the presence of the polymerization agent. The length of the primer depends on many factors, including application, temperature to be employed, template reaction conditions, other reagents, and source of primers. For example, depending on the complexity of the target sequence, the primer may be at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, 500, to one base shorter in length than the template sequence at the 3' end of the primer to allow extension of a nucleic acid chain, though the 5' end of the primer may extend in length beyond the 3' end of the template sequence. In certain embodiments, primers can be large polynucleotides, such as from about 35 nucleotides to several kb or more. Primers can be selected to be "substantially complementary" to the sequence on the template to which it is designed to hybridize and serve as a site for the initiation of synthesis. By "substantially complementary", it is meant that the primer is sufficiently complementary to hybridize with a target polynucleotide. Desirably, the primer contains no mismatches with the template to which it is designed to hybridize but this is not essential. For example, non-complementary nucleotide residues can be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the template. Alternatively, non-complementary nucleotide residues or a stretch of non-complementary nucleotide residues can be interspersed into a primer, provided that the primer sequence has sufficient complementarity with the sequence of the template to hybridize therewith and thereby form a template for synthesis of the extension product of the primer.

[0194] "Probe" refers to a molecule that binds to a specific sequence or sub-sequence or other moiety of another molecule. Unless otherwise indicated, the term "probe" typically refers to a polynucleotide probe that binds to another polynucleotide, often called the "target polynucleotide", through complementary base pairing. Probes can bind target polynucleotides lacking complete sequence complementarity with the probe, depending on the stringency of the hybridization conditions. Probes can be labeled directly or indirectly and include primers within their scope.

[0195] The term "promoter" refers to a nucleic acid which directs expression of another nucleic acid to which it is operably linked, by initiating, regulating or otherwise controlling transcription of the nucleic acid. Promoters usually comprise a TATA box and often a "CAAT" box, capable of directing RNA polymerase to initiate RNA synthesis at the appropriate transcription initiation site for a particular coding sequence. They may additionally comprise other recognition sequences generally positioned upstream or 5' to the

TATA box, referred to as upstream promoter elements, which may influence the transcription initiation rate, tissue expression and/or temporal expression of an operably connected nucleic acid sequence. As used herein, a “core promoter” is intended to mean a promoter sequence consisting only of all basal elements needed for transcription initiation, *e.g.*, a TATA box and/or an initiator, without ancillary (*e.g.*, upstream) promoter elements.

[0196] “Constitutive promoter” refers to a promoter that directs transcription in many or all tissues of a plant.

[0197] By “stem-specific promoter” is meant a promoter that preferentially directs transcription in stem tissue of a plant. “Preferentially directs transcription” means that the rate of transcription of an operably linked nucleic acid is higher in the nominated tissue or developmental stage than in another tissue or developmental stage used for comparison.

[0198] The term “recombinant” as used herein refers to a nucleic acid or polypeptide resulting from in vitro manipulation into a form not normally found in nature. As used in the art, “recombinant” usually refers to the products of recombinant DNA technology.

[0199] Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include “reference sequence”, “comparison window”, “sequence identity”, “percentage of sequence identity” and “substantial identity”. These relationships are commonly analysed by use of sequence comparison programs such as BESTFIT (Deveraux et al. 1984, Nucleic Acids Research **12**, 387-395) which is incorporated herein by reference. Sequences of a similar or substantially different length may be aligned and compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by BESTFIT.

[0200] As used herein, the term “regulatory element” refers to a sequence of DNA, usually, but not always, upstream (5') to the coding sequence of a structural gene, which includes sequences that control the expression of the coding region by providing the recognition for RNA polymerase and/or other factors required for transcription to start at a particular site. An example of a complex regulatory element that provides for the recognition for RNA polymerase or other transcriptional factors to ensure initiation at a particular site is a promoter. A promoter generally comprises a core promoter region, responsible for the initiation of transcription, and optionally other regulatory elements that modify gene expression. It is to be understood that nucleotide sequences, located within introns, or 3' of the coding region sequence may also contribute to the regulation of expression of a coding region

of interest. Examples of suitable introns include, but are not limited to, the maize IVS6 intron, or the maize actin intron. A regulatory element may also include those elements located downstream (3') to the site of transcription initiation, or within transcribed regions, or both. In the context of this disclosure, a post-transcriptional regulatory element may include elements that are active following transcription initiation, for example translational and transcriptional enhancers, translational and transcriptional repressors, and mRNA stability determinants.

[0201] A "reference sequence" is at least 6 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence of nucleotide bases that is similar between the two polynucleotides, and (2) a sequence of nucleotide bases that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for comparison may be conducted by computerized implementations of algorithms (such as EMBOSS programs NEEDLE and WATER accessible on the EMBL-EBI website <http://www.ebi.ac.uk/Tools/emboss/align/index.html>, and GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA, incorporated herein by reference) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage sequence identity over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., 1997, Nucl. Acids Res. **25**:3389, which is incorporated herein by reference. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel et al., "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998, Chapter 15.

[0202] The term "sequence identity" as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis over a comparison window. Thus, a "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the

identical nucleic acid base (e.g., A, T, C, G, I) 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 window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The percentage of sequence identity can be
5 determined using the EMBOSS programs NEEDLE or WATER or other algorithms such as those described above. In specific embodiments, "sequence identity" will be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the
10 software, which is incorporated herein by reference in its entirety. In relation to polypeptide sequences, "sequence similarity" refers to the extent that optimally aligned sequences show the same amino acid or conservatively substituted amino acids according to a matrix or algorithm specified in the sequence comparison program, for example the Blossum or PAM matrices used with multiple sequence alignment program Clustal.

15 **[0203]** "Stringency" as used herein, refers to the temperature and ionic strength conditions, and presence or absence of certain organic solvents, during hybridization. The higher the stringency, the higher will be the degree of complementarity between immobilized nucleotide sequences and the labeled polynucleotide sequences that remain bound to them following the hybridization procedure.

20 **[0204]** "Stringent conditions" refers to temperature and ionic conditions under which only nucleotide sequences having a high frequency of complementary bases will hybridize. The stringency required is nucleotide sequence dependent and depends upon the various components present during hybridization and subsequent washes, and the time allowed for these processes. Generally, in order to maximize the hybridization rate, non-
25 stringent hybridization conditions are selected; about 20 to 25° C lower than the thermal melting point (T_m). The T_m is the temperature at which 50% of specific target sequence hybridizes to a perfectly complementary probe in solution at a defined ionic strength and pH. Generally, in order to require at least about 85% nucleotide complementarity of hybridized sequences, highly stringent washing conditions are selected to be about 5 to 15° C lower than
30 the T_m . In order to require at least about 70% nucleotide complementarity of hybridized sequences, moderately stringent washing conditions are selected to be about 15 to 30° C lower than the T_m . Highly permissive (low stringency) washing conditions may be as low as 50° C below the T_m , allowing a high level of mis-matching between hybridized sequences.

Those skilled in the art will recognise that other physical and chemical parameters in the hybridization and wash stages can also be altered to affect the outcome of a detectable hybridization signal from a specific level of similarity between target and probe sequences. Other examples of stringency conditions are described in Section 4 below.

5 **[0205]** The term “transcribable DNA sequence” or “transcribed DNA sequence”, excludes the non-transcribed regulatory sequence that drives transcription. Depending on the aspect of the invention, the transcribable sequence may be derived in whole or in part from any source known to the art, including a plant, a fungus, an animal, a bacterial genome or episome, eukaryotic, nuclear or plasmid DNA, cDNA, viral DNA or chemically synthesized
10 DNA. A transcribable sequence may contain one or more modifications in either the coding or the untranslated regions which could affect the biological activity or the chemical structure of the expression product, the rate of expression or the manner of expression control. Such modifications include, but are not limited to, insertions, deletions and substitutions of one or more nucleotides. The transcribable sequence may contain an uninterrupted coding sequence
15 or it may include one or more introns, bound by the appropriate splice junctions. The transcribable sequence may also encode a fusion protein. It is contemplated that introduction into plant tissue of chimeric nucleic acid constructs of the invention will include constructions wherein the transcribable sequence and its promoter are each derived from different species.

[0206] The term “transformation” means alteration of genotype by introduction of
20 genetic material (*e.g.*, the chimeric construct of the present invention) into an organism.

[0207] As used herein, the term “transgenic” or “transformed” with respect to a plant cell, plant part (including seed), plant tissue or plant means a plant cell, plant part, plant tissue or plant which comprises an isolated chimeric DNA construct according to the invention which has been introduced into the genome of a plant cell, plant part, plant tissue or
25 plant.

[0208] By “vector” is meant a polynucleotide molecule, suitably a DNA molecule derived, for example, from a plasmid, bacteriophage, yeast, virus, mammal, avian, reptile or fish into which a polynucleotide can be inserted or cloned. A vector preferably contains one or more unique restriction sites and can be capable of autonomous replication in a defined
30 host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector can be an autonomously replicating vector, *i.e.*, a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal

replication, e.g., a linear or closed circular plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector can contain any means for assuring self-replication. Alternatively, the vector can be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. A vector system can comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector can also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants. Examples of such resistance genes are known to those of skill in the art.

[0209] The terms “wild type,” “native” or “non-transgenic” refers to an untransformed plant cell, plant part, plant tissue or plant, *i.e.*, one where the genome has not been altered by the presence of a chimeric nucleic acid construct as defined herein.

2. *Abbreviations*

[0210] The following abbreviations are used throughout the application:

nt =nucleotide

nts =nucleotides

kb =kilobase(s) or kilobase pair(s)

ORF =open reading frame

SNPs =single nucleotide polymorphisms

3. *Transcribable DNA sequences useful for isolating promoter sequences*

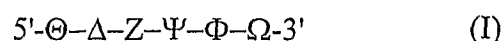
[0211] The promoter sequences of the present invention were first isolated through their linkage to a transcribable sequence, which comprises an ORF whose sequence is set forth in SEQ ID NO: 59, and which was found to be transcribed at high levels in mature stem tissues of sugarcane (*Saccharum* sp.). In accordance with the present invention, nucleotide sequences that correspond or are complementary to at least a portion of the sequence set forth in SEQ ID NO: 59 may be useful as probes for isolating homologous transcribable sequences from other plants, especially from other sugarcane plants and more broadly other monocotyledonous plants such as cereals, turf, turf grass, rice, orchid, iris, lily, onion, banana,

pineapples, sugarcane, sorghum, and palm and to, in turn, permit the isolation of promoter sequences with analogous qualities to those described herein.

[0212] The probes may be used in any suitable screening procedure. For example, a microarray screening procedure, as described below, may be used to identify genes expressed differentially in various tissues. However, it will be understood that the present invention is not restricted to use of any particular method for identifying such differentially expressed genes. For instance, alternative procedures for identifying genes expressed differentially in various tissues include, but are not restricted to: hybridization screening as for example described in patent specification PCT/AU99/01033; cDNA and genomic subtractive hybridization as for example described by Bulman and Neill (1996, In "*Plant Gene Isolation: Principles and Practice*", G.D. Foster and D. Twell, eds Chichester, UK, Wiley, pp 369-397); multi-probe fluorescent analysis of microscopic cDNA arrays as for example described by Schena (1996 *BioEssays* **18**:427-431); mRNA differential display as for example described by Liang and Pardee (1992, *Science* **257**:967-970) and by Callard *et al.* (1994, *BioTechniques* **16**:1096-1103); computer analysis of mRNA abundance based on frequency of occurrence of identical sequences emerging from large-scale sequencing of cDNA ends (ESTs) as for example taught by Cooke *et al.* (1996, *EST and genomic sequencing projects*. In *Plant Gene Isolation: Principles and Practice*, *supra*, pp. 410-419); or promoter tagging by insertional mutagenesis with promoterless reporter genes as for example disclosed by Lindsey and Topping (1996, *T-DNA-mediated insertional mutagenesis*. In *Plant Gene Isolation: Principles and Practice*, *supra*, pp. 275-300) and Mudge and Birch (1998, *Austral. J. Plant Physiol.* **25**:637-643).

4. Promoter sequences of the present invention

[0213] The present invention provides promoter sequences useful for expression of transcribable sequences in plants. In specific embodiments, stem-specific promoters for expression of chimeric or heterologous nucleic acid sequences in plants, especially monocotyledonous plants are provided. Representative examples of such promoter sequences may be selected from the sequences set forth in SEQ ID NOS: 47 to 58. Sequence analysis has revealed that these promoter sequences share a common segmental architecture, which can be represented by the following formula:



in which segment Ω is more conserved across the class of promoter sequences than segment Φ , segment Φ is more conserved than segment Z , segment Z is more conserved than segment Δ , segment Δ is more conserved than segment Θ , and Ψ is an optional spacer or intervening segment, as illustrated in Figure 2. Segment Ω appears to comprise a core promoter region containing a TATA box and (AGGA)CAAT box and it has been experimentally determined that this segment is transcriptionally active in plant cells. In accordance with the present invention, it is predicted that segments Φ , Z , Δ and Θ as well as segment Λ , which represents the portion of segment Ω , which is upstream of the TATA box, comprise promoter elements, which preferentially direct transcription in plant sink tissue, including stem tissue. In certain embodiments, transcription is directed in stem tissue, including mature stem tissue, of monocotyledonous plants, illustrative examples of which include turf, turf grass, cereal, maize, rice, oat, wheat, barley, orchid, iris, lily, onion, banana, pineapple, sugarcane, sorghum, and palm. In some embodiments, the promoter sequences of the present invention are particularly useful for preferentially expressing nucleic acids in carbohydrate storage tissue, and more particularly in mature plant carbohydrate storage tissue, illustrative examples of which include the sucrose-storage tissue of plants that store sugars, such as but not limited to sugarcane, sugarbeet and sweet sorghum.

[0214] The promoter sequences of the present invention can be used to prepare biologically active fragments that have promoter activity, to isolate corresponding sequences from other organisms, particularly other plants and more particularly other monocotyledonous plants, or to synthesize synthetic sequences. They can also be used in combination with native or heterologous core promoter regions, control elements or other regulatory sequences to modulate transcription and/or translation.

[0215] Accordingly, the present invention contemplates that biologically-active fragments of any one of SEQ ID NOS: 47 to 58, which comprise less than the entire promoter sequences disclosed herein, may be utilized to drive expression of an operably linked nucleotide sequence of interest, such as a nucleotide sequence encoding a heterologous protein. It is within skill in the art to determine whether such fragments decrease or increase expression levels or alter the nature of expression, *i.e.*, constitutive or inducible expression. Such fragments should retain promoter activity, or the ability to modulate the activity of a coupled core promoter region, particularly the ability to control expression of operably linked nucleotide sequences.

[0216] Biologically active fragments of promoters can be readily identified by randomly preparing and assaying deletion mutants of the promoter sequences of the invention (e.g., SEQ ID NOS: 47 to 58). With this strategy, a series of constructs is prepared, wherein each construct contains a different portion of the clone (a subclone), and these constructs are then screened for activity. The activity of a promoter can be determined by standard methods known in the art, as disclosed for example in Medberry *et al.* (1992, *Plant Cell* **4**:185; 1993, *The Plant J.* **3**:619), Sambrook *et al.* (1989, *supra*) and McPherson *et al.* (U.S. Patent No. 5,164,316). For instance, a suitable means for screening for activity is to operably link a deleted promoter construction to a selectable or screenable marker, and to isolate only those cells or tissues or plants which express the marker gene. In this way, a number of different, deleted promoter constructs are identified which still retain the desired, or even enhanced, activity. The smallest segment which is required for activity is thereby identified through comparison of the selected constructs. This segment may then be used for the construction of vectors for the expression of heterologous genes. Alternatively, biologically active fragments may be identified by fusion to a core promoter region that is coupled to a reporter gene, and screening transformed plant cells for reporter gene expression at developmental stages of interest, as disclosed for example in Puente *et al.* (1996, *EMBO J.* **15**: 3732). The combination of segments most effective for particular developmental patterns of promoter activity is thereby identified through comparison of the selected constructs. These segments may then be used for the construction of vectors for the expression of heterologous genes.

[0217] Based on sequence analysis of the promoter sequences set forth in SEQ ID NO: 47-58, it is predicted that promoter elements positioned upstream or 5' to the TATA box in segment Ω (e.g., promoter elements in any one or more of segments Λ , Φ , Ψ , Z , Δ and Θ) influence the transcription initiation rate and/or tissue (e.g., stem-specific) expression of operably connected nucleic acid sequences. Accordingly, the present invention also contemplates fusing any one or more of segments Λ , Φ , Ψ , Z , Δ , Θ to a heterologous core promoter (e.g., a core promoter region of any promoter operative in the host organism into which it is desired to express a transcribable sequence of interest) to thereby produce a chimeric promoter sequence. Suitable core promoter sequences are well known in the art and are generally derived from plant-operative promoters, such as but not limited to the CaMV35S, nopaline synthase, ferredoxin-RolD, maize ubiquitin and rice actin promoters. Reference also may be made to Puente *et al.* (1996, *EMBO J.* **15**: 3732) and Klimyuk *et al.* (1995 *Molecular & General Genetics* **249**: 357), who disclose illustrative plant core

promoters. Alternatively the core promoter may correspond to a consensus core promoter sequence. Non-limiting examples of core promoter sequences are set forth in SEQ ID NO: 61-63.

[0218] Representative biologically-active fragments of the promoter sequences set forth in SEQ ID NOS: 47 to 58 may comprise at least about 125, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500 nucleotides, or almost up to the number of nucleotides present in a full-length promoter sequence. Non-limiting examples of such biologically active fragments are set forth in SEQ ID NO: 2 to 7, which correspond to segment Ω , respectively, of alleles 77, 5, 51, A, B and 49, as described herein. Longer fragments can be produced by adding corresponding upstream sequences such as but not limited to Φ , Ψ , Z , Δ and Θ . Alternatively, biologically active fragments may include any one or more of segments Λ , Φ , Ψ , Z , Δ and Θ , which can be fused to core promoter sequences, to create chimeric promoter sequences with expression patterns, in some embodiments, reflecting the activity of the promoter sequences set forth in SEQ ID NO: 47-58. Illustrative examples of biologically active fragments according to the present invention are listed in Table 1 below.

TABLE 1

Fragment	77	5	51	A	B	49
A	Ω	Ω	Ω	Ω	Ω	Ω
B	$\Phi-\Omega$	$\Phi-\Omega$	$\Phi-\Omega$	$\Phi-\Omega$	$\Phi-\Omega$	$\Phi-\Omega$
C	$Z-\Phi-\Omega$	$Z-\Phi-\Omega$	$Z-\Phi-\Omega$	$Z-\Phi-\Omega$	$Z-\Phi-\Omega$	$Z-\Phi-\Omega$
D	$\Delta-Z-\Phi-\Omega$	$\Delta-Z-\Phi-\Omega$	$\Delta-Z-\Phi-\Omega$	$\Delta-Z-\Phi-\Omega$	$\Delta-Z-\Phi-\Omega$	
E	$\Theta-\Delta-Z-\Phi-\Omega$	$\Theta-\Delta-Z-\Phi-\Omega$	-	$\Theta-\Delta-Z-\Phi-\Omega$	$\Theta-\Delta-Z-\Phi-\Omega$	
F	$\Psi-\Phi-\Omega$			$\Psi-\Phi-\Omega$	$\Psi-\Phi-\Omega$	
G	$Z-\Psi-\Phi-\Omega$			$Z-\Psi-\Phi-\Omega$	$Z-\Psi-\Phi-\Omega$	
H	$\Delta-Z-\Psi-\Phi-\Omega$			$\Delta-Z-\Psi-\Phi-\Omega$	$\Delta-Z-\Psi-\Phi-\Omega$	
I	$\Theta-\Delta-Z-\Psi-\Phi-\Omega$			$\Theta-\Delta-Z-\Psi-\Phi-\Omega$	$\Theta-\Delta-Z-\Psi-\Phi-\Omega$	
J	Λ	Λ	Λ	Λ	Λ	Λ
K	$\Phi-\Lambda$	$\Phi-\Lambda$	$\Phi-\Lambda$	$\Phi-\Lambda$	$\Phi-\Lambda$	$\Phi-\Lambda$
L	$Z-\Phi-\Lambda$	$Z-\Phi-\Lambda$	$Z-\Phi-\Lambda$	$Z-\Phi-\Lambda$	$Z-\Phi-\Lambda$	$Z-\Phi-\Lambda$
M	$\Delta-Z-\Phi-\Lambda$	$\Delta-Z-\Phi-\Lambda$	$\Delta-Z-\Phi-\Lambda$	$\Delta-Z-\Phi-\Lambda$	$\Delta-Z-\Phi-\Lambda$	
N	$\Theta-\Delta-Z-\Phi-\Lambda$	$\Theta-\Delta-Z-\Phi-\Lambda$	$\Theta-\Delta-Z-\Phi-\Lambda$	$\Theta-\Delta-Z-\Phi-\Lambda$	$\Theta-\Delta-Z-\Phi-\Lambda$	
O	$Z-\Psi-\Phi-\Lambda$			$Z-\Psi-\Phi-\Lambda$	$Z-\Psi-\Phi-\Lambda$	
P	$\Delta-Z-\Psi-\Phi-\Lambda$			$\Delta-Z-\Psi-\Phi-\Lambda$	$\Delta-Z-\Psi-\Phi-\Lambda$	
Q	$\Theta-\Delta-Z-\Psi-\Phi-\Lambda$			$\Theta-\Delta-Z-\Psi-\Phi-\Lambda$	$\Theta-\Delta-Z-\Psi-\Phi-\Lambda$	
R	Φ	Φ	Φ	Φ	Φ	Φ
S	$Z-\Phi$	$Z-\Phi$	$Z-\Phi$	$Z-\Phi$	$Z-\Phi$	$Z-\Phi$
T	$\Delta-Z-\Phi$	$\Delta-Z-\Phi$	$\Delta-Z-\Phi$	$\Delta-Z-\Phi$	$\Delta-Z-\Phi$	
U	$\Theta-\Delta-Z-\Phi$	$\Theta-\Delta-Z-\Phi$	$\Theta-\Delta-Z-\Phi$	$\Theta-\Delta-Z-\Phi$	$\Theta-\Delta-Z-\Phi$	
V	$Z-\Psi-\Phi$			$Z-\Psi-\Phi$	$Z-\Psi-\Phi$	
W	$\Delta-Z-\Psi-\Phi$			$\Delta-Z-\Psi-\Phi$	$\Delta-Z-\Psi-\Phi$	
X	$\Theta-\Delta-Z-\Psi-\Phi$			$\Theta-\Delta-Z-\Psi-\Phi$	$\Theta-\Delta-Z-\Psi-\Phi$	

[0219] The present invention also encompasses promoter sequence variants that are substantially complementary to a reference sequence. Such variants are identified by blotting techniques that include a step whereby nucleic acids are immobilized on a matrix (preferably a synthetic membrane such as nitrocellulose), followed by a hybridization step, and a
5 detection step. Southern blotting is used to identify a complementary DNA sequence; northern blotting is used to identify a complementary RNA sequence. Dot blotting and slot blotting can be used to identify complementary DNA/DNA, DNA/RNA or RNA/RNA polynucleotide sequences. Such techniques are well known by those skilled in the art, and have been described in Ausubel *et al.* (1994-1998, *supra*) at pages 2.9.1 through 2.9.20.

10 [0220] According to such methods, Southern blotting involves separating DNA molecules according to size by gel electrophoresis, transferring the size-separated DNA to a synthetic membrane, and hybridizing the membrane-bound DNA to a complementary nucleotide sequence labeled radioactively, enzymatically or fluorochromatically. In dot blotting and slot blotting, DNA samples are directly applied to a synthetic membrane prior to
15 hybridization as above.

[0221] An alternative blotting step is used when identifying complementary polynucleotides in a cDNA or genomic DNA library, such as through the process of plaque or colony hybridization. A typical example of this procedure is described in Sambrook *et al.* ("Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Press, 1989) Chapters 8-
20 12.

[0222] Typically, the following general procedure can be used to determine hybridization conditions. Polynucleotides are blotted/transferred to a synthetic membrane, as described above. A reference polynucleotide such as a polynucleotide of the invention is labeled as described above, and the ability of this labeled polynucleotide to hybridize with an
25 immobilized polynucleotide is analyzed.

[0223] A skilled person will recognize that a number of factors influence hybridization. The specific activity of radioactively labeled polynucleotide sequence should typically be greater than or equal to about 10^8 dpm/mg to provide a detectable signal. A radiolabeled nucleotide sequence of specific activity 10^8 to 10^9 dpm/mg can detect
30 approximately 0.5 pg of DNA. It is well known in the art that sufficient DNA must be immobilized on the membrane to permit detection. It is desirable to have excess immobilized DNA, usually 10 μ g. Adding an inert polymer such as 10% (w/v) dextran sulfate (MW

500,000) or polyethylene glycol 6000 during hybridization can also increase the sensitivity of hybridization (see Ausubel *supra* at 2.10.10).

[0224] To achieve meaningful results from hybridization between a polynucleotide immobilized on a membrane and a labeled polynucleotide, a sufficient amount of the labeled polynucleotide must be hybridized to the immobilized polynucleotide following washing. Washing ensures that the labeled polynucleotide is hybridized only to the immobilized polynucleotide with a desired degree of complementarity to the labeled polynucleotide.

[0225] It will be understood that polynucleotide sequence variants according to the invention will hybridize to a reference polynucleotide under at least low stringency conditions. Reference herein to low stringency conditions include and encompass from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization at 42°C, and at least about 1 M to at least about 2 M salt for washing at 42°C. Low stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M NaHPO₄ (pH 7.2), 7% SDS for hybridization at 65°C, and (i) 2 × SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM NaHPO₄ (pH 7.2), 5% SDS for washing at room temperature.

[0226] Suitably, the polynucleotide variants hybridize to a reference polynucleotide under at least medium stringency conditions. Medium stringency conditions include and encompass from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization at 42°C, and at least about 0.1 M to at least about 0.2 M salt for washing at 55°C. Medium stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M NaHPO₄ (pH 7.2), 7% SDS for hybridization at 65°C, and (i) 2 × SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM NaHPO₄ (pH 7.2), 5% SDS for washing at 60-65°C.

[0227] In specific embodiments, the polynucleotide variants hybridize to a reference polynucleotide under high stringency conditions. High stringency conditions include and encompass from at least about 31% v/v to at least about 50% v/v formamide and from about 0.01 M to about 0.15 M salt for hybridization at 42°C, and about 0.01 M to about 0.02 M salt for washing at 55°C. High stringency conditions also may include 1% BSA, 1 mM EDTA, 0.5 M NaHPO₄ (pH 7.2), 7% SDS for hybridization at 65°C, and (i) 0.2 × SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM NaHPO₄ (pH 7.2), 1% SDS for washing at a temperature in excess of 65°C.

[0228] Other stringent conditions are well known in the art. A skilled addressee will recognize that various factors can be manipulated to optimize the specificity of the hybridization. Optimization of the stringency of the final washes can serve to ensure a high degree of hybridization. For detailed examples, see Ausubel *et al.*, *supra* at pages 2.10.1 to 2.10.16 and Sambrook *et al.* (1989, *supra*) at sections 1.101 to 1.104, which are incorporated herein by reference.

[0229] While stringent washes are typically carried out at temperatures from about 42°C to 68°C, one skilled in the art will appreciate that other temperatures may be suitable for stringent conditions. Maximum hybridization typically occurs at about 20°C to 25°C below the T_m for formation of a DNA-DNA hybrid. It is well known in the art that the T_m is the melting temperature, or temperature at which two complementary polynucleotide sequences dissociate. Methods for estimating T_m based on the length and sequence composition of duplex DNA and the composition of the hybridization solution are well known in the art (see Ausubel *et al.*, *supra* at page 2.10.8).

[0230] In general, the T_m of a perfectly matched duplex of DNA may be predicted as an approximation by the formula:

[0231] $T_m = 81.5 + 16.6 (\log_{10} M) + 0.41 (\%G+C) - 0.63 (\% \text{ formamide}) - (600/\text{length})$

[0232] wherein: M is the concentration of Na^+ , preferably in the range of 0.01 molar to 0.4 molar; %G+C is the sum of guanosine and cytosine bases as a percentage of the total number of bases, within the range between 30% and 75% G+C; % formamide is the percent formamide concentration by volume; length is the number of base pairs in the DNA duplex.

[0233] The T_m of a duplex DNA decreases by approximately 1°C with every increase of 1% in the number of randomly mismatched base pairs. Washing is generally carried out at $T_m - 15^\circ\text{C}$ for high stringency, or $T_m - 30^\circ\text{C}$ for moderate stringency.

[0234] In a preferred hybridization procedure, a membrane (*e.g.*, a nitrocellulose membrane or a nylon membrane) containing immobilized DNA is hybridized overnight at 42°C in a hybridization buffer (50% deionized formamide, 5 × SSC, 5 × Denhardt's solution (0.1% ficoll, 0.1% polyvinylpyrrolidone and 0.1% bovine serum albumin), 0.1% SDS and 200 mg/mL denatured salmon sperm DNA) containing labeled probe. The membrane is then subjected to two sequential medium stringency washes (*i.e.*, 2 × SSC, 0.1% SDS for 15 min at

45°C, followed by $2 \times$ SSC, 0.1% SDS for 15 min at 50°C), followed by two sequential higher stringency washes (*i.e.*, $0.2 \times$ SSC, 0.1% SDS for 12 min at 55°C followed by $0.2 \times$ SSC and 0.1% SDS solution for 12 min at 65-68°C).

[0235] Methods for detecting a labeled polynucleotide hybridized to an immobilized polynucleotide are well known to practitioners in the art. Such methods include autoradiography, phosphorimaging, chemiluminescent, fluorescent and colorimetric detection.

[0236] In general, variants will comprise regions that show at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence identity over a reference promoter sequence of identical size ("comparison window") or when compared to an aligned sequence in which the alignment is performed by a computer program known in the art, as described in the definitions section on sequence relationships. What constitutes suitable variants may be determined by conventional techniques. For example, polynucleotides according to any one of SEQ ID NO: 47-58 can be mutated using random mutagenesis (*e.g.*, transposon mutagenesis), oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis and cassette mutagenesis of an earlier prepared variant or non-variant version of an isolated natural promoter according to the invention. Thus, in addition to the unmodified promoter sequences set forth in SEQ ID NO: 47-58, the current invention encompasses functional variants of these sequences. For example, based on the information provided herein with respect to functional elements, one of skill in the art could derive functional promoters by deleting or modifying non-essential portions of the sequence without disrupting the essential functional elements. Such mutants may potentially have enhanced or altered function relative to the native sequence or alternatively, may be silent with regard to function.

[0237] Oligonucleotide-mediated mutagenesis is a preferred method for preparing nucleotide substitution variants of a promoter of the invention. This technique is well known in the art as, for example, described by Adelman *et al.* (1983, *DNA* 2:183). Briefly, promoter DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a template DNA, where the template is the single-stranded form of a plasmid or bacteriophage containing the unaltered or native DNA sequence of the promoter of interest. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that will thus incorporate the oligonucleotide primer, and will code for the selected alteration in the promoter of interest.

[0238] Generally, oligonucleotides of at least 25 nucleotides in length are used. An optimal oligonucleotide will have 12 to 15 nucleotides that are completely complementary to the template on either side of the nucleotide(s) coding for the mutation. This ensures that the oligonucleotide will hybridize properly to the single-stranded DNA template molecule.

5 [0239] The DNA template can be generated by those vectors that are either derived from bacteriophage M13 vectors, or those vectors that contain a single-stranded phage origin of replication as described by Viera *et al.* (1987, *Methods Enzymol.* **153**:3). Thus, the DNA that is to be mutated may be inserted into one of the vectors to generate single-stranded template. Production of single-stranded template is described, for example, in Sections 4.21-
10 4.41 of Sambrook *et al.* (1989, *supra*).

[0240] Alternatively, the single-stranded template may be generated by denaturing double-stranded plasmid (or other DNA) using standard techniques.

[0241] For alteration of the native DNA sequence, the oligonucleotide is hybridized to the single-stranded template under suitable hybridization conditions. A DNA polymerizing
15 enzyme, usually the Klenow fragment of DNA polymerase I, is then added to synthesize the complementary strand of the template using the oligonucleotide as a primer for synthesis. A heteroduplex molecule is thus formed such that one strand of DNA encodes the mutated form of the promoter under test, and the other strand (the original template) encodes the native unaltered sequence of the promoter under test. This heteroduplex molecule is then
20 transformed into a suitable host cell, usually a prokaryote such as *E. coli*. After the cells are grown, they are plated onto agarose plates and screened using the oligonucleotide primer having a detectable label to identify the bacterial colonies having the mutated DNA. The resultant mutated DNA fragments are then cloned into suitable expression hosts such as *E. coli* using conventional technology and clones that retain the desired promoter activity are
25 detected. Where the clones have been derived using random mutagenesis techniques, positive clones would have to be sequenced in order to detect the mutation.

[0242] Alternatively, linker-scanning mutagenesis of DNA may be used to introduce clusters of point mutations throughout a sequence of interest that has been cloned into a plasmid vector. For example, reference may be made to Ausubel *et al.*, *supra*, (in
30 particular, Chapter 8.4, incorporated herein by reference) which describes a first protocol that uses complementary oligonucleotides and requires a unique restriction site adjacent to the region that is to be mutagenized. A nested series of deletion mutations is first generated in the region. A pair of complementary oligonucleotides is synthesized to fill in the gap in the

sequence of interest between the linker at the deletion endpoint and the nearby restriction site. The linker sequence actually provides the desired clusters of point mutations as it is moved or “scanned” across the region by its position at the varied endpoints of the deletion mutation series. An alternate protocol is also described by Ausubel *et al.*, *supra*, which makes use of site directed mutagenesis procedures to introduce small clusters of point mutations throughout the target region. Briefly, mutations are introduced into a sequence by annealing a synthetic oligonucleotide containing one or more mismatches to the sequence of interest cloned into a single-stranded M13 vector. This template is grown in an *Escherichia coli* *dut⁻ ung⁻* strain, which allows the incorporation of uracil into the template strand. The oligonucleotide is annealed to the template and extended with T4 DNA polymerase to create a double-stranded heteroduplex. Finally, the heteroduplex is introduced into a wild-type *E. coli* strain, which will prevent replication of the template strand due to the presence of apurinic sites (generated where uracil is incorporated), thereby resulting in plaques containing only mutated DNA.

[0243] Region-specific mutagenesis and directed mutagenesis using PCR may also be employed to construct promoter variants according to the invention. In this regard, reference may be made, for example, to Ausubel *et al.*, *supra*, in particular Chapters 8.2A and 8.5.

[0244] The activity of promoter sequence variants thus obtained can be determined by conventional methods known to those of skill in the art, illustrative examples of which are discussed above.

5. *Chimeric nucleic acid constructs and expression vectors*

[0245] An isolated nucleic acid promoter sequence or variant according to the invention can be fused to a heterologous nucleic acid to form a chimeric construct. The heterologous nucleic acid may be a foreign or endogenous DNA sequence. For the purposes of transformation and expression of the heterologous nucleic acid in plants, it is generally desirable that the chimeric construct includes regulatory sequences which influence expression of the heterologous nucleic acid in plants. Suitably, the chimeric construct is present in an expression vector which includes regulatory sequences that enable selective propagation in bacteria.

5.1 3' Non-translated region

[0246] A 3' non-translated sequence refers to that portion of a gene comprising a DNA segment that contains a polyadenylation signal and any other regulatory signals capable

of effecting mRNA processing or gene expression. The polyadenylation signal is characterized by effecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. Polyadenylation signals are commonly recognized by the presence of homology to the canonical form 5' AATAAA-3' although variations are not uncommon.

5 [0247] The 3' non-translated regulatory DNA sequence typically includes from about 50 to 1,000 base pairs and contains plant transcriptional and translational termination sequences. Examples of suitable 3' non-translated sequences are the 3' transcribed non-translated regions containing a polyadenylation signal from the nopaline synthase (*nos*) gene of *Agrobacterium tumefaciens* (Bevan *et al.*, 1983, *Nucl. Acid Res.*, **11**:369) and the
10 terminator for the T7 transcript from the octopine synthase gene of *Agrobacterium tumefaciens*. Alternatively, suitable 3' non-translated sequences may be derived from plant genes such as the 3' end of the protease inhibitor I or II genes from potato or tomato, the soybean storage protein genes and the pea E9 small subunit of the ribulose-1,5-bisphosphate carboxylase (ssRUBISCO) gene, although other 3' elements known to those of skill in the art
15 can also be employed. Alternatively, 3' non-translated regulatory sequences can be obtained *de novo* as, for example, described by An (1987, *Methods in Enzymology*, **153**:292).

5.2 Optional sequences

 [0248] The chimeric construct of the present invention can further include enhancers, either translation or transcription enhancers, as may be required. These enhancer
20 regions are well known to persons skilled in the art, and can include an ATG initiation codon and adjacent sequences. The initiation codon must be in phase with the reading frame of the coding sequence relating to the foreign or endogenous DNA sequence to ensure translation of the entire sequence. The translation control signals and initiation codons can be of a variety of origins, both natural and synthetic. Translational initiation regions may be provided from the
25 source of the transcriptional initiation region, or from the foreign or endogenous DNA sequence. The sequence can also be derived from the source of the promoter selected to drive transcription, and can be specifically modified so as to increase translation of the mRNA.

 [0249] Examples of transcriptional enhancers include, but are not restricted to, elements from the CaMV 35S promoter and octopine synthase genes as for example described
30 by Last *et al.* (U.S. Patent No. 5,290,924, which is incorporated herein by reference). It is proposed that the use of an enhancer element such as the *ocs* element, and particularly

multiple copies of the element, will act to increase the level of transcription from adjacent promoters when applied in the context of plant transformation.

[0250] As the DNA sequence inserted between the transcription initiation site and the start of the coding sequence, *i.e.*, the untranslated leader sequence, can influence gene expression, one can also employ a particular leader sequence. Preferred leader sequences include those that comprise sequences selected to direct optimum expression of the foreign or endogenous DNA sequence. For example, such leader sequences include a preferred consensus sequence which can increase or maintain mRNA stability and prevent inappropriate initiation of translation as for example described by Joshi (1987, *Nucl. Acid Res.*, **15**:6643), which is incorporated herein by reference. However, other leader sequences, *e.g.*, the leader sequence of RTBV, have a high degree of secondary structure that is expected to decrease mRNA stability and/or decrease translation of the mRNA. Thus, leader sequences (i) that do not have a high degree of secondary structure, (ii) that have a high degree of secondary structure where the secondary structure does not inhibit mRNA stability and/or decrease translation, or (iii) that are derived from genes that are highly expressed in plants, will be most preferred.

[0251] Regulatory elements such as the sucrose synthase intron as, for example, described by Vasil *et al.* (1989, *Plant Physiol.*, **91**:5175), the Adh intron I as, for example, described by Callis *et al.* (1987, *Genes Develop.*, **II**), or the TMV omega element as, for example, described by Gallie *et al.* (1989, *The Plant Cell*, **1**:301) can also be included where desired. Other such regulatory elements useful in the practice of the invention are known to those of skill in the art.

[0252] Additionally, targeting sequences may be employed to target a protein product of the foreign or endogenous DNA sequence to an intracellular compartment within plant cells or to the extracellular environment. For example, a DNA sequence encoding a transit or signal peptide sequence may be operably linked to a sequence encoding a desired protein such that, when translated, the transit or signal peptide can transport the protein to a particular intracellular or extracellular destination, respectively, and can then be post-translationally removed. Transit or signal peptides act by facilitating the transport of proteins through intracellular membranes, *e.g.*, vacuole, vesicle, plastid and mitochondrial membranes, whereas signal peptides direct proteins through the extracellular membrane. For example, the transit or signal peptide can direct a desired protein to a particular organelle such as a plastid (*e.g.*, a chloroplast), rather than to the cytoplasm. Thus, the chimeric DNA construct can

further comprise a plastid transit peptide encoding DNA sequence operably linked between a promoter region or promoter variant according to the invention and the foreign or endogenous DNA sequence. For example, reference may be made to Heijne *et al.* (1989, *Eur. J. Biochem.*, **180**:535) and Keegstra *et al.* (1989, *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **40**:471), which
5 are incorporated herein by reference.

[0253] The chimeric construct can also be introduced into a vector, such as a plasmid. Plasmid vectors include additional DNA sequences that provide for easy selection, amplification, and transformation of the expression cassette in prokaryotic and eukaryotic cells, *e.g.*, pUC-derived vectors, pSK-derived vectors, pGEM-derived vectors, pSP-derived
10 vectors, or pBS-derived vectors. Additional DNA sequences include origins of replication to provide for autonomous replication of the vector, selectable marker genes, preferably encoding antibiotic or herbicide resistance, unique multiple cloning sites providing for multiple sites to insert DNA sequences or genes encoded in the chimeric construct, and sequences that enhance transformation of prokaryotic and eukaryotic cells.

15 [0254] In general, the vector suitably contains an element(s) that permits stable integration of the vector into the host cell genome or autonomous replication of the vector in the cell independent of the genome of the cell. The vector may be integrated into the host cell genome when introduced into a host cell. For integration, the vector may rely on the foreign or endogenous DNA sequence or any other element of the vector for stable integration of the
20 vector into the genome by homologous recombination. Alternatively, the vector may contain additional nucleic acid sequences for directing integration by homologous recombination into the genome of the host cell. The additional nucleic acid sequences enable the vector to be integrated into the host cell genome at a precise location in the chromosome. To increase the likelihood of integration at a precise location, the integrational elements should preferably
25 contain a sufficient number of nucleic acids, such as 100 to 1,500 base pairs, preferably 400 to 1,500 base pairs, and most preferably 800 to 1,500 base pairs, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be
30 non-encoding or encoding nucleic acid sequences.

[0255] For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322,

pUC19, pACYC177, and pACYC184 permitting replication in *E. coli*, and pUB110, pE194, pTA1060, and pAM.beta.1 permitting replication in *Bacillus*. The origin of replication may be one having a mutation to make its function temperature-sensitive in a *Bacillus* cell (see, *e.g.*, Ehrlich, 1978, *Proc. Natl. Acad. Sci. USA* **75**:1433).

5.3 Marker genes

[0256] To facilitate identification of transformants, the chimeric nucleic acid construct desirably comprises a selectable or screenable marker gene as, or in addition to, the expressible foreign or endogenous DNA sequence. The actual choice of a marker is not crucial as long as it is functional (*i.e.*, selective) in combination with the plant cells of choice. The marker gene and the foreign or endogenous DNA sequence of interest do not have to be linked, since co-transformation of unlinked genes as, for example, described in U.S. Pat. No. 4,399,216 is also an efficient process in plant transformation.

[0257] Included within the terms selectable or screenable marker genes are genes that encode a "secretable marker" whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers that encode a secretable antigen that can be identified by antibody interaction, or secretable enzymes that can be detected by their catalytic activity. Secretable proteins include, but are not restricted to, proteins that are inserted or trapped in the cell wall (*e.g.*, proteins that include a leader sequence such as that found in the expression unit of extensin or tobacco PR-S); small, diffusible proteins detectable, *e.g.* by ELISA; and small active enzymes detectable in extracellular solution (*e.g.*, α -amylase, β -lactamase, phosphinothricin acetyltransferase).

5.3.1 *Selectable markers*

[0258] Examples of bacterial selectable markers are the *dal* genes from *Bacillus subtilis* or *Bacillus licheniformis*, or markers that confer antibiotic resistance such as ampicillin, kanamycin, erythromycin, chloramphenicol or tetracycline resistance. Exemplary selectable markers for selection of plant transformants include, but are not limited to, a *hyg* gene which encodes hygromycin B resistance; a neomycin phosphotransferase (*neo*) gene conferring resistance to kanamycin, paromomycin, G418 and the like as, for example, described by Potrykus *et al.* (1985, *Mol. Gen. Genet.* **199**:183); a glutathione-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides as, for example, described in EP-A 256 223; a glutamine synthetase gene conferring, upon overexpression, resistance to glutamine synthetase inhibitors such as phosphinothricin as, for example,

described WO87/05327, an acetyl transferase gene from *Streptomyces viridochromogenes* conferring resistance to the selective agent phosphinothricin as, for example, described in EP-A 275 957, a gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine as, for example, described by Hinchee *et al.* (1988, *Biotech.*, **6**:915), a *bar* gene conferring resistance against bialaphos as, for example, described in WO91/02071; a nitrilase gene such as *bxn* from *Klebsiella ozaenae* which confers resistance to bromoxynil (Stalker *et al.*, 1988, *Science*, **242**:419); a dihydrofolate reductase (DHFR) gene conferring resistance to methotrexate (Thillet *et al.*, 1988, *J. Biol. Chem.*, **263**:12500); a mutant acetolactate synthase gene (ALS), which confers resistance to imidazolinone, sulfonylurea or other ALS-inhibiting chemicals (EP-A-154 204); a mutated anthranilate synthase gene that confers resistance to 5-methyl tryptophan; or a dalapon dehalogenase gene that confers resistance to the herbicide.

5.3.2 Screenable markers

[0259] Representative screenable markers include, but are not limited to, a *uidA* gene encoding a β -glucuronidase (GUS) enzyme for which various chromogenic substrates are known; a β -galactosidase gene encoding an enzyme for which chromogenic substrates are known; an aequorin gene (Prasher *et al.*, 1985, *Biochem. Biophys. Res. Comm.*, **126**:1259), which may be employed in calcium-sensitive bioluminescence detection; a green fluorescent protein gene (Niedz *et al.*, 1995 *Plant Cell Reports*, **14**:403); a luciferase (*luc*) gene (Ow *et al.*, 1986, *Science*, **234**:856), which allows for bioluminescence detection; a β -lactamase gene (Sutcliffe, 1978, *Proc. Natl. Acad. Sci. USA* **75**:3737), which encodes an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin); an R-locus gene, encoding a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, 1988, in *Chromosome Structure and Function*, pp. 263-282); an α -amylase gene (Ikuta *et al.*, 1990, *Biotech.*, **8**:241); a tyrosinase gene (Katz *et al.*, 1983, *J. Gen. Microbiol.*, **129**:2703) which encodes an enzyme capable of oxidizing tyrosine to dopa and dopaquinone which in turn condenses to form the easily detectable compound melanin; or a *xylE* gene (Zukowsky *et al.*, 1983, *Proc. Natl. Acad. Sci. USA* **80**:1101), which encodes a catechol dioxygenase that can convert chromogenic catechols.

6. Uses of the promoter sequences of the invention

[0260] The isolated promoters sequences of the invention may be used, *inter alia*, to drive expression of a foreign or endogenous nucleic acid sequence. Illustrative agronomic

properties encoded by the foreign or endogenous sequence include, but are not limited to, increased yield of a desired endogenous plant component, production of additional compounds by reactions involving endogenous plant components, decreased production of an undesired plant component, insect resistance or tolerance, herbicide resistance or tolerance, disease resistance or tolerance, tolerance to other stresses (*e.g.* drought, salinity, cold).

[0261] The foreign or endogenous nucleic acid sequence may comprise a region transcribed into a molecule that modulates the expression of a corresponding target gene. The molecule may be an antisense RNA or a ribozyme or other transcript aimed at downregulation of expression of the corresponding target gene.

[0262] Anti-sense regulation, co-suppression and the use of ribozymes and hairpin RNA in plants are well known in the art. However, the skilled person is referred to United States Patent 5,759,829 for an example of antisense technology; and to U. S. Patent 5,283,184, U. S. Patent 5,686,649, and WIPO PCT specification WO9853083 for examples of co-suppression technology; and to U.S. patent 5,707,835, U.S. patent 5,747,335 and U.S. patent 5,840,874 which each provide examples of ribozyme technology; and to USA patent application 2008/0104732 for examples of hairpin RNA technology for RNAi induction. Each of these patent documents is incorporated herein by reference.

[0263] Alternatively, the foreign or endogenous nucleic acid sequence may encode a molecule which is readily detectable or measurable, *e.g.* β -glucuronidase or luciferase; a selectable product, *e.g.*, neomycin phosphotransferase (*nptII*) conferring resistance to aminoglycosidic antibiotics such as geneticin and paramomycin; a product conferring herbicide tolerance, *e.g.* glyphosate resistance or glufosinate resistance; a product affecting starch biosynthesis or modification *e.g.* starch branching enzyme, starch synthases, ADP-glucose pyrophosphorylase; a product involved in fatty acid biosynthesis, *e.g.* desaturase or hydroxylase; a product conferring insect resistance, *e.g.* crystal toxin protein of *Bacillus thuringiensis*; a product conferring viral resistance, *e.g.* viral coat protein; a product conferring fungal resistance, *e.g.* chitinase, β -1,3-glucanase or phytoalexin; a product altering sucrose metabolism, *e.g.* invertase or sucrose synthase; a gene encoding valuable pharmaceuticals, *e.g.* antibiotics, secondary metabolites, pharmaceutical peptides or vaccines.

7. *Introduction of chimeric genes into plant cells*

[0264] There are many plant transformation techniques well known to workers in the art, and new techniques are continually becoming known. The particular choice of a

transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the particular choice of a transformation system to introduce a chimeric DNA construct into plant cells is not essential to or a limitation of the invention, provided it achieves an acceptable level of nucleic acid transfer. Guidance in the practical implementation of transformation systems for plant improvement is provided by Birch (1997, *Annu. Rev. Plant Physiol. Plant Molec. Biol.* 48: 297-326), which is incorporated herein by reference.

[0265] In principle both dicotyledonous and monocotyledonous plants that are amenable to transformation, can be modified by introducing a chimeric DNA construct according to the invention into a recipient cell and growing a new plant that harbors and expresses the foreign or endogenous DNA sequence.

[0266] Introduction and expression of foreign or chimeric nucleic acid sequences in dicotyledonous (broad-leafed) plants such as tobacco, potato and alfalfa has been shown to be possible using the T-DNA of the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* (See, for example, Umbeck, U.S. Patent No. 5,004,863, and International application PCT/US93/02480). A construct of the invention may be introduced into a plant cell utilizing *A. tumefaciens* containing the Ti plasmid. In using an *A. tumefaciens* culture as a transformation vehicle, it is most advantageous to use a non-oncogenic strain of the *Agrobacterium* as the vector carrier so that normal non-oncogenic differentiation of the transformed tissues is possible. It is preferred that the *Agrobacterium* harbors a binary Ti plasmid system. Such a binary system comprises (1) a first Ti plasmid having a virulence region essential for the introduction of transfer DNA (T-DNA) into plants, and (2) a chimeric plasmid. The chimeric plasmid contains at least one border region of the T-DNA region of a wild-type Ti plasmid flanking the nucleic acid to be transferred. Binary Ti plasmid systems have been shown effective to transform plant cells as, for example, described by De Framond (1983, *Biotechnology*, 1:262) and Hoekema *et al.* (1983, *Nature*, 303:179). Such a binary system is preferred *inter alia* because it does not require integration into the Ti plasmid in *Agrobacterium*.

[0267] Methods involving the use of *Agrobacterium* include, but are not limited to: (a) co-cultivation of *Agrobacterium* with cultured isolated protoplasts; (b) transformation of plant cells or tissues with *Agrobacterium*; or (c) transformation of seeds, apices or meristems with *Agrobacterium*.

[0268] Recently, rice, corn, pineapple and sugarcane, which are monocots, have been shown to be susceptible to transformation by *Agrobacterium*, for example as described in United States Patent No. 6,037,522, International Publication WO99/36637 and Arencibia *et al.* (1998, *Transgenic Res.* 7:213). However, some monocot crop plants have not yet been
5 successfully transformed using *Agrobacterium*-mediated transformation. The Ti plasmid, however, may be manipulated in the future to act as a vector for these other monocot plants. Additionally, using the Ti plasmid as a model system, it may be possible to artificially construct transformation vectors for these plants. Ti plasmids might also be introduced into monocot plants by artificial methods such as microinjection, or fusion between monocot
10 protoplasts and bacterial spheroplasts containing the T-region, which can then be integrated into the plant nuclear DNA.

[0269] In addition, gene transfer can be accomplished by *in situ* transformation by *Agrobacterium*, as described by Bechtold *et al.* (1993, *C.R. Acad. Sci. Paris*, **316**:1194). This approach is based on the vacuum infiltration of a suspension of *Agrobacterium* cells.

15 [0270] Alternatively, nucleic acids may be introduced using root-inducing (Ri) plasmids of *Agrobacterium* as vectors.

[0271] Cauliflower mosaic virus (CaMV) may also be used as a vector for introducing of exogenous nucleic acids into plant cells (U.S. Pat. No. 4,407,956). CaMV DNA genome is inserted into a parent bacterial plasmid creating a recombinant DNA
20 molecule that can be propagated in bacteria. After cloning, the recombinant plasmid again may be cloned and further modified by introduction of the desired nucleic acid sequence. The modified viral portion of the recombinant plasmid is then excised from the parent bacterial plasmid, and used to inoculate the plant cells or plants.

[0272] Nucleic acids can also be introduced into plant cells by electroporation as,
25 for example, described by Fromm *et al.* (1985, *Proc. Natl. Acad. Sci., U.S.A.*, **82**:5824) and Shimamoto *et al.* (1989, *Nature* **338**:274-276). In this technique, plant protoplasts are electroporated in the presence of vectors or nucleic acids containing the relevant nucleic acid sequences. Electrical impulses of high field strength reversibly permeabilize membranes allowing the introduction of nucleic acids. Electroporated plant protoplasts reform the cell
30 wall, divide and form a plant callus.

[0273] Another method for introducing nucleic acids into a plant cell is high velocity ballistic penetration by small particles (also known as particle bombardment or

microprojectile bombardment) with the nucleic acid to be introduced contained either within the matrix of small beads or particles, or on the surface thereof as, for example described by Klein *et al.* (1987, *Nature* **327**:70). Although typically only a single introduction of a new nucleic acid sequence is required, this method particularly provides for multiple introductions.

5 [0274] Alternatively, nucleic acids can be introduced into a plant cell by contacting the plant cell using mechanical or chemical means. For example, a nucleic acid can be mechanically transferred by microinjection directly into plant cells by use of micropipettes. Alternatively, a nucleic acid may be transferred into the plant cell by using polyethylene glycol which forms a precipitation complex with genetic material that is taken up by the cell.

10 [0275] Also contemplated are silicon carbide or tungsten whiskers, for example as described in United States Patent No. 5,302,523.

 [0276] There are a variety of methods known currently for transformation of monocotyledonous plants. Presently, preferred methods for transformation of monocots are microprojectile bombardment of explants or suspension cells, and direct DNA uptake or
15 electroporation as, for example, described by Shimamoto *et al.* (1989, *supra*). Transgenic maize plants have been obtained by introducing the *Streptomyces hygroscopicus bar* gene into embryogenic cells of a maize suspension culture by microprojectile bombardment (Gordon-Kamm, 1990, *Plant Cell*, **2**:603-618). The introduction of genetic material into aleurone protoplasts of other monocotyledonous crops such as wheat and barley has been reported
20 (Lee, 1989, *Plant Mol. Biol.* **13**:21-30). Wheat plants have been regenerated from embryogenic suspension culture by selecting only the aged compact and nodular embryogenic callus tissues for the establishment of the embryogenic suspension cultures (Vasil, 1990, *Bio/Technol.* **8**:429-434). The combination with transformation systems for these crops enables the application of the present invention to monocots. These methods may also be
25 applied for the transformation and regeneration of dicots. Transgenic sugarcane plants have been regenerated from embryogenic callus as, for example, described by Bower *et al.* (1996, *Molecular Breeding* **2**:239-249).

 [0277] Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, *e.g.*, bombardment with *Agrobacterium*
30 coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with *Agrobacterium* (EP-A-486233).

8. *Strategies to avoid transgene silencing*

[0278] Transgenes driven by various promoter sequences can be efficiently silenced during the regeneration and growth of mature plants. To avoid undesired transgene silencing, constructs are usually designed and transferred so as to minimize insofar as possible features likely to contribute to the production of aberrant transcripts and associated sequence-specific gene silencing responses in plants.

8.1 Simple integration events and targeted integration sites

[0279] Simple integrations at a known favorable site for stable transgene expression may be obtained by homologous recombination in plants. Accessory components such as site-specific recombinases, transposases or rare cutting nucleases can be used to facilitate simple and/or targeted integration as described below.

8.2 Recombinase-mediated resolution of complex integration events

[0280] Site-specific recombinase systems, such as Cre-*lox* from bacteriophage P1, can be employed for resolution of complex integration events into simple patterns (Srivastava 1999, 2001; De Buck 2001a, 2001b, 2007).

8.3 Recombinase-mediated site-specific integration

[0281] Site-specific recombination systems can also be used to bring about targeted integration of transgenes into predetermined genomic loci through a two-round transformation procedure. The first round of transformation introduces a construct containing a *lox* site. Single-copy lines are selected as recipients for the next step, in which Cre recombinase activity integrates a *lox*-flanked gene of interest specifically into the single-copy *lox* 'landing pad'. The three necessary components (favorable *lox* landing site, Cre recombinase enzyme activity and *lox*-flanked gene for integration) can be brought together either by crossing transgenic plant lines with separate components, or by a second round of transformation into the selected recipient lines. Several site-specific recombinase systems including FLP-*FRT* and R-*RS* have shown promise in plants, but Cre-*lox* is the best characterized (Ow 2002; Ow 2007).

[0282] Direct gene transfer and *Agrobacterium*-mediated gene transfer systems have been applied for Cre-*lox* site-specific integration (Albert 1995, Vergunst 1998, Srivastava 2002). The excision by site-specific recombinases is intrinsically more favorable than integration. Therefore, the strategy to obtain stable integrants typically involves use of (i)

mutant *lox* sites that are compatible for targeted integration but less effective for excision of the integrated molecule, (ii) transient recombinase activity and (iii) selection based on reconstitution of a promoter - selectable marker expression unit through targeted integration. Conditions (ii) and (iii) can be accomplished together if the landing pad is a promoter-*lox-cre* cassette, so that targeted integration abolishes Cre production (provided the recipient line was hemizygous for the landing pad) and commences integrated marker gene expression. Counter-selection has been essential to eliminate lines with non-targeted integration events in most attempts at homologous recombination in plants, but not when using an efficient recombinase strategy. With an inducible *cre* gene and appropriate vector design it should be possible to achieve both single-copy site-specific integration and removal of unwanted selectable marker genes following selection of transformed cells (Ow 2007).

[0283] *Agrobacterium*-mediated gene transfer can be used with a recombinase-mediated cassette exchange (RMCE) strategy in which (i) the landing pad and replacement gene are each flanked by a wild-type and incompatible mutant *lox* site, (ii) Cre is provided by transient or integrative transformation using a separate T-DNA, and (iii) stringent selection for site-specific integration is based on expression of a replacement selectable marker gene from a promoter in the landing pad (Nanto 2005; Louwerse 2007).

8.4 Transposon-mediated integration

[0284] In contrast with random integration following *Agrobacterium*-mediated or direct gene transfer, maize *Ac/Ds* transpositions show a bias to regions that might be more amenable to transgene expression (Zhao 2006). When barley transformed using T-DNA that contained a *bar* selectable marker within a non-autonomous *Ds* element was crossed with *Ac* transposase-expressing plants, the *bar*-containing *Ds* element transposed to different sites in the genome and segregated away from the initial T-DNA insertions in subsequent generations. The resulting plants that harboured a single copy of the *bar*-containing *Ds* element showed greatly enhanced *bar* transgene expression stability over several generations relative to the source lines (Koprek 2001). The requirement for crossing has subsequently been avoided by including the transposase gene and a negative selectable marker (cytosine deaminase) in the same T-DNA as the *Ds* :: positive selectable marker element, and selecting against cells that incorporate the full T-DNA (Yan and Rommens 2007).

8.5 Restriction-site-directed integration

[0285] Co-expression of a rare-cutting restriction enzyme or a zinc finger nuclease, which induces double-stranded breaks in DNA, has been developed for site-specific integration of transgenes into a corresponding recognition site following protoplast or microprojectile or *Agrobacterium*- mediated transfer. The recognition site can be native to the recipient genome, or in a previously engineered 'landing pad' (Wright 2005, D'Halluin 2008).

8.6 Use of S/MAR elements

[0286] Nuclear scaffold or matrix attachment regions (S/MARs) are sequences of about 300 base pairs to several kilobases that play a structural role in anchoring chromatin to the framework of the nuclear scaffold (Allen 2000, Chernov 2004). The inclusion of flanking S/MARs in constructs has been reported to substantially increase transgene expression in plants (Allen 1996, Ulker 1999), Vain 1999,,Brouwer 2002, Mankin 2003).

8.7 Elimination of silencing triggers in the transgene sequence

[0287] Duplicated T-DNA borders can be used to minimise the incidence of integrated sequences from the flanking binary or Ti vector (Thole 2007). Constructs employing tandem terminators can be used to reduce read-through (Luo 2007).

8.8 Minimal expression cassettes

[0288] It is useful to transfer the minimum DNA sequence needed to confer the desired transgenic trait. This eliminates the possibility that vector sequences might be unintended sources of signals to trigger silencing responses that can spread into the transgene. In the case of *Agrobacterium*-mediated transfer this is accomplished by use of minimal distance between the T-DNA borders. In the case of DGT, the transgene expression cassette may be isolated without vector backbone by either restriction digestion or PCR amplification (Agrawal 2005; Kumar 2006).

8.9 Codon Optimisation

[0289] Although the genetic code is universal, the frequency of use of particular codons in the redundant set encoding any amino acid varies between organisms and even between protein classes (for example those expressed at high or low levels). This is thought to be related to the abundance of corresponding tRNAs in cells, which might in turn limit translation of transgenes containing rarely used codons. Synonymous mutations may be

introduced into transgene sequences, either to use the most abundant codons in recipient cell genes or to more closely match the overall codon usage frequencies of the recipient (De Rocher 1998). Potential poly(A) addition signals can be removed simultaneously (Diehn 1998, Misztal 2004). Recently, Li (2007) reported that replacement of uncommon codons while maintaining codon diversity gave superior expression relative to use of the most abundant codon throughout a synthetic gene.

[0290] Heterologous genes may simultaneously be modified to eliminate signals for other processing events that limit transgene expression, for example intron splice signals, sequence context around the start codon, or mRNA destabilizing elements (Gutierrez 1999, Haseloff 1997, Holmberg 2001, Khanna 2006).

8.10 Inhibiting RNAi pathways

[0291] One role for RNAi in plants is in antiviral defense, and many viruses encode proteins that interfere with RNAi mechanisms. Diverse viral suppressors act at several different steps in the RNAi pathway (Roth 2004; Voinnet 2005; Scholthof 2007). These suppressors can inhibit silencing of transgenes in addition to viral RNAs, as first demonstrated for the potyvirus helper component-protease, HC-Pro. Co-transformation with silencing suppressors or crossing to combine the transgene with a silencing suppressor may be used to inhibit silencing (Anandalakshmi 1998, Johansen 2001, Lim 2005, Lewsey 2007).

9. Production and characterization of differentiated transgenic plants

9.1 Regeneration

[0292] The methods used to regenerate transformed cells into differentiated plants are not critical to this invention, and any method suitable for a target plant can be employed. Normally, a plant cell is regenerated to obtain a whole plant following a transformation process.

[0293] Regeneration from protoplasts varies from species to species of plants, but generally a suspension of protoplasts is first made. In certain species, embryo formation can then be induced from the protoplast suspension, to the stage of ripening and germination as natural embryos. The culture media will generally contain various amino acids and hormones, necessary for growth and regeneration. Examples of hormones utilized include auxins and cytokinins. It is sometimes advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the

medium, on the genotype, and on the history of the culture. If these variables are controlled, regeneration is reproducible. Regeneration also occurs from plant callus, explants, organs or parts. Transformation can be performed in the context of organ or plant part regeneration as, for example, described in *Methods in Enzymology*, Vol. 118 and Klee *et al.* (1987, *Annual Review of Plant Physiology*, **38**:467), which are incorporated herein by reference. Utilizing the leaf disk-transformation-regeneration method of Horsch *et al.* (1985, *Science*, **227**:1229, incorporated herein by reference), disks are cultured on selective media, followed by shoot formation in about 2-4 weeks. Shoots that develop are excised from calli and transplanted to appropriate root-inducing selective medium. Rooted plantlets are transplanted to soil as soon as possible after roots appear. The plantlets can be repotted as required, until reaching maturity.

[0294] In vegetatively propagated crops, the mature transgenic plants are propagated by the taking of cuttings or by tissue culture techniques to produce multiple identical plants. Selection of desirable transgenics is made and new varieties are obtained and propagated vegetatively for commercial use.

[0295] In seed propagated crops, the mature transgenic plants can be self-crossed to produce a homozygous inbred plant. The inbred plant produces seed containing the newly introduced foreign gene(s). These seeds can be grown to produce plants that would produce the selected phenotype, *e.g.*, early flowering.

[0296] Parts obtained from the regenerated plant, such as flowers, seeds, leaves, branches, fruit, and the like are included in the invention, provided that these parts comprise cells that have been transformed as described. Progeny and variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise the introduced nucleic acid sequences.

[0297] It will be appreciated that the literature describes numerous techniques for regenerating specific plant types and more are continually becoming known. Those of ordinary skill in the art can refer to the literature for details and select suitable techniques without undue experimentation.

9.2 Characterization

[0298] To confirm the presence of the heterologous nucleic acid in the regenerating plants, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern

blotting and PCR; a protein expressed by the heterologous DNA may be analysed by western blotting, high performance liquid chromatography or ELISA (*e.g.*, nptII) as is well known in the art.

5 **[0299]** Representative examples of various methods applicable to characterization of transgenic plants are provided in Chapters 9 and 11 of PLANT MOLECULAR BIOLOGY A Laboratory Manual Ed. M.S. Clark (Springer-Verlag, Heidelberg, 1997).

[0300] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting example.

EXAMPLES

EXAMPLE 1

IDENTIFICATION OF MULTIPLE *ScCIPK1* PROMOTER ALLELES IN SUGARCANE

Results

5 Identification of a gene that is up-regulated in mature stem

[0301] The sequence of the *ScCIPK1* coding region is set forth in SEQ ID NO: 59. Transcripts hybridizing to a probe derived from SEQ ID NO: 59 were shown, by northern analysis, to be strongly up-regulated in mature *vs.* immature stem (Figure 1a), and also detectable in mature roots (Figure 1b), but not in leaf tissue. Northern analysis using RNA
10 from vascular, parenchyma and rind tissue dissected from internode eight of field-grown Q117 (Rae *et al.*, 2005) revealed the presence of transcript in all three tissue types within the stem (Figure 1c).

Sugarcane is highly heterozygous at the *ScCIPK1* locus

[0302] Sequence analysis of 13 Q200 genomic BAC clones hybridizing to the
15 *ScCIPK1* coding region revealed the presence of at least eight haplotypes of *ScCIPK1* in this cultivar.

Multiple *ScCIPK1* alleles are differentially expressed in sugarcane

[0303] Sequence analysis of RT-PCR products indicates that at least four haplotypes are expressed in internode 12 of Q117. One haplotype was shown to comprise
20 approximately 20% of RT-PCR clones from internode 12, and 100% (30/30) of RT-PCR clones in internode 30, consistent with this allele (designated as allele A) being most strongly expressed in mature stem tissues.

ScCIPK1 promoter alleles

[0304] Using the GenomeWalker single-specific-primer PCR procedure, in
25 combination with BAC clone sequencing, we identified six distinct promoter variants for *ScCIPK1*. These sequences are represented by SEQ ID NOS: 53, 54, 55, 56, 57 and 58.

[0305] These six promoter alleles show at least 85% identity for 168 bp upstream of the start codon. A putative TATA box predicted using the program HCTata (Milanesi *et al.* 1996) occurs approximately 133-135 bp upstream of the start codon, and 23 bp upstream of

the transcription start site determined by 5'-RACE. Beyond 168 bp upstream, the sequences diverge into 2 sub-families with many conserved motifs.

[0306] Sequencing of a BAC clone containing *ScCIPK1* allele A revealed that this allele is linked to the *ScCIPK1* A promoter allele (SEQ ID NO 56). Therefore this promoter is potentially useful for driving strong, mature-stem expression of transgenes. Other *ScCIPK1* promoter alleles may be used to drive different transgene expression levels and developmental patterns within sink tissues.

[0307] RT-PCR analysis in transgenic sugarcane cell lines showed reporter gene expression driven from the Ω fragment of the *ScCIPK1* A promoter, providing functional confirmation that the Ω sequence comprises a core promoter region. Multiple independent transgenic sugarcane lines containing the *ScCIPK1* A or B promoter alleles fused to the LUC reporter gene showed LUC activity in regenerating shoots in tissue culture, and in stem and root tissues of transgenic plants grown in a containment glasshouse for 4 months. This confirmed the ability of the isolated *ScCIPK1* promoters to drive heterologous gene expression in transgenic plant cells.

Discussion

[0308] Sugarcane is highly heterozygous at the *ScCIPK1* locus, with eight coding region variants and six promoter variants identified in cultivar Q200. We have shown that the upstream genomic sequences isolated from *ScCIPK1* alleles are functional as promoters for heterologous reporter gene expression in transgenic sugarcane plants. It is reported that transgenes driven by various promoter sequences can be efficiently silenced during the regeneration and growth of mature plants in sugarcane (Hansom et al. 1999; Ingelbrecht *et al.*, 1999; Wei *et al.*, 2003). To avoid undesired transgene silencing, constructs should be designed and transferred so as to minimise insofar as possible any features likely to contribute to the production of aberrant transcripts and associated sequence-specific gene silencing responses in plants.

[0309] In particular, it will be advantageous to use the promoters described in the present invention in gene constructs designed to (i) preclude read-through into or out of the transgene (*e.g.* through the use of flanking transcriptional terminators); (ii) eliminate known silencing signals including sequence features (such as inverted repeats) or motifs (such as targets for recipient plant small regulatory RNAs); (iii) exclude unnecessary sequences that might be sources of unknown silencing signals; and (iv) ensure efficient transcriptional

termination. It will also be advantageous to use gene transfer parameters designed to (i) maximise the proportion of simple, intact integration events of the transferred expression cassette; and (ii) preferably integrate at a known locus that has been shown experimentally to be neutral for transgene expression (*e.g.* away from heterochromatin, outside of any endogenous transcript and not unintentionally influenced by endogenous *cis*-acting enhancer or repressor elements). Following gene transfer into plants, it is generally necessary to screen transformants for those showing the desired expression pattern and lack of developmental silencing in primary transformants and in subsequent generations tested under typical commercial growing conditions. These considerations are well known in the art, as discussed in Section 8 above, and for example by Finnegan and McElroy 1994, Matzke 1994, Meyer 1995, Birch 1997, Matzke and Birchler 2005, Brodersen and Voinnet 2006, Vaucheret 2006, Chapman and Carrington 2007, Ossowski 2008.

Materials and Methods

Choice of Target Locus

[0310] Sugarcane cDNA clones that are potentially differentially regulated in immature (sucrose catabolising) and maturing (sucrose loading) stems of sugarcane were identified initially by microarray analysis of EST libraries (Casu *et al.*, 2004). There are many uncertainties in the interpretation of microarray analyses, including differential expression of cross-hybridizing multi-gene family members, the presence of fusion events in EST clones and EST sequences too short to assign tentative gene functions. Further experimentation is required to determine which of many candidate EST clones indeed represent genes with particular expression patterns inferred from a microarray analysis. The present studies were conducted to search for genes expressed preferentially within the mature (sucrose storing) stems of sugarcane, a pattern not reported by Casu *et al.* (2004). Analysis of many sugarcane EST clones in this context led the present inventors to a particular interest in a sugarcane gene locus designated below as *ScCIPK1*.

RNA Extraction and Northern Analysis

[0311] RNA was extracted from field- or glasshouse-grown sugarcane cultivar Q117 tissues by CsCl ultracentrifugation (Chirgwin *et al.*, 1979). Following denaturation, approximately 10 µg RNA was fractionated on a 1.2% TBE agarose gel, and transferred onto Hybond N⁺ membrane (Amersham) using capillary transfer with 10×SSC. Hybridization was done for 16 h at 65°C in 0.5 M NaH₂PO₄, 1 mM EDTA, 7% SDS; using ³²P-dCTP-labelled

probes prepared using a Megaprime kit (Amersham). Following hybridization, membranes were washed to high stringency (0.1×SSC; 0.1% SDS at 65°C), and analyzed using a PhosphorImager SI (Molecular Dynamics).

DNA Extraction and Southern Analysis

- 5 **[0312]** Sugarcane genomic DNA was isolated using a CTAB-based method (Rogers and Bendich 1988). Ten µg of each sample was digested with restriction enzymes, fractionated on a 0.8% agarose TAE gel, and transferred onto Hybond N⁺ membrane (Amersham) using capillary transfer with either 0.4 M NaOH or 10 × SSC. Labeling of probes and hybridization conditions were as described for northern analysis.

10 DNA Sequencing

- [0313]** DNA sequencing of plasmid templates was done using a BigDye Terminator 3.1 DNA sequencing kit (Applied Biosystems), and separations by the Australian Genomic Research Facility (Brisbane, Queensland, Australia).

Analysis of Allelic Variation and Expression

- 15 **[0314]** The degree of allelic variation for *ScCIPK1* was assessed by sequencing homologous clones from a genomic BAC library prepared from sugarcane cultivar Q200. To identify expressed alleles, RT-PCR products derived from mature stem were amplified using the primers 5'-GCCATAACACTAAGACGAGCGCCAACC-3' and 5'-CGACACCCACTCCGGGAAGTCGAACTCC-3' and sequenced. For RT-PCR, cDNA was
20 synthesized using Superscript III (Invitrogen) and oligo dT primer using RNA from internodes 11-12 or 30. In all cases, the PCR was done using Expand High Fidelity polymerase (Roche) according to the manufacturer's instructions, and all amplified products were cloned into pGEM-T Easy (Promega) prior to sequencing.

Isolation of Upstream Promoter Regions and PCR Amplification

- 25 **[0315]** Promoter alleles corresponding to *ScCIPK1* were amplified using a PCR-based Genome Walker strategy (Clontech), using primers GSP1 (5'-CCTCCTCTGTCGGTTCGCTGGTCGAACAAGCCG-3') and GSP3B (5'-GTGCGTCGTCAGCGTCGCGAGGGCAAC -3') derived from the *ScCIPK1* coding region, or primers GW1 (5'-CCTTGGCGAACGTGCCTCGTC-3') and GW2 (5'-CGTGCCTCGTCCCAGCAAGC-3') derived from the 5' end of the *ScCIPK1* transcript. To
30 maximize the chances of amplifying all promoter alleles in the polyploid genome of cultivar

Q117, we used a total of eight GenomeWalker libraries (prepared using restriction enzymes *DraI*, *EcoRV*, *PvuII*, *ScaI*, *StuI*, *BsrBI*, *Ecl136II*, and *SspI*). These and all other PCR amplification reactions used Expand High Fidelity polymerase (Roche) according to the manufacturer's instructions. Amplified products were cloned into pGem-T Easy (Promega) prior to sequencing. Additionally, promoter alleles were obtained by subcloning from clones in a Q200 genomic BAC library. The transcriptional start site was determined by 5'RACE using the GeneRacer Kit (Invitrogen) according to the manufacturer's instructions.

Construction of Promoter-Reporter Gene Fusions

[0316] Promoter sequences with native 5'UTRs were excised from GenomeWalker and BAC clones using *PstI* and *NcoI* and cloned in front of the *luc*⁺NF (Promega) reporter gene. Constructs containing the same reporter gene driven by the maize *Ubi-1* promoter (Christensen *et al.*, 1992) were included as positive controls. All constructs included the *nos* terminator.

Sugarcane Transformation

[0317] Transformation and regeneration of sugarcane cultivar Q117 (obtained from BSES Meringa Sugar Experiment Station, Gordonvale, Qld) was done as previously described (Bower *et al.* 1996). Plants were grown in a containment glasshouse under natural light intensity, at 28°C and watered twice a day. Each plant was grown as a single stalk in a pot of 20 cm diameter and a density of 18 pots/m², fertilized with Osmocote[®] at 5 g/month for the first and the second months, then 10 g/month. Leaves were numbered from one for the top visual dewlap (TVD) with higher numbers for older leaves. Internodes were numbered according to the leaf attached to the node immediately above.

Reporter Gene Assays

[0318] LUC assays were done as described (Luehrsen and Walbot 1993), using a BMG POLARstar OPTIMA luminometer.

[0319] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0320] The citation of any reference herein should not be construed as an admission
5 that such reference is available as "Prior Art" to the instant application.

[0321] Throughout the specification the aim has been to describe the preferred
embodiments of the invention without limiting the invention to any one embodiment or
specific collection of features. Those of skill in the art will therefore appreciate that, in light
of the instant disclosure, various modifications and changes can be made in the particular
10 embodiments exemplified without departing from the scope of the present invention. All such
modifications and changes are intended to be included within the scope of the appended
claims.

BIBLIOGRAPHY

- Agrawal P.K., Kohli A., Twyman R. M. and Christou P. 2005. Transformation of plants with multiple cassettes generates simple transgene integration patterns and high expression levels. *Molecular Breeding* 16: 247-260.
- 5 Albert, H., Dale, E. C., Lee, E. and Ow, D. W. 1995. Site-specific integration of DNA into wild-type and mutant lox sites placed in the plant genome. *Plant Journal* 7(4):649-659.
- Allen, G. C., Hall, G., Michalowski, S., Newman, W., Spiker, S., Weissinger, A. K. and Thompson, W. F. 1996. High-level transgene expression in plant cells: Effects of a strong scaffold attachment region from tobacco. *Plant Cell* 8(5):899-913.
- 10 Allen, G. C., Spiker, S. and Thompson, W. F. 2000. Use of matrix attachment regions (MARS) to minimize transgene silencing. *Plant Molecular Biology* 43(2-3):361-376.
- Anandalakshmi, R., Pruss, G. J., Ge, X., Marathe, R., Mallory, A. C., Smith, T. H. and Vance, V. B. 1998. A viral suppressor of gene silencing in plants. *Proceedings of the National Academy of Sciences of the United States of America* 95(22):13079-13084.
- 15 Bower, R., Elliott, A.R., Potier, B.A.M., and Birch R.G. 1996. High-efficiency, microprojectile-mediated cotransformation of sugarcane, using visible or selectable markers. *Molecular Breeding* 2: 239-249.
- Brouwer, C., Bruce, W., Maddock, S., Avramova, Z. and Bowen, B. 2002. Suppression of transgene silencing by matrix attachment regions in maize: A dual role for the maize 5' *Adhl* matrix attachment region. *Plant Cell* 14(9):2251-2264.
- 20 Casu, R.E., Dimmock, C.M., Chapman, S.C., Grof, C.P.L., McIntyre, C.L., Bonnett, G.D, and Manners, J.M. 2004. Identification of differentially expressed transcripts from maturing stem of sugarcane by in silico analysis of stem expressed sequence tags and gene expression profiling. *Plant Molecular Biology* 54: 503-517.
- 25 Chernov, I. P., Akopov, S. B. and Nikolaev, L. G. 2004. Structure and functions of nuclear matrix associated regions (S/MARS). *Russian Journal of Bioorganic Chemistry* 30(1):1-11.

Chirgwin, J.M., Przybyla, A.E., Macdonald, R.J., and Rutter, W.J. 1979. Isolation of biologically-active ribonucleic-acid from sources enriched in ribonuclease. *Biochemistry* 18: 5294-5299.

Clarke, L., and Carbon, J. 1976. Colony bank containing synthetic Col El hybrid plasmids representative of entire *Escherichia Coli* genome. *Cell* 9: 91-99.

Christensen, A.H., Sharrock, R.A., and Quail, P.H. 1992. Maize polyubiquitin genes - Structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. *Plant Molecular Biology* 18: 675-689.

Cronn, R., Cedroni, M., Haselkorn, T., Grover, C., and Wendel, J.F. 2002. PCR-mediated recombination in amplification products derived from polyploid cotton. *Theoretical and Applied Genetics* 104: 482-489.

D'Halluin, K., Vanderstraeten, C., Stals, E., Cornelissen, M. and Ruiter, R. 2008. Homologous recombination: A basis for targeted genome optimization in crop species such as maize. *Plant Biotechnology Journal* 6(93-102).

De Buck, S., Van Montagu, M. and Depicker, A. 2001a. Transgene silencing of invertedly repeated transgenes is released upon deletion of one of the transgenes involved. *Plant Molecular Biology* 46(4):433-445.

De Buck, S. and Depicker, A. 2001b. Disruption of their palindromic arrangement leads to selective loss of DNA methylation in inversely repeated *gus* transgenes in *Arabidopsis*. *Molecular Genetics and Genomics* 265(6):1060-1068.

De Buck, S., Peck, I., De Wilde, C., Marjanac, G., Nolf, J., De Paepe, A. and Depicker, A. 2007. Generation of single-copy T-DNA transformants in *Arabidopsis* by the *cre/loxP* recombination-mediated resolution system. *Plant Physiology* 145(4):1171-1182.

De Rocher, E. J., Vargo-Gogola, T. C., Diehn, S. H. and Green, P. J. 1998. Direct evidence for rapid degradation of *Bacillus thuringiensis* toxin mRNA as a cause of poor expression in plants. *Plant Physiology* 117(4):1445-1461.

Diehn, S. H., Chiu, W. L., De Rocher, E. J. and Green, P. J. 1998. Premature polyadenylation at multiple sites within a *Bacillus thuringiensis* toxin gene-coding region. *Plant Physiology* 117(4):1433-1443.

Edme, S.J., Comstock, J.C., Miller, J.D., and Tai, P.Y.P. 2005. Determination of DNA content and genome size in sugarcane. *Journal of the American Society of Sugar Cane Technologists* 25: 1-16.

5 Gutierrez, R. A., MacIntosh, G. C. and Green, P.J. 1999. Current perspectives on mRNA stability in plants: multiple levels and mechanisms of control. *Trends in Plant Science* 4: 429-438.

Haseloff, J., Siemering, K. R., Prasher, D. C. and Hodge, S. 1997. Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. *Proceedings of the National Academy of Sciences of the USA* 94: 10 2122-2127.

Holmberg, N., Farres, J., Bailey, J. E. and Kallio, P. T. 2001. Targeted expression of a synthetic codon optimized gene, encoding the spruce budworm antifreeze protein, leads to accumulation of antifreeze activity in the apoplasts of transgenic tobacco. *Gene* 275(1):115-124.

15 Jefferson, R. 1987. Assaying chimeric genes in plants: The GUS gene fusion system. *Plant Molecular Biology Reporter* 5: 387-405.

Johansen, L. K. and Carrington, J. C. 2001. Silencing on the spot. Induction and suppression of RNA silencing in the *Agrobacterium*-mediated transient expression system. *Plant Physiology* 126(3):930-938.

20 Khanna, H. K. and Daggard, G. E. 2006. Targeted expression of redesigned and codon optimised synthetic gene leads to recrystallisation inhibition and reduced electrolyte leakage in spring wheat at sub-zero temperatures. *Plant Cell Reports* 25(12):1336-1346.

Koprek, T., Rangel, S., McElroy, D., Louwerse, J. D., Williams-Carrier, R. E. and Lemaux, P. G. 2001. Transposon-mediated single-copy gene delivery leads to increased 25 transgene expression stability in barley. *Plant Physiology* 125(3):1354-1362.

Kumar, S., Arul, L., Talwar, D. and Raina, S. K. 2006. PCR amplification of minimal gene expression cassette: an alternative, low cost and easy approach to 'clean DNA' transformation. *Current Science* 91: 930-934.

Lewsey, M., Robertson, F. C., Canto, T., Palukaitis, P. and Carr, J. P. 2007. Selective 30 targeting of miRNA-regulated plant development by a viral counter-silencing protein. *Plant Journal* 50: 240-252.

Li, X. Q., Wei, J. Z., Tan, A. and Aroian, R. V. 2007. Resistance to root-knot nematode in tomato roots expressing a nematocidal *Bacillus thuringiensis* crystal protein. *Plant Biotechnology Journal* 5(4):455-464.

5 Lim, H. S., Ko, T. S., Lambert, K. N., Kim, H. G., Korban, S. S., Hartman, G. L. and Domier, L. L. 2005. Soybean mosaic virus helper component-protease enhances somatic embryo production and stabilizes transgene expression in soybean. *Plant Physiology and Biochemistry* 43(10-11):1014-1021.

10 Louwerse, J. D., van Lier, M. C. M., van der Steen, D. M., de Vlaam, C. M. T., Hooykaas, P. J. J. and Vergunst, A. C. 2007. Stable recombinase-mediated cassette exchange in *Arabidopsis* using *Agrobacterium tumefaciens*. *Plant Physiology* 145(4):1282-1293.

Luehrsen, K., and Walbot, V. 1993. Firefly luciferase as a reporter for plant gene expression studies. *Promega Notes* 44: 24-29.

15 Luo, Z. H. and Chen, Z. X. 2007. Improperly terminated, unpolyadenylated mRNA of sense transgenes is targeted by *RDR6*-mediated RNA silencing in *Arabidopsis*. *Plant Cell* 19(3):943-958.

Mankin, S. L., Allen, G. C., Phelan, T., Spiker, S. and Thompson, W. F. 2003. Elevation of transgene expression level by flanking matrix attachment regions (MAR) is promoter dependent: A study of the interactions of six promoters with the *RB7* 3' MAR. *Transgenic Research* 12(1):3-12.

20 Milanesi, L., Muselli, M., and Arrigo, P. 1996. Hamming Clustering method for signals prediction in 5' and 3' regions of eukaryotic genes. *Computing Applications in the Biosciences* 12: p399-404.

25 Misztal, L. H., Mostowska, A., Skibinska, M., Bajsa, J., Musial, W. G. and Jarmolowski, A. 2004. Expression of modified *cryIac* gene of *Bacillus thuringiensis* in transgenic tobacco plants. *Molecular Biotechnology* 26(1):17-26.

Nanto, K., Yamada-Watanabe, K., Ebinuma, H. 2005. *Agrobacterium*-mediated RMCE approach for gene replacement. *Plant Biotechnology Journal* 3: 203-214.

Ow, D. W. 2002. Recombinase-directed plant transformation for the post-genomic era. *Plant Molecular Biology* 48: 183-200.

Ow, D. W. 2007. GM maize from site-specific recombination technology, what next? *Current Opinion in Biotechnology* 18: 115-120.

Rae, A.L., Grof, C.P.L., Casu, R.E., and Bonnett, G.D. 2005. Sucrose accumulation in the sugarcane stem: pathways and control points for transport and compartmentation. *Field Crops Research* 92: 159-168.

Rogers, S., and Bendich, A. 1988. Extraction of DNA from plant tissues. In: *Plant molecular biology manual*. Kluwer Academic Publishers, Dordrecht, pp 1-10.

Roth, B. M., Pruss, G. J. and Vance, V. B. 2004. Plant viral suppressors of RNA silencing. *Virus Research* 102(1):97-108.

10 Scholthof, H. B. 2007. Heterologous expression of viral RNA interference suppressors: Risk management. *Plant Physiology* 145:1110-1117.

Srivastava, V., Anderson, O. D. and Ow, D. W. 1999. Single-copy transgenic wheat generated through the resolution of complex integration patterns. *Proceedings of the National Academy of Sciences of the United States of America* 96(20):11117-11121.

15 Srivastava, V. and Ow, D. W. 2001. Single-copy primary transformants of maize obtained through the co-introduction of a recombinase-expressing construct. *Plant Molecular Biology* 46(5):561-566.

Srivastava, V. and Ow, D. W. 2002. Biolistic mediated site-specific integration in rice. *Molecular Breeding* 8: 345-350.

20 Thole, V., Worland, B., Snape, J. W. and Vain P 2007. The pCLEAN dual binary vector system for *Agrobacterium*-mediated plant transformation. *Plant Physiology* 145: 1211-1219.

Ulker, B., Allen, G. C., Thompson, W. F., Spiker, S. and Weissinger, A. K. 1999. A tobacco matrix attachment region reduces the loss of transgene expression in the progeny of transgenic tobacco plants. *Plant Journal* 18(3):253-263.

25 Vain, P., Worland, B., Kohli, A., Snape, J. W., Christou, P., Allen, G. C. and Thompson, W. F. 1999. Matrix attachment regions increase transgene expression levels and stability in transgenic rice plants and their progeny. *Plant Journal* 18(3):233-242.

Vergunst, A. C., Jansen, L. E. T. and Hooykaas, P. J. J. 1998. Site-specific integration of *Agrobacterium* T-DNA in *Arabidopsis thaliana* mediated by *cre* recombinase. *Nucleic Acids Research* 26(11):2729-2734.

5 Voinnet, O. 2005. Induction and suppression of RNA silencing: Insights from viral infections. *Nature Reviews Genetics* 6(3):206-U201.

Wright, D. A., Townsend, J. A., Winfrey, R. J., Irwin, P. A., Rajagopal, J., Lonosky, P. M., Hall, B. D., Jondle, M. D. and Voytas, D. F. 2005. High-frequency homologous recombination in plants mediated by zinc-finger nucleases. *Plant Journal* 44: 693-705.

10 Yan, H. and Rommens, C. M. 2007. Transposition-based plant transformation. *Plant Physiology* 143(2):570-578.

Yu, W., Rusterholtz, K.J., Krummel, A.T., and Lehman, N. 2006. Detection of high levels of recombination generated during PCR amplification of RNA templates. *Biotechniques* 40: 499-507.

15 Zhao, T., Palotta, M., Langridge, P., Prasad, M., Graner, A., Schulze-Lefert, P. and Koprek, T. 2006. Mapped ds/T-DNA launch pads for functional genomics in barley. *Plant Journal* 47(5):811-826.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a promoter sequence that is operable in plant cells, wherein the promoter sequence comprises a nucleotide sequence selected from the group consisting of:

- 5 (a) YGASMTTKAYWAKTSGCTARRRARN_aKTYRRAKRMRWARWTGKYR
SRRN_bHTTYN_cDMYTYKTN_dTTCCAASRGYTWRKMAAAYYAHRMTMCN_eRARGTAT
AKRWMAHAN_fWRMATKWGSRRTYYTRRRHKA WTTHYWRMTYAMYMYAWTCAK
YTYWAYWCWWTTYYYN_gMKKWYWTN_hAWMYYSYMTTWTRRGWACMYKATT
WRWRWKCYAAYCN_iYRRTTRRN_jAARYSCYARTYN_kTYGRATTTASWAACAAAAA
10 YGKCAAATTGCATACCCAACTCAN_lCYCATATAAAAMGKYACCAACCCAACATN_m
TN_nTTYC [SEQ ID NO: 1];

(b) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 1 or a complement thereof; and

- 15 (c) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 1 or a complement thereof, under at least medium or high stringency conditions,

wherein:

A, C, G and T represent the nucleic acid bases adenine, cytosine, guanine and thymine, respectively;

- 20 M is A or C;

R is A or G;

W is A or T;

S is C or G;

Y is C or T

- 25 K is G or T;

V is A or C or G;

H is A or C or T;

D is A or G or T;

B is C or G or T;

N is G or A or C or T;

each of a, b, c, d, e, g, i, k and m is an integer from 0-1;

each of h, j and n is an integer from 0-2;

l is an integer from 0-3; and

5 f is an integer from 0-5.

2. A nucleic acid sequence according to claim 1, wherein N_a comprises 0 bases or is
A.

3. A nucleic acid sequence according to claim 1, wherein N_b comprises 0 bases or is
K.

10 4. A nucleic acid sequence according to claim 1, wherein N_c comprises 0 bases or is
K.

5. A nucleic acid sequence according to claim 1, wherein N_d comprises 0 bases or is
A.

15 6. A nucleic acid sequence according to claim 1, wherein N_e comprises 0 bases or is
T.

7. A nucleic acid sequence according to claim 1, wherein N_f comprises 0 bases or a
sequence selected from ATTT or ACT.

8. A nucleic acid sequence according to claim 1, wherein N_g comprises 0 bases or is
T.

20 9. A nucleic acid sequence according to claim 1, wherein N_h comprises 0 bases or is
K.

10. A nucleic acid sequence according to claim 1, wherein N_i comprises 0 bases or is
A

25 11. A nucleic acid sequence according to claim 1, wherein N_j comprises 0 bases or is
the sequence TC.

12. A nucleic acid sequence according to claim 1, wherein N_j comprises 0 bases or is
W.

13. A nucleic acid sequence according to claim 1, wherein N_k comprises 0 bases or is a
sequence selected from GA or CT.

30 14. A nucleic acid sequence according to claim 1, wherein N_l comprises 0 bases or is
the sequence TGC.

15. A nucleic acid sequence according to claim 1, wherein N_m comprises 0 bases or is A.

16. A nucleic acid sequence according to claim 1, wherein N_n comprises 0 bases or is the sequence AT.

5 17. A nucleic acid sequence according to claim 1, which is selected from the group consisting of:

TGAGATTTACAAGTCGCTAAAAAAGTTGGAGGCGTAATTGTTGGGGCTTT
TGACTTTTTTCCAACAGTTTATAAAATCACGCTCCTAAAGTATAGAAAATAATTTT
GCATTAGGAATCTTAAACTATTTTCAAATTACCCTAATCATTTTTTATACTTTTTTTT
10 CTTTCTTGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATC
AAACCCAGTTTTCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCAT
CGCCCATATAAAACGTTACCAACCCAACATATATTTC [SEQ ID NO: 2];

TGAGATTTACAAGTCGCTAAAAAGGTTGGAGGCATAATTGTTGGGGCTTTG
ACTTTTTTTCCAACAGTTTATAAAATCACGCTCCTAAAGTATAGAAAACATGCATT
15 AGGAATCTTAAACTATTTTCAAATTACTCTAATCATTTTTTATACTTTTTTTCTTTCT
TGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCC
CAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCATCGCCCA
TATAAAACGGCACCAACCCAACATATATTTC [SEQ ID NO: 3];

TGAGATTTACAAGTCGCTAAAAAAGTTGGAGGCGTATTTGTTGGGGGTTT
20 TTGACTTTTTTCCAACAGTTTATAAAATCATGCTCCTAAAGTATAGAAAACAATTT
TGCATTAGGAATCCTAAACTATTTCTAAATTACCCTAATCATTTTTTATACTTTTTTT
TCTTTCTTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCA
AACCCAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCATC
GCCCATATAAAACGGCACCAACCCAACATATATTTC [SEQ ID NO: 4];

CGACCTTGATTATTGGCTAGGGAAATTCAAATAAAAAGATGGCACAATAT
TCTTACTCGTATTCCAAGGGCTAGGCAAATAAAATACGAGGTATATGTCAAAC
TCAAATGTGCGGTTTTGGGTGAATTATTGCTCAACACATTCAGCTCAACTCAATT
CCCTAGGATATTTAACCTCTCTTATAAGTACATTATTTATGTGCCAACCCAATTA
GAAGTGCTAATCTTGAATTTACTAACAAAAATGGCAAATTGCATACCCAACTCAC
30 CCATATAAAAAGGCACCAACCCAACATTATTTTC [SEQ ID NO: 5];

CGACCTTGATTATTGGCTAGGGAAATTCAAATAAAAAGATGGCACAATAT
TCTTACTCGTATTCCAAGGGCTAGGCAAATAAAATACGAGGTATATGTCAAAC
TCAAATGTGCGGTTTTGGGTGAATTATTGCTCAACACATTCAGCTCAACTCAATT

CCCTAGGATATTTAACCCTCTCTTATAAGTACATTATTTATGTGCCAACCCAATTG
GAAGTGCTAATCTTGAATTTACTAACAAAAATGTCAAATTGCATACCCAACCTCAC
CCATATAAAAAGGCACCAACCCAACATTATTTTC [SEQ ID NO: 6]; and

5 TGAGATTTACAAGTCGCTAAAAAAGTTGGAGGCGTAATTGTTGGGGCTTTG
ACTTTTTTCCCCGTTTATAAAATCACGCTCCTAAAGTATAGAAAACAATTTTGCAT
TAGGAATCTTAAAATATTTTCAAATTACCCTAATCATTTTTTATACTTTTTTTCTTTC
TTGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACC
CCAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACCTCATCGCTC
ATATAAACGGCACCAACCCAACATATTTCC [SEQ ID NO: 7].

10 18. A nucleic acid sequence according to claim 1, wherein the promoter sequence further comprises upstream of (a), (b) or (c) a nucleotide sequence selected from the group consisting of:

(d) YRWYWRWTAYS YCTWDGAWMRTYKTYRAWARAYMAN_oRVCARAKM
TSRARWTTGTTGASKYN_pYRYWRYAASCRTKGN_qCACTYRAAYDVRYRKRYHSCW
15 TAYTRWWGAAWMRMGAASMWRTSWWKMATWWRYYYAADMAMAN_rWMYKA
GCWAGMRMRYYYWWTCSARKACTTKMCMRAYKRRRTRN_sRAYATRYTCMAYN_tCA
YAAKWGKWGMDSN_uAYCMAMMYYYMCYKARTAWMTTATWMYTTWGAGCN_vY
AATWGTTTN_wAWAMAAN_xWYAYMRCWMAMAARN_yGYTWTCAAARMWN_zYARTT
GGYWN_{aa} [SEQ ID NO: 8];

20 (e) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 8 or a complement thereof; and

(f) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 8 or a complement thereof, under at least medium or high stringency conditions,

25 wherein:

A, C, G, T, M, R, W, S, Y, K, V, H, D, B and N are as defined above;

each of *p*, *s*, *t*, *x*, *y* and *z* is an integer from 0-1;

each of *q* and *r* is an integer from 0-2;

o is an integer from 0-4;

30 *w* is an integer from 0-5;

each of *v* and *aa* is an integer from 0-6; and

u is an integer from 0-23.

19. A nucleic acid sequence according to claim 18, wherein N_o is the sequence AGAC.

20. A nucleic acid sequence according to claim 18, wherein N_p is G.

21. A nucleic acid sequence according to claim 18, wherein N_q is the sequence GC.

5 22. A nucleic acid sequence according to claim 18, wherein N_r is a sequence selected from AA or TG.

23. A nucleic acid sequence according to claim 18, wherein N_s is T.

24. A nucleic acid sequence according to claim 18, wherein N_t is A.

25. A nucleic acid sequence according to claim 18, wherein N_u is a sequence selected
10 from AATTTCTAATCATGGTCAATATA [SEQ ID NO: 9],
AATTTATAATCATGGCTAATATA [SEQ ID NO: 10], TATATATATATTAATCTATC
[SEQ ID NO: 11], TATATATATTAATCTATC [SEQ ID NO: 12] or
AATTTCTAATCATGGCTAATATA [SEQ ID NO: 13].

26. A nucleic acid sequence according to claim 18, wherein N_v is the sequence
15 ATCTC.

27. A nucleic acid sequence according to claim 18, wherein N_w is A or a sequence selected from TCAA, GGCAC or TTTT.

28. A nucleic acid sequence according to claim 18, wherein N_x is D.

29. A nucleic acid sequence according to claim 18, wherein N_y is A.

20 30. A nucleic acid sequence according to claim 18, wherein N_z is A.

31. A nucleic acid sequence according to claim 18, wherein N_{aa} is the sequence AATCAA.

32. A nucleic acid sequence according to claim 18, wherein any one or more of N_o , N_p , N_q , N_r , N_s , N_t , N_u , N_v , N_w , N_x , N_y , N_z or N_{aa} comprises 0 bases.

25 33. A nucleic acid sequence according to claim 18, which is selected from the group consisting of:

CAACTAATATCTCTATGAGCATCTTCGAAAGACAAAGACGGCAGATCTCA
AAATTGTTGAGGTCATAATAAGCATTGCACTCAAACAGGCATGTTGCTTATTA
GAATAGCGAAGCAATGAAGAATAAATCCAAGAAAATATGAGCAAGCACGTCAAT
30 CGAGCACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGTGCTCAATTTCT
AATCATGGTCAATATAATCAAACCCACCGAGTATATTACTTAGAGCATCTC
CAATAGTTTTCAAAAAAAAAAATCATCGCAAAAAAAGTTTTCAAAAAAATAATTG
GTAAATCAA [SEQ ID NO: 14];

CAACTAATATCTCTAGGAGCATCTTCAAAAAGACAAAGACGACAGATCTCA
AGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTCGCTTATTTAA
GAATAGCGAAGCAATCAAGAATAAATTCAAAAAAAAAATATGAGCAAGCACATCA
ATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGCTCAATTT
5 ATAATCATGGCTAATATAATCAAACCCACCGAGTATATTATTACTTAGAGCATC
TCCAATAGTTTTTCAAAAAAAAAATTCATCGCAAAAAAAAAAGTTTTTCAAAAAATAAT
TGGTAAATCAA [SEQ ID NO: 15];

CAACTAATATCTCTATGAGCATCTTTAAAAGACAAAGACAACAGATCTCA
AGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGAGCATGTCGCTTATTTAA
10 GAATAGCGAAGCAATCAAGAATAAATTCAAGAAAATATGAGCAAGCACATCAAT
CGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGCTCATCAA
CCCCACCGAGTATATTATTACTTAGAGCATCTCCAATAGTTTTAAAAAAATCATC
GCAAAAAAGGTTTTTCAAAAAATAATTGGTAAATCAA [SEQ ID NO: 16];

TGTTAGTTACCCCTTAGAAAAGTTGTTAATAAATCAGCCAAAGATGGAATTT
15 GTTGACTCGTGCTACAACCATGGGCCACTTGAATTCATGGACACCATACTGTTGA
AACAAGAACATGTGTTTTCATTTGCTTAATCACATGACCTAGCTAGAGAGCTTTTCC
AATACTTTCCAGATGAAATGTAATATACTCCACACATAATTGTTGAAGTATATAT
ATATTAATCTATCACCCACATTTCCCTTAATAACTTATACTTTTGAGCTAATTGTTTG
GCACATACAAGATACAACCTCACAATGCTATCAAAGCTCAGTTGGCT [SEQ ID NO:
20 17];

TGTTAGTTACCCCTTAGAAAAGTTGTTAATAAATCAGCCAAAGATGGAATTT
GTTGACTCGTGCTACAACCATGGGCCACTTGAATTCATGGACACCATACTGTTGA
AACAAGAACATGTGTTTTCATTTGCTTAATCACATGACCTAGCTAGAGAGCTTTTCC
AATACTTTCCAGATGAAATGTAATATACTCCACACATAATTGTTGAGGTATATAT
25 ATTAATCTATCACCCACATTTCCCTTAATAACTTATACTTTTGAGCTAATTGTTTGG
CACATACAAGATACAACCTCACAATGCTATCAAAGCTCAGTTGGCT [SEQ ID NO:
18]; and

CAACTAATATGTCTATGAGCATCTTCAAAAAGACAAAGACGACAGATCTCA
AGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTCGCTTATTTAA
30 GAATAGCGAAGCAATCAAGAATAAATTCAAAAAAAAAATATGAGCAAGCACATCAAT
CGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGCTCAATTTCT
AATCATGGCTAATATAATCAAACCCACCCAGTATATTATTACTTAGAGCATCTC
CAATAGTTTTTTTTTAAAAAATCATCGCAAAAAAAAAAGCTTTCAAAAAATAATTGG
TAAATCAA [SEQ ID NO: 19].

34. A nucleic acid sequence according to claim 18, wherein the promoter sequence further comprises upstream of (d), (e) or (f) a nucleotide sequence selected from the group consisting of:

(g) WYHAWCRKTAYWWRAN_{ab}AGRAGYRWSN_{ac}YATGASAGCATRWGTRTM
 5 MCWMATGTRN_{ad}YWTTTCWTWRAAWATARYWWYYTWYRRARWTRKTATA
 KYAATAAATSARWCWA WRAKKRAWTWRTWKWTGAMTMRHKHTWYAAAYAT
 CKRYCAMTYRN_{ae}AKWMATRTRASWMCMTAMWRKTWWAAAAYRRAMWMAWGT
 RTTTGAWWN_{af}RHKTTTTMYWTYMATRAATN_{ag}AYAWYWWRWWTAYRSWHAWT
 CAWRWKMRAYYWYWYMCMTYATGRW [SEQ ID NO: 20];

10 (h) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 20 or a complement thereof; and

(i) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 20 or a complement thereof, under at least medium or high stringency conditions,

15 wherein:

A, C, G, T, M, R, W, S, Y, K, V, H, D, B and N are as defined above;

each of *ae*, *af* and *ag* is an integer from 0-1;

ab is an integer from 0-3;

ac is an integer from 0-8; and

20 *ad* is an integer from 0-9.

35. A nucleic acid sequence according to claim 34, wherein N_{ab} is the sequence CAT.

36. A nucleic acid sequence according to claim 34, wherein N_{ac} is the sequence AATAAAA.

25 37. A nucleic acid sequence according to claim 34, wherein N_{ad} is the sequence GATAAAGAT.

38. A nucleic acid sequence according to claim 34, wherein N_{ae} is G.

39. A nucleic acid sequence according to claim 34, wherein N_{af} is C.

40. A nucleic acid sequence according to claim 34, wherein N_{ag} is C.

30 41. A nucleic acid sequence according to claim 34, wherein any one or more of N_{ab}, N_{ac}, N_{ad}, N_{ae}, N_{af} or N_{ag} comprises 0 nucleotides

42. A nucleic acid sequence according to claim 34, which is selected from the group consisting of:

TCTACCGGTACTCGAAGGAGCGTCCATGAGAGCATGTGTGTCCCTAATGTG
 TTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCAAACAAA

GATGGAATTTGTTGATGACTCGCTTTACAAACATCGGCCACTTGGATTCATGGAC
ACCATACTGTTATAAAACAGAAACATGTGTTTGATTTCGTGTTTTCTTTTCATGAAT
CACAATATAAATATAGATATTCATGTGCGCACTCACCCATTATGAA [SEQ ID NO:
21];

5 TCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCTAATGTG
TTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCAAACAAA
GATGGAATTTGTTGATGACTCGTGCTACAAACATCGGCCACTTGGAGTCATGGAC
ACCCTACTGTTATAAAACGGAAACATGTGTTTGATTTCGCTTTTTCTTTTCATGAAT
CACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGA [SEQ ID NO:
10 22];

TCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCTAATGTG
TTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCAAACAAA
GATGGAATTTGTTGATGACTCGCGCTACAAACATCGGCCACTTGGATTCATGGAC
ACCATACTGTTATAAAACGGAAACATGTGTTTGATTTCGCTTTTTCTTTTCATGAAT
15 CACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGA [SEQ ID NO:
23];

ATAATCATTATATAACATAGAAGTAAGGAATAAAATATGACAGCATAAGT
ATAACACATGTAGATAAAGATCATTTCATAGAAAATAACATATTTACAAATAAATA
GTATAGCAATAAATGAGTCTATAAGTAATTAAATATTTGAATAAATATTTAAATA
20 TCTATCAATCAATAAATAAAGTACATAAAAGTTAAAAATGAACTAAAGTATTTGA
AAAAGTTTTACATCAATAAATATATCTAGTTTACGCTAAATCAAATAATATATTT
ACATCATGGT [SEQ ID NO: 24];

ATAATCATTATATAACATAGAAGTAAGGAATAAAATATGACAGCATAAGT
ATAACACATGTAGATAAAGATCATTTCATAGAAAATAACATATTTACAAATAAATA
25 GTATAGCAATAAATGAGTCTATAAGTAATTAAATATTTGAATAAATATTTAAATA
TCTATCAATCAATAAATAAAGTACATAAAAGTTAAAAATGAACTAAAGTATTTGA
AAAAGTTTTACATCAATAAATATATCTAGTTTACGCTAAATCAAATAATATATTT
ACATCATGGT [SEQ ID NO: 25]; and

TCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCTAATGTG
30 TTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCAAACAAA
GATGGAATTTGTTGATGACTCGCGCTACAAACATCGGCCACTTGGATTCATGGAC
ACCATACTGTTATAAAACGGAAACATGTGTTTGATTTCGCTTTTTCTTTTCATGAAT
CACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGA [SEQ ID NO:
26].

43. A nucleic acid sequence according to claim 34, wherein the promoter sequence further comprises upstream of (g), (h) or (i) a nucleotide sequence selected from the group consisting of:

(j) RRSKWMWAKYTYWRKAMWTRGTAYAKSRRTRMWYRWRTYTWTMAR
 5 TAWTTAWWTWWRTAARWAWCTAYN_{ah}YDATWYAWWTARWDRN_{ai}RAGKWSMY
 WSRWGWYAMMRAKGWRTTWATAKGWMTTYGWMAAYSTYWASATYRRTMARY
 WTAMTMTYTYAN_{aj}GGYWRRRKCMMAAKMAN_{ak}SATYATGGTYWRMWWWGKWK
 YYWKATWSTAKWMWWWMTMCRKATWWTAWTKMAWATMCWWWMTWMRWT
 WMTYTCAKATRYRRATMYAAATYMAGN_{al}TARTKWRAAWTSAAWTMWGMRWT
 10 AWSMATTTTAKWTWCATACYYGRWWTGGRTGKKAGMAAKWWMKGAWTMRY
 WTWWWTYRRWSRKYTTSAWRWMCRKW [SEQ ID NO: 27];

(k) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 27 or a complement thereof; and

15 (l) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 27 or a complement thereof, under at least medium or high stringency conditions,

wherein:

A, C, G, T, M, R, W, S, Y, K, V, H, D, B and N are as defined above;

each of *ah* and *al* is an integer from 0-1;

20 *aj* is an integer from 0-2;

ai is an integer from 0-5; and

ak is an integer from 0-8.

44. A nucleic acid sequence according to claim 43, wherein N_{ah} is G.

25 45. A nucleic acid sequence according to claim 43, wherein N_{ai} is a sequence selected from AATAA, AATA or TAGGT.

46. A nucleic acid sequence according to claim 43, wherein N_{aj} is the sequence AA.

47. A nucleic acid sequence according to claim 43, wherein N_{ak} is a sequence selected from TATGTTTA or AATTAAAC.

48. A nucleic acid sequence according to claim 43, wherein N_{al} is C.

30 49. A nucleic acid sequence according to claim 43, wherein any one or more of N_{ah}, N_{ai}, N_{aj}, N_{ak} or N_{al} comprises 0 bases.

50. A nucleic acid sequence according to claim 43, which is selected from the group consisting of:

AACTAATATCTCAGTAAATAGTATAGCAATAAATGAGTCTATAAGTATTTA
AATATATAAATATCTACTGATATAAATAAATAAAAGTACATACAAGTTACA
AATGAATTAATAAGGTATTTGAAAACGTTTACATCAATAAATATACTATTTTAGG
CTAAATCAAATAAATATGTTTACATCATGGTTTACATAGTTGCTTTATTCTATACA
5 AATACGGATAATATTGAATATCCATCGTACGTTTATTTTCATATACGGATCCAAATT
CAGCTAATGAAAAATGAATTCAGAAATATCTATTTTATTTTCATACCTGAAATGG
ATGTGAGAAATATATGAATAATATAATTCAGCCAGCTTCATATCCATT [SEQ ID
NO: 28];

AACGAATATATCAATAAATAGTATAGCAATAAATGAGTCTATAAGTAATT
10 AAATATATAAATATCTATCAATATAAATAAATAAAGTACATACAAGTTACAAATG
AATTAATAAGGTATTTGAAAACGTTTAAATCAATAAATATACTATTTTAGGCTAA
ATCAAATAACATCATGGTTTACATAGTTGCTTTATTCTATACAAATACGGATAAT
ATTGAATATCCTTCATACGTTTATTTTCATATATGAATCCAAATTCAGCTAATGAAA
AATGAATTCAGAGATATCCATTTTATTTTCATACCTGAAATGGATGTGAGAAATA
15 TATGAATAATATAATTCAGACAGCTTCATATCCATT [SEQ ID NO: 29];

AACGAATATCTCAATAAATAGTATAGCAATAAATGAGTCTATAAGTAATT
AAATATATAAATATCTATCGATATAAATAAATAAATAAAGTACATACAAGTTACA
AATGAATTAATAAGGTATTTGAAAACGTTTACATTAATAAATATACTATTTTAGG
CTAAATCAAATAACATCATGGTTTACATAGTTGCTTTATTCTATACAAATACGG
20 ATAATATTGAATATCCTTCATACGTTTATTTTCATATATGAATCCAAATTCAGCTAA
TGAAAAATGAATTCAGAGATATCCATTTTATTTTCATACCTGAAATGGATGTGAG
AAATATATGAATAATATAATTCAGACAGCTTCATATCCATT [SEQ ID NO: 30];

GGGTTCAAGTTTTAGACTTGGTACATGGGTGCTCATATTTTCAATATTTAT
TTTAGTAAGAACTATGTTATTCATTTAGTAGTAGGTGAGGTGCCTGGTGACAAC
25 GAGGTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCTCTCAAAG
GTAGGGGCCAAAGCAAATTAACGATTATGGTCAGATATGGATTCAGATAGTAG
TATTTTCCATATTTTAATTCAAATACTAATTTAAATACTCTCAGATGCAAATATAA
ATCAAGTAGTTTGAATTCAAATATGCATTAAGAATTTTAGATACATACTCGGTTTG
GGTGGTAGCAAGTACGGATTCGCTTTTATTGATGGTTTTGAAGAACGGA [SEQ ID
30 NO: 31]; and

GGGTTCAAGTTTTAGACTTGGTACATGGGTGCTCATATTTTCAATATTTAT
TTTAGTAAGAACTATGTTATTCATTTAGTGGTAGGTGAGGTGCCTGGTGACAAC
GAGGTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCTCTCAAAG
GTAGGGGCCAAAGCAAATTAACGATTATGGTCAGATATGGATTCAGATAGTAG

TATTTTCCATATTTTAATTCAAATACTAATTTAAATACTCTCAGATGCAAATATAA
ATCAAGTAGTTTGAATTCAAATATGCATTAAGAATTTTAGATACTCGGTTTG
GGTGGTAGCAAGTACGGATTGCTTTTATTGATGGTTTTGAAGAACGGA [SEQ ID
NO: 32].

- 5 51. A nucleic acid sequence according to claim 18, wherein the promoter sequence further comprises upstream of (j), (k) or (l) a nucleotide sequence selected from the group consisting of:

(m) YWYTYWRWKTGKSYK WYWDMAAGWRWSYATW_{N_{am}}RMKWTRWRRT
BTWGAAGMWTBKAATWRYSMCATRTATAN_{N_{an}}TAAAGAAWARN_{N_{ao}}AN_{N_{ap}}TKACAYTA
10 YWWGWAYAWYAYATSTARRYAMWKGTCATWYATRRAMMMTWYMYAWWK
AKWN_{N_{aq}} [SEQ ID NO: 33];

(n) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 33 or a complement thereof; and

- 15 (o) a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 33 or a complement thereof, under at least medium or high stringency conditions,

wherein:

A, C, G, T, M, R, W, S, Y, K, V, H, D, B and N are as defined above;

each of *am* and *ao* is an integer from 0-1;

20 *ap* is an integer from 0-2;

an is an integer from 0-11; and

aq is an integer from 0-26.

52. A nucleic acid sequence according to claim 51, wherein N_{am} is W.

53. A nucleic acid sequence according to claim 51, wherein N_{an} is a sequence selected
25 from AAACGCAAAAT [SEQ ID NO: 34], AAACGCAAAAC [SEQ ID NO: 35] or
TAAAGAGTATA [SEQ ID NO: 36].

54. A nucleic acid sequence according to claim 51, wherein N_{ao} is T.

55. A nucleic acid sequence according to claim 51, wherein N_{ap} is a sequence selected from TA or AA.

56. A nucleic acid sequence according to claim 51, wherein N_{aq} is a sequence selected from GCAATAAATGAGTCTATAATATGCAC [SEQ ID NO: 37] or GGGGGTGGAACCTTCCTTCAC [SEQ ID NO: 38].

57. A nucleic acid sequence according to claim 51, wherein any one or more of N_{am} , N_{an} , N_{ao} , N_{ap} or N_{aq} comprises 0 nucleotides.

58. A nucleic acid sequence according to claim 51, which is selected from the group consisting of:

TATTCAATTTGGGTGATAGCAAGTATGCATTGCTTTATGGTTTTGAAGAAT
GGAATAATCACATATATAAAACGCAAAATTAAAGAATAAATATGACACTATATG
10 TATAATACATGTAGACAATGGTCATTCATAGAAAATAACATATTTATAGCAATAA
ATGAGTCTATAATATGCAC [SEQ ID NO: 39];

TATTCAATTTGGGCGATATCAAGTATGCATTTGCTTTATGGTTTTGAAGAA
TGGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACTATAAGT
ATAATACATGTAGACAATGGTCATTCATAGAACATAACATATTTAGA [SEQ ID
15 NO: 40];

TATTCAATTTGGGCGATAGCAAGTATGCATTTGCTTTATGGTTTTGAAGAA
TTGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACTATAAGT
ATAATACATGTAGGCAATGGTCATTCATAGAACATAACATATTTAGA [SEQ ID
NO: 41];

20 CTCTTTGAGTGTCTTTCTAAAAGAGACTATAAAAGATGAAATGTAGAAGCT
TCTAATTGCGCCATGTATATAAAGAGTATATAAAGAAAAGTAAATTACATTACTT
GAATATCATATCTAAATACATGTCATATATGAACCCTTTTCCAAAGAGTGGGGGT
GGAACCTTCCTTCAC [SEQ ID NO: 42]; and

CTCTTTGAGTGTCTTTCTAAAAGAGACTATAAAAGATGAAATCTAGAAGCT
25 TCTAATTGCGCCATGTATATAAAGAAAAGTAAATTACATTACTTGAACATCATAT
CTAAATACATGTCATATATGAACCCTTTTCCAAAGAGTGGGGGTGGAACCTTCCT
TCAC [SEQ ID NO: 43].

59. A nucleic acid sequence according to claim 51, wherein an intervening sequence is interposed between the nucleotide sequence according to any one of (d), (e) or (f) and the
30 nucleotide sequence according to the nucleotide sequence according to any one of (g), (h) or (i).

60. A nucleic acid sequence according to claim 59, wherein the intervening sequence is selected from the group consisting of:

TCTATACCTAATAATAAAGAGGCAAAATTTCTCTTCACCTGTTTTTTTGGG
 TCCGGCCATCCCTTAACCTTTGCGAATGTGAAAACTGCTTATAGCCCTTCTC
 TTTATATAATTAGGAATCATAATCCAATTAGATCTTTCTGATTTCTGGGTGAATAGG
 AATATTAATCCAAATAGAAAAAATATAATAATATGCA [SEQ ID NO: 44], or a

5 nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 44 or a complement thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 44 or a complement thereof, under at least medium or high stringency conditions;

TTCATTAGGTCCTTTATTCTATACAAATACGAATAATATTGAATATCCATCC
 10 CATATTGATTTTCATATATAGATCCAAATTTAGCTAATAAAAATGAATTCAGATAT
 ATCCAGCTTCAGATCCATTTCCACCGTTACTTGAAGATGCTAATAGGGGTGTTTAT
 AGGGGTGAGTATGCGTGAGAGCGTGTATGTCTGTACTT [SEQ ID NO: 45], or a

nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 45 or a
 15 complement thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 45 or a complement thereof, under at least medium or high stringency conditions; and

TTCATTAGGTCCTTTATTCTATACAAATACGAATAATATTGAATATCCATCC
 CATATTGATTTTCATATATAGATCCAAATTTAGCTAATAAAAATGAATTCAGATAT
 ATCCAGCTTCAGATCCATTTCCACCGTTACTTGAAGATGCTAATAGGGGTGTTTAT
 20 AGGGGTGAGTATGCGTGAGAGCGTGTATGTCTGTACTT [SEQ ID NO: 46], or a

nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO: 46 or a
 complement thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ
 ID NO: 46 or a complement thereof, under at least medium or high stringency conditions.

25 61. A nucleic acid sequence according to any one of claim 1 to 60, wherein the promoter sequence has a structure represented by formula (I):



wherein:

Θ is any one of (m), (n) or (o);

30 Δ is any one of (j), (k) or (l);

Z is any one of (g), (h) or (i);

Ψ is an optional spacer or intervening sequence;

Φ is any one of (d), (e) or (f); and

Ω is any one of (a), (b) or (c).

62. A nucleic acid sequence according to claim 61, wherein the promoter sequence comprises a nucleotide sequence selected from the group consisting of:

5 TATTCAATTTGGGTGATAGCAAGTATGCATTGCTTTATGGTTTTGAAGAAT
 GGAATAATCACATATATAAAACGCAAAATTAAAGAATAAATATGACACTATATG
 TATAATACATGTAGACAATGGTCATTCATAGAAAATAACATATTTATAGCAATAA
 ATGAGTCTATAATATGCACAATAATATCTCAGTAAATAGTATAGCAATAAATGA
 GTCTATAAGTATTTAAATATATAAATATCTACTGATATAAATAAATAAAAG
 10 TACATACAAGTTACAAATGAATTAATAAGGTATTTGAAAACGTTTACATCAATAA
 ATATACTATTTTAGGCTAAATCAAATAATATGTTTACATCATGGTTTACATAGTT
 GCTTTATTCTATACAAATACGGATAATATTGAATATCCATCGTACGTTTATTTTCAT
 ATACGGATCCAAATTCAGCTAATGAAAAATGAATTCAGAAATATCTATTTTATTT
 TCATACCTGAAATGGATGTGAGAAATATATGAATAATATAATTCAGCCAGCTTCA
 15 TATCCATTTCTACCGGTACTCGAAGGAGCGTCCATGAGAGCATGTGTGTCCCTAA
 TGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCAAA
 CAAAGATGGAATTTGTTGATGACTCGCTTTACAAACATCGGCCACTTGGATTCAT
 GGACACCATACTGTTATAAAACAGAAACATGTGTTTGATTTCGTGTTTTCTTTTCAT
 GAATCACAATATAAATATAGATATTCATGTGCGCACTCACCCATTATGAATCTAT
 20 ACCTAATAATAAAGAGGGCAAAATTTCTCTTCACCTGTTTTTTTTGGGTCCGGCCAT
 CCCTTAACTAACTTTGCGAATGTGAAAAACTGCTTATAGCCCTTCTCTTTATATAA
 TTAGGAATCATAATCCAATTAGATCTTTCTGATTTCTGGGTGAATAGGAATATTAAT
 CCAAATAGAAAAAATATAATAATATGCACAATAATATCTCTATGAGCATCTTCG
 AAAGACAAAGACGGCAGATCTCAAATTTGTTGAGGTCATAATAAGCATTGCACT
 25 CAAACAGGCATGTTGCTTATTAAAGAATAGCGAAGCAATGAAGAATAAATCCAA
 GAAATATGAGCAAGCACGTCAATCGAGCACTTGACCAACTGGGTAGACATGTT
 CAATCACAAGAGGTGCTCAATTTCTAATCATGGTCAATATAATCAAACCCACCG
 AGTATATTATTACTTAGAGCATCTCCAATAGTTTTCAAAAAAAAAAATCATCGCAA
 AAAAAGTTTTCAAAAAAATAATTGGTAAATCAATGAGATTTACAAGTCGCTAAAA
 30 AAAGTTGGAGGCGTAATTGTTGGGGCTTTTGACTTTTTTCCAACAGTTTATAAAAT
 CACGCTCCTAAAGTATAGAAAATAATTTTGCATTAGGAATCTTAAACTATTTTCA
 AATTACCCTAATCATTTTTTATACTTTTTTTCTTTCTTGTATATTTGCATTTTGGGA
 ACCCGATTAGAAATCTAATCATGGTTAATCAAACCCAGTTTTTCGGATTTAGAAA

CAAAAACGGCAAATTGCATACCCAACTCATCGCCCATATAAAACGTTACCAACCC
 AACATATATTTCC [SEQ ID NO: 47], or a nucleotide sequence that shares at least 80%
 (and at least 81% to at least 99% and all integer percentages in between) sequence identity
 with the sequence set forth in SEQ ID NO: 47 or a complement thereof, or a nucleotide
 5 sequence that hybridizes to the sequence set forth in SEQ ID NO: 47 or a complement thereof,
 under at least medium or high stringency conditions;

TATTCAATTTGGGCGATATCAAGTATGCATTTGCTTTATGGTTTTGAAGAA
 TGGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACTATAAGT
 ATAATACATGTAGACAATGGTCATTCATAGAACATAACATATTTAGAAACGAATA
 10 TATCAATAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAAATATATAAATA
 TCTATCAATATAAATAAATAAAGTACATACAAGTTACAAATGAATTAATAAGGTA
 TTTGAAAACGTTTAAATCAATAAATATACTATTTTAGGCTAAATCAAATAACAT
 CATGGTTTACATAGTTGCTTTATTCTATACAAATACGGATAATATTGAATATCCTT
 CATACGTTTATTTTCATATATGAATCCAAATTCAGCTAATGAAAAATGAATTCAGA
 15 GATATCCATTTTATTTTCATACCTGAAATGGATGTGAGAAATATATGAATAATAT
 AATTCAGACAGCTTCATATCCATTTCCACCGTTACTTGAAGGAGTGTCCATGAGA
 GCATGTGTGTCCCTAATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTT
 ATATTAATAAATCAAACAAAGATGGAATTTGTTGATGACTCGTGCTACAAACATC
 GGCCACTTGGAGTCATGGACACCCTACTGTTATAAAACGGAAACATGTGTTTGAT
 20 TCGCTTTTTCTTTTCATGAATCACAATATAAATATAGACATTCATGTGCGCACTCA
 CACCTTATGGACAATAATATCTCTAGGAGCATCTTCAAAAGACAAAGACGACAG
 ATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTGCGCTT
 ATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAAAAAAAAATATGAGCAAGC
 ACATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGC
 25 TCAATTTATAATCATGGCTAATATAATCAAACCCACCGAGTATATTATTACTTAG
 AGCATCTCCAATAGTTTTCAAAAAAAAAATTCATCGCAAAAAAAAAAGTTTTCAAAAA
 AATAATTGGTAAATCAATGAGATTTACAAGTCGCTAAAAAGGTTGGAGGCATAAT
 TGTGTTGGGGCTTTGACTTTTTTTCCAACAGTTTATAAAATCACGCTCCTAAAGTATA
 GAAAACATGCATTAGGAATCTTAACTATTTTCAAATTACTCTAATCATTTTTATA
 30 CTTTTTTTCTTTCTTGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATG
 GTTAATCAAACCCAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCC
 AACTCATCGCCCATATAAAACGGCACCAACCCAACATATTTCC [SEQ ID NO: 48],
 or a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all
 integer percentages in between) sequence identity with the sequence set forth in SEQ ID NO:

48 or a complement thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 48 or a complement thereof, under at least medium or high stringency conditions;

TATTCAATTTGGGCGATAGCAAGTATGCATTTGCTTTATGGTTTTGAAGAA
 5 TTGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACTATAAGT
 ATAATACATGTAGGCAATGGTCATTCATAGAACATAACATATTTAGAAACGAATA
 TCTCAATAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAAATATATAAATA
 TCTATCGATATAAATAAATAAATAAAGTACATACAAGTTACAAATGAATTAATAA
 GGTATTTGAAAACGTTTACATTAATAAATATACTATTTTAGGCTAAATCAAAATA
 10 ACATCATGGTTTACATAGTTGCTTTATTCTATACAAATACGGATAATATTGAATAT
 CCTTCATACGTTTATTTTCATATATGAATCCAAATTCAGCTAATGAAAAATGAATTC
 AGAGATATCCATTTTATTTTCATACCTGAAATGGATGTGAGAAATATATGAATAA
 TATAATTCAGACAGCTTCATATCCATTTCCACCGTTACTTGAAGGAGTGTCCATGA
 GAGCATGTGTGTCCCTAATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTG
 15 TTATATTAATAAATCAAACAAAGATGGAATTTGTTGATGACTCGCGCTACAAACA
 TCGGCCACTTGGATTCATGGACACCATACTGTTATAAAACGGAAACATGTGTTTG
 ATTCGCTTTTTCTTTTCATGAATCACAATATAAATATAGACATTCATGTGCGCACT
 CACACCTTATGGACAACATAATCTCTATGAGCATCTTTAAAAGACAAAGACAAC
 AGATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGAGCATGTTCG
 20 TTATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAGAAAATATGAGCAAGC
 ACATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGC
 TCATCAAACCCACCGAGTATATTACTTAGAGCATCTCCAATAGTTTAAAAA
 AAATCATCGCAAAAAAGGTTTTCAAAAAAATAATTGGTAAATCAATGAGATTTAC
 AAGTCGCTAAAAAAAGTTGGAGGCGTATTTGTTGGGGGTTTTTGACTTTTTTCCAA
 25 CAGTTTATAAAATCATGCTCCTAAAGTATAGAAAACAATTTTGCATTAGGAATCC
 TAAACTATTTCTAAATTACCCTAATCATTTTTTATACTTTTTTTCTTTCTTATATTG
 CATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCACGTTATCG
 GATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCATCGCCCATATAAAAC
 GGCACCAACCCAACATATATTTCC [SEQ ID NO: 49], or a nucleotide sequence that
 30 shares at least 80% (and at least 81% to at least 99% and all integer percentages in between)
 sequence identity with the sequence set forth in SEQ ID NO: 49 or a complement thereof, or a
 nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 49 or a
 complement thereof, under at least medium or high stringency conditions;

CTCTTTGAGTGTCTTTCTAAAAGAGACTATAAAAGATGAAATGTAGAAGCT
 TCTAATTGCGCCATGTATATAAAGAGTATATAAAGAAAAGTAAATTACATTACTT
 GAATATCATATCTAAATACATGTCATATATGAACCCTTTTCCAAAGAGTGGGGGT
 GGAACCTTCCTTCACGGGTTCAGTTTTAGACTTGGTACATGGGTGCTCATATTTT
 5 TCAATATTTATTTTAGTAAGAACTATGTTATTCATTTAGTAGTAGGTGAGGTGCC
 TGGTGACAACGAGGTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTA
 ATCTCTCAAAGGTAGGGGCCAAAGCAAATTAAACGATTATGGTCAGATATGGATT
 CAGATAGTAGTATTTTCCATATTTTAATTCAAATACTAATTTAAATACTCTCAGAT
 GCAAATATAAATCAAGTAGTTTGAATTCAAATATGCATTAAGAATTTTAGATACA
 10 TACTCGGTTTGGGTGGTAGCAAGTACGGATTTCGCTTTTATTGATGGTTTTGAAGAA
 CGGAATAATCATTATATAACATAGAAGTAAGGAATAAAATATGACAGCATAAGT
 ATAACACATGTAGATAAAGATCATTTCATAGAAAATAACATATTTACAAATAAATA
 GTATAGCAATAAATGAGTCTATAAGTAATTAAATATTTGAATAAATATTTAAATA
 TCTATCAATCAATAAATAAAGTACATAAAAGTTAAAAATGAACTAAAGTATTTGA
 15 AAAAGTTTTACATCAATAAATATATCTAGTTTACGCTAAATCAAATAATATATTT
 ACATCATGGTTTCATTAGGTCCTTTATTCTATACAAATACGAATAATATTGAATAT
 CCATCCCATATTGATTTTCATATATAGATCCAAATTTAGCTAATAAAAATGAATTCA
 GATATATCCAGCTTCAGATCCATTTCCACCGTTACTTGAAGATGCTAATAGGGGT
 GTTTATAGGGGTGAGTATGCGTGAGAGCGTGTATGTCTGTACTTTGTTAGTTACCC
 20 CTTAGAAAGTTGTTAATAAATCAGCCAAAGATGGAATTTGTTGACTCGTGCTACA
 ACCATGGGCCACTTGAATTCATGGACACCATACTGTTGAAACAAGAACATGTGTT
 TCATTTGCTTAATCACATGACCTAGCTAGAGAGCTTTTCCAATACTTTCCAGATGA
 AATGTAATATACTCCACACATAATTGTTGAAGTATATATATATTAATCTATCACC
 ACATTTCTTAATAACTTATACTTTTGAGCTAATTGTTTGGCACATACAAGATACA
 25 ACTCACAATGCTATCAAAGCTCAGTTGGCTCGACCTTGATTATTGGCTAGGGAAA
 TTCAAATAAAAAGATGGCACAATATTCTTACTCGTATTCCAAGGGCTAGGCAAAC
 TAAAATACGAGGTATATGTCAAACTCAAAATGTGCGGTTTTGGGTGAATTATTG
 CTCAACACATTCAGCTCAACTCAATTCCCTAGGATATTTAACCCTCTCTTATAAGT
 ACATTATTTATGTGCCAACCCAATTAGAAGTGCTAATCTTGAATTTACTAACAAA
 30 AATGGCAAATTGCATACCCAACCTCACCCATATAAAAAGGCACCAACCCAACATTA
 TTTTC [SEQ ID NO: 50], or a nucleotide sequence that shares at least 80% (and at least
 81% to at least 99% and all integer percentages in between) sequence identity with the
 sequence set forth in SEQ ID NO: 50 or a complement thereof, or a nucleotide sequence that

hybridizes to the sequence set forth in SEQ ID NO: 50 or a complement thereof, under at least medium or high stringency conditions;

CTCTTTGAGTGTCTTTCTAAAAGAGACTATAAAAGATGAAATCTAGAAGCT
TCTAATTGCGCCATGTATATAAAGAAAAGTAAATTACATTACTTGAACATCATAT
5 CTAAATACATGTCATATATGAACCCTTTTCCAAAGAGTGGGGGTGGAACCTTCCT
TCACGGGTTC AAGTTTTAGACTTGGTACATGGGTGCTCATATTTTTCAATATTTAT
TTAGTAAGAACTATGTTATTCATTTAGTGGTAGGTGAGGTGCCTGGTGACAAC
GAGGTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCTCTCAAAG
GTAGGGGCCAAAGCAAATTAACGATTATGGTCAGATATGGATTCAGATAGTAG
10 TATTTTCCATATTTTAATTCAAATACTAATTTAAATACTCTCAGATGCAAATATAA
ATCAAGTAGTTTGAATTCAAATATGCATTAAGAATTTTAGATACATACTCGGTTTG
GGTGGTAGCAAGTACGGATTCGCTTTTATTGATGGTTTTGAAGAACGGAATAATC
ATTATATAACATAGAAGTAAGGAATAAAATATGACAGCATAAGTATAACACATG
TAGATAAAGATCATTTCATAGAAAATAACATATTTACAAATAAATAGTATAGCAAT
15 AAATGAGTCTATAAGTAATTAATATTTGAATAAATATTTAAATATCTATCAATC
AATAAATAAAGTACATAAAAAGTTAAAAATGAACTAAAGTATTTGAAAAAGTTTT
ACATCAATAAATATATCTAGTTTACGCTAAATCAAATAATATATTTACATCATG
GTTTCATTAGGTCCTTTATTCTATACAAATACGAATAATATTGAATATCCATCCCA
TATTGATTTTCATATATAGATCCAAATTTAGCTAATAAAAATGAATTCAGATATATC
20 CAGCTTCAGATCCATTTCCACCGTACTTGAAGATGCTAATAGGGGTGTTTATAG
GGGTGAGTATGCGTGAGAGCGTGTATGTCTGTACTTTGTTAGTTACCCCTTAGAA
AGTTGTTAATAAATCAGCCAAAGATGGAATTTGTTGACTCGTGCTACAACCATGG
GCCACTTGAATTCATGGACACCATACTGTTGAAACAAGAACATGTGTTTCATTTG
CTTAATCACATGACCTAGCTAGAGAGCTTTTCCAATACTTTCCAGATGAAATGTA
25 ATATACTCCACACATAATTGTTGAGGTATATATATTAATCTATCACCCACATTTCC
TTAATAACTTATACTTTTGAGCTAATTGTTTGGCACATACAAGATACAACCTCACAA
TGCTATCAAAGCTCAGTTGGCTCGACCTTGATTATTGGCTAGGGAAATTCAAATA
AAAAGATGGCACAATATTCTTACTCGTATTCCAAGGGCTAGGCAAACTAAAATAC
GAGGTATATGTCAAACTCAAAATGTGCGGTTTTTGGGTGAATTATTGCTCAACAC
30 ATTCAGCTCAACTCAATTCCCTAGGATATTTAACCCTCTCTTATAAGTACATTATT
TATGTGCCAACCCAATTGGAAGTGCTAATCTTGAATTTACTAACAAAAATGTCAA
ATTGCATACCCAACCTCACCCATATAAAAAGGCACCAACCCAACATTATTTTC

[SEQ ID NO: 51], or a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set

forth in SEQ ID NO: 51 or a complement thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 51 or a complement thereof, under at least medium or high stringency conditions; and

TCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCTAATGTG
 5 TTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCAAACAAA
 GATGGAATTTGTTGATGACTCGCGCTACAAACATCGGCCACTTGGATTCATGGAC
 ACCATACTGTTATAAAACGGAAACATGTGTTTGATTGCTTTTTCTTTTCATGAAT
 CACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGACAATAATA
 TGTCTATGAGCATCTTCAAAAGACAAAGACGACAGATCTCAAGATTGTTGAGGTC
 10 ATAGCAAGCGTTGCACTCAAACGGGCATGTCGCTTATTAAAGAATAGCGAAGCA
 ATCAAGAATAAATTCAAAAAAATATGAGCAAGCACATCAATCGAGGACTTGACC
 AACTGGGTAGACATGTTCAATCACAAGAGGAGCTCAATTTCTAATCATGGCTAAT
 ATAATCAAACCCACCCAGTATATTACTTAGAGCATCTCCAATAGTTTTTTTT
 AAAAAATCATCGCAAAAAAAGCTTTCAAAAAAATAATTGGTAAATCAATGAGA
 15 TTTACAAGTCGCTAAAAAAGTTGGAGGCGTAATTGTTGGGGCTTTGACTTTTTTCC
 CCGTTTATAAAATCACGCTCCTAAAGTATAGAAAACAATTTTGCATTAGGAATCT
 TAAAATATTTTCAAATTACCCTAATCATTTTTTATACTTTTTTTCTTCTTGTATATTT
 GCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCAGTTATC
 GGATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCATCGCTCATATAAAAC
 20 GGCACCAACCCAACATATTTCC [SEQ ID NO: 52], or a nucleotide sequence that shares
 at least 80% (and at least 81% to at least 99% and all integer percentages in between)
 sequence identity with the sequence set forth in SEQ ID NO: 52 or a complement thereof, or a
 nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 52 or a
 complement thereof, under at least medium or high stringency conditions.

25 63. A nucleic acid sequence according to claim 61, wherein the promoter sequence
 comprises a nucleotide sequence selected from the group consisting of:

TATTCAATTTGGGTGATAGCAAGTATGCATTGCTTTATGGTTTTGAAGAAT
 GGAATAATCACATATATAAAACGCAAAATTAAAGAATAAATATGACACTATATG
 TATAATACATGTAGACAATGGTCATTCATAGAAAATAACATATTTATAGCAATAA
 30 ATGAGTCTATAATATGCACAACTAATATCTCAGTAAATAGTATAGCAATAAATGA
 GTCTATAAGTATTTAAATATATAAATATCTACTGATATAAATAAATAAAAG
 TACATACAAGTTACAAATGAATTAATAAGGTATTTGAAAACGTTTACATCAATAA
 ATATACTATTTTAGGCTAAATCAAAATAATATGTTTACATCATGGTTTACATAGTT
 GCTTTATTCTATACAAATACGGATAATATTGAATATCCATCGTACGTTTATTTTCAT

ATACGGATCCAAATTCAGCTAATGAAAAATGAATTCAGAAATATCTATTTTATTT
 TCATACCTGAAATGGATGTGAGAAATATATGAATAATATAATTCAGCCAGCTTCA
 TATCCATTTCTACCGGTACTCGAAGGAGCGTCCATGAGAGCATGTGTGTCCCTAA
 TGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCAAA
 5 CAAAGATGGAATTTGTTGATGACTCGCTTTACAAACATCGGCCACTTGGATTTCAT
 GGACACCATACTGTTATAAAACAGAAACATGTGTTTGATTTCGTGTTTTCTTTTCAT
 GAATCACAATATAAATATAGATATTCATGTGCGCACTCACCCATTATGAATCTAT
 ACCTAATAATAAAGAGGCAAAATTTCTCTTCACCTGTTTTTTTTTGGGTCCGGCCAT
 CCCTTAACCTAATTTGCGAATGTGAAAACTGCTTATAGCCCTTCTCTTTATATAA
 10 TTAGGAATCATAATCCAATTAGATCTTTCTGATTTCGGGTGAATAGGAATATTAAT
 CCAAATAGAAAAAATATAATAATATGCACAATAATATCTCTATGAGCATCTTCG
 AAAGACAAAGACGGCAGATCTCAAATTTGTTGAGGTCATAATAAGCATTGCACT
 CAAACAGGCATGTTGCTTATTAAAGAATAGCGAAGCAATGAAGAATAAATCCAA
 GAAATATGAGCAAGCACGTCAATCGAGCACTTGACCAACTGGGTAGACATGTT
 15 CAATCACAAGAGGTGCTCAATTTCTAATCATGGTCAATATAATCAAACCCACCG
 AGTATATTATTACTTAGAGCATCTCCAATAGTTTTCAAAAAAAAAAATCATCGCAA
 AAAAAGTTTTCAAAAAAATAATTGGTAAATCAATGAGATTTACAAGTCGCTAAAA
 AAAGTTGGAGGCGTAATTGTTGGGGCTTTTGACTTTTTTCCAACAGTTTATAAAAT
 CACGCTCCTAAAGTATAGAAAATAATTTTGCATTAGGAATCTTAAACTATTTTCA
 20 AATTACCCTAATCATTTTTTATACTTTTTTTTTCTTCTTGTATATTTGCATTTTGGGA
 ACCCGATTAGAAATCTAATCATGGTTAATCAAACCCAGTTTTTCGGATTTAGAAA
 CAAAAACGGCAAATTGCATACCCAACTCATCGCCCATATAAAACGTTACCAACCC
 AACATATATTTCCACCTAATGCCGTCGCCCACTAATTGCCATAACACTAAGACG
 AGCGCCAACCTTCGGTGCTCTGCTTGCCTCGCTGTTGCTGTTGCCCATGG [SEQ ID
 25 NO: 53], or a nucleotide sequence that shares at least 80% (and at least 81% to at least 99%
 and all integer percentages in between) sequence identity with the sequence set forth in SEQ
 ID NO: 53 or a complement thereof, or a nucleotide sequence that hybridizes to the sequence
 set forth in SEQ ID NO: 53 or a complement thereof, under at least medium or high
 stringency conditions;
 30 TCAAATAAATATAAATCAGGTAGTTCAAATTCAAATATACATTTAGATATG
 TATTCAATTTGGGCGATATCAAGTATGCATTTGCTTTATGGTTTTGAAGAATGGAA
 TAATCACATATATAAAACGCAAACTAAAGAATAAATGACACTATAAGTATAAT
 ACATGTAGACAATGGTCATTCATAGAACATAACATATTTAGAAACGAATATATCA
 ATAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAAATATATAAATATCTAT

CAATATAAATAAATAAAGTACATACAAGTTACAAATGAATTAATAAGGTATTTGA
 AAACGTTTAAATCAATAAATATACTATTTTAGGCTAAATCAAAATAACATCATGG
 TTTACATAGTTGCTTTATTCTATACAATAACGGATAATATTGAATATCCTTCATAC
 GTTTATTTTCATATATGAATCCAAATTCAGCTAATGAAAAATGAATTCAGAGATAT
 5 CCATTTTATTTTCATACCTGAAATGGATGTGAGAAATATATGAATAATATAATTCA
 GACAGCTTCATATCCATTTCCACCGTTACTTGAAGGAGTGTCCATGAGAGCATGT
 GTGTCCTTAATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTA
 ATAAATCAAACAAAGATGGAATTTGTTGATGACTCGTGCTACAAACATCGGCCAC
 TTGGAGTCATGGACACCCTACTGTTATAAAACGGAAACATGTGTTTGATTGCTTT
 10 TTCTTTTCATGAATCACAATATAAATATAGACATTCATGTGCGCACTCACACCTTA
 TGGACAATAATATCTCTAGGAGCATCTTCAAAAGACAAAGACGACAGATCTCA
 AGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGGGCATGTCGCTTATTAAA
 GAATAGCGAAGCAATCAAGAATAAATTCAAAAAAAAAATATGAGCAAGCACATCA
 ATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGCTCAATTT
 15 ATAATCATGGCTAATATAATCAAACCCACCGAGTATATTATTACTTAGAGCATC
 TCCAATAGTTTTCAAAAAAAAAATTCATCGCAAAAAAAAAAGTTTTCAAAAAATAAT
 TGGTAAATCAATGAGATTTACAAGTCGCTAAAAAGGTTGGAGGCATAATTGTTGG
 GGCTTTGACTTTTTTTTCCAACAGTTTATAAAATCACGCTCCTAAAGTATAGAAAAC
 ATGCATTAGGAATCTTAACTATTTTCAAATTACTCTAATCATTTTTTATACTTTTTT
 20 TCTTTCTTGTATATTTGCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAAT
 CAAACCCAGTTATCGGATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCA
 TCGCCCATATAAAACGGCACCAACCCAACATATTTCCACCTAATGCCGTGCCCCA
 ACTAATTGCCATAACACTAAGACGAGCGCCAACCTTGCGGTGCTCTGCTTGCCCTC
 GCTGTTGCTGTTGCCCATGG [SEQ ID NO: 54], or a nucleotide sequence that shares at
 25 least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence
 identity with the sequence set forth in SEQ ID NO: 54 or a complement thereof, or a
 nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 54 or a
 complement thereof, under at least medium or high stringency conditions;

TTGGATCAAATAAATATAAATCAAGTAGTTCAAATTCAAATATACATTTAG
 30 ATATGTATTCAATTTGGGCGATAGCAAGTATGCATTTGCTTTATGGTTTTGAAGAA
 TTGAATAATCACATATATAAAACGCAAACTAAAGAATAAATGACACTATAAGT
 ATAATACATGTAGGCAATGGTCATTCATAGAACATAACATATTTAGAAACGAATA
 TCTCAATAAATAGTATAGCAATAAATGAGTCTATAAGTAATTAAATATATAAATA
 TCTATCGATATAAATAAATAAATAAAGTACATACAAGTTACAAATGAATTAATAA

GGTATTTGAAAACGTTTACATTAATAAATATACTATTTTAGGCTAAATCAAATA
 ACATCATGGTTTACATAGTTGCTTTATTCTATACAAATACGGATAATATTGAATAT
 CCTTCATACGTTTATTTTCATATATGAATCCAAATTCAGCTAATGAAAAATGAATTC
 AGAGATATCCATTTTATTTTCATACCTGAAATGGATGTGAGAAATATATGAATAA
 5 TATAATTCAGACAGCTTCATATCCATTTCCACCGTTACTTGAAGGAGTGTCCATGA
 GAGCATGTGTGTCCCTAATGTGTTTTCTTTAAATATAGTTATCCTTTGGAAAGTTG
 TTATATTAATAAATCAAACAAAGATGGAATTTGTTGATGACTCGCGCTACAAACA
 TCGGCCACTTGGATTCATGGACACCATACTGTTATAAAACGGAAACATGTGTTTG
 ATTCGCTTTTTCTTTTCATGAATCACAATATAAATATAGACATTCATGTGCGCACT
 10 CACACCTTATGGACAATAATATCTCTATGAGCATCTTTAAAAGACAAAGACAAC
 AGATCTCAAGATTGTTGAGGTCATAGCAAGCGTTGCACTCAAACGAGCATGTGCGC
 TTATTAAAGAATAGCGAAGCAATCAAGAATAAATTCAAGAAAATATGAGCAAGC
 ACATCAATCGAGGACTTGACCAACTGGGTAGACATGTTCAATCACAAGAGGAGC
 TCATCAAACCCACCGAGTATATTACTTAGAGCATCTCCAATAGTTTAAAAA
 15 AAATCATCGCAAAAAAGGTTTTCAAAAAAATAATTGGTAAATCAATGAGATTTAC
 AAGTCGCTAAAAAAAGTTGGAGGCGTATTTGTTGGGGGTTTTTGACTTTTTTCCAA
 CAGTTTATAAAATCATGCTCCTAAAGTATAGAAAACAATTTTGCATTAGGAATCC
 TAAACTATTTCTAAATTACCCTAATCATTTTTTATACTTTTTTTCTTTCTTATATTTG
 CATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCAGTTATCG
 20 GATTTAGAAACAAAAACGGCAAATTGCATACCCAACTCATCGCCCATATAAAAC
 GGCACCAACCCAACATATATTTCCACCTAATGCCGTCGCCCCGACTAATTGCCATA
 ACTCTTAAAGACGAGCGCCAACCTTGCGGTGCTCTGCTTGCTCGCTGTTGCTGTT
 GCCCATGG [SEQ ID NO: 55], or a nucleotide sequence that shares at least 80% (and at
 least 81% to at least 99% and all integer percentages in between) sequence identity with the
 25 sequence set forth in SEQ ID NO: 55 or a complement thereof, or a nucleotide sequence that
 hybridizes to the sequence set forth in SEQ ID NO: 55 or a complement thereof, under at least
 medium or high stringency conditions;

CTGCAGGCGGCCGCGAATTCAGTAGTGATTACTATAGGGCACGCGTGGTC
 GACGGCCCGGGCTGGTATCGGGGCGTAGATTCCTCTCAACATATCAGCGTTGGAA
 30 TATGTTTGCCTTTTGAGTGGCCAGCACCAAGTACCATAGCCCCAGCTAACTAGCT
 CGAGTGGCCCTCGAGAATATCAGGGGTAGTTGCTATATACCAACATCATGGTGAA
 TTAACCTCAATGTCTTTCCTCACCAAAATATTTAAAGGTGTTTACAGGTGTCCCTT
 TCCCTGCTTTCATGTTGTAATCTTCAAGCTCTAGCAGCCAGGCGAACAACCGAAG
 GAATTAAGAAGACGACTAGAAAGGCCTTGGTTGTATTTTTATTTTTGGATGTTATT

TTGTGTCAAGTTTCTCTCTTTTCGTA CTGGCTATTTACATATGAGACACTCTTTGAGT
GTCTTTCTAAAAGAGACTATAAAAGATGAAATGTAGAAGCTTCTAATTGCGCCAT
GTATATAAAGAGTATATAAAGAAAAGTAAATTACATTACTTGAATATCATATCTA
AATACATGTCATATATGAACCCTTTTCCAAAGAGTGGGGGTGGAACCTTCCTTCA
5 CGGGTTCAAGTTTTAGACTTGGTACATGGGTGCTCATATTTTTCAATATTTATTTT
AGTAAGAACTATGTTATTCATTTAGTAGTAGGTGAGGTGCCTGGTGACAACGAG
GTGTTTATAGTGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCTCTCAAAGGTA
GGGGCCAAAGCAAATTAACGATTATGGTCAGATATGGATTCAGATAGTAGTATT
TTCCATATTTTAATTCAAATACTAATTTAAATACTCTCAGATGCAAATATAAATCA
10 AGTAGTTTGAATTCAAATATGCATTAAGAATTTTAGATACATACTCGGTTTGGGT
GGTAGCAAGTACGGATTCGCTTTTATTGATGGTTTTGAAGAACGGAATAATCATT
ATATAACATAGAAGTAAGGAATAAAATATGACAGCATAAGTATAACACATGTAG
ATAAAGATCATT CATAGAAAATAACATATTTACAAATAAATAGTATAGCAATAAA
TGAGTCTATAAGTAATTAAATATTTGAATAAATATTTAAATATCTATCAATCAATA
15 AATAAAGTACATAAAAGTTAAAAATGAACTAAAGTATTTGAAAAAGTTTACATC
AATAAATATATCTAGTTTACGCTAAATCAAATAATATATTTACATCATGGTTTCA
TTAGGTCCTTTATTCTATACAAATACGAATAATATTGAATATCCATCCCATATTGA
TTTCATATATAGATCCAAATTTAGCTAATAAAAATGAATTCAGATATATCCAGCTT
CAGATCCATTTCCACCGTTACTTGAAGATGCTAATAGGGGTGTTTATAGGGGTGA
20 GTATGCGTGAGAGCGTGTATGTCTGTACTTTGTTAGTTACCCCTTAGAAAGTTGTT
AATAAATCAGCCAAAGATGGAATTTGTTGACTCGTGCTACAACCATGGGCCACTT
GAATTCATGGACACCATACTGTTGAAACAAGAACATGTGTTTCATTTGCTTAATC
ACATGACCTAGCTAGAGAGCTTTTCCAATACTTTCCAGATGAAATGTAATATACT
CCACACATAATTGTTGAAGTATATATATATTAATCTATCACCCACATTTCCCTTAAT
25 AACTTATACTTTTGAGCTAATTGTTTGGCACATACAAGATACAACCTCACAATGCT
ATCAAAGCTCAGTTGGCTCGACCTTGATTATTGGCTAGGGAAATTCAAATAAAAA
GATGGCACAATATTCTTACTCGTATTCCAAGGGCTAGGCAAACTAAAATACGAGG
TATATGTCAAAACTCAAAATGTGCGGTTTTGGGTGAATTATTGCTCAACACATTC
AGCTCAACTCAATTCCTAGGATATTTAACCTCTCTTATAAGTACATTATTTATG
30 TGCCAACCCAATTAGAAGTGCTAATCTTGAATTTACTAACAAAAATGGCAAATTG
CATACCCAACCTACCCATATAAAAAGGCACCAACCCAACATTATTTTCACCTAGT
GTCGTCGCCGAACCTCATTGCCATAACACTAAGACGAGCGCCAACCTTGTGGTGCT
CTTGCCTCGCTGTTGCTGTCGCCCCATGG [SEQ ID NO: 56], or a nucleotide sequence
that shares at least 80% (and at least 81% to at least 99% and all integer percentages in

between) sequence identity with the sequence set forth in SEQ ID NO: 56 or a complement thereof, or a nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 56 or a complement thereof, under at least medium or high stringency conditions;

```
CTGCAGGCGGCCGCGAATTCAGTAGTGATTACTATAGGGCACGCGTGGTC
5 GACGGCCCCGGGCTGGTATCGGGGCGTAGATTCCTCTCAACATATCAGCGTTGGAA
TATGTTTGCCTATTGAGTGGCCAGCACCAAGTACCATAGCCCCAGCTAACTAGCT
CGAGTGGCCCTCGAGAATATCAGAGGTAGTTGCTATATACCAACATCATGGTGAA
TTAACCTCAATGTCTTTCCTCACCAAAATATTTAAAGGTGTTTACAGGTGTCCCTT
TCCCTGCTTTCATGGTGTAATCTTCAAGCTCTAGCAGGCAGGCGAACAACCGAAG
10 GAATTAAGAAGACGACTAGAAAGGCCTTGGTTGTATTTTTATTTTTGGATGTTATT
TTGTGTCAAGTTTCTCTCTTTCGTACTGGCTATTTAGATATGAGACACTCTTTGAG
TGTCTTTCTAAAAGAGACTATAAAAGATGAAATCTAGAAGCTTCTAATTGCGCCA
TGTATATAAAGAAAAGTAAATTACATTACTTGAACATCATATCTAAATACATGTC
ATATATGAACCCTTTTCCAAAGAGTGGGGGTGGAACCTTCCTTCACGGGTTCAG
15 TTTTAGACTTGGTACATGGGTGCTCATATTTTTCAATATTTATTTTAGTAAGAAAC
TATGTTATTCATTTAGTGGTAGGTGAGGTGCCTGGTGACAACGAGGTGTTTATAG
TGACTTCGTCAATCTCAAGATTGGTCAGCTTAATCTCTCAAAGGTAGGGGCCAAA
GCAAATTAAACGATTATGGTCAGATATGGATTCAGATAGTAGTATTTTCCATATTT
TAATTCAAATACTAATTTAAATACTCTCAGATGCAAATATAAATCAAGTAGTTTG
20 AATTCAAATATGCATTAAGAATTTTAGATACATACTCGGTTTGGGTGGTAGCAAG
TACGGATTTCGCTTTTATTGATGGTTTTGAAGAACGGAATAATCATTATATAACATA
GAAGTAAGGAATAAAATATGACAGCATAAGTATAACACATGTAGATAAAGATCA
TTCATAGAAAATAACATATTTACAAATAAATAGTATAGCAATAAATGAGTCTATA
AGTAATTAAATATTTGAATAAATATTTAAATATCTATCAATCAATAAATAAAGTA
25 CATAAAAGTTAAAAATGAACTAAAGTATTTGAAAAAGTTTTACATCAATAAATAT
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TTTCCACCGTTACTTGAAGATGCTAATAGGGGTGTTTATAGGGGTGAGTATGCGT
30 GAGAGCGTGTATGTCTGTACTTTGTTAGTTACCCCTTAGAAAGTTGTTAATAAATC
AGCCAAAGATGGAATTTGTTGACTCGTGCTACAACCATGGGCCACTTGAATTCAT
GGACACCATACTGTTGAAACAAGAACATGTGTTTCATTTGCTTAATCACATGACC
TAGCTAGAGAGCTTTTCCAATACTTTCCAGATGAAATGTAATATACTCCACACAT
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TTTGAGCTAATTGTTTGGCACATACAAGATACAACCTCACAATGCTATCAAAGCTC
 AGTTGGCTCGACCTTGATTATTGGCTAGGGAAATTCAAATAAAAAGATGGCACAA
 TATTCTTACTCGTATTCCAAGGGCTAGGCAAACCTAAAATACGAGGTATATGTCAA
 AACTCAAAATGTGCGGTTTTTGGGTGAATTATTGCTCAACACATTTCAGCTCAACTC
 5 AATTCCCTAGGATATTTAACCCTCTCTTATAAGTACATTATTTATGTGCCAACCCA
 ATTGGAAGTGCTAATCTTGAATTTACTAACAAAAATGTCAAATTGCATACCCAAC
 TCACCCATATAAAAAGGCACCAACCCAACATTATTTTCGCCTAGTGTGCTCGCCG
 AACTCATTGCCATAACACTAAGACGAGCGCCAACCTTGTGGTGCTCTTGCCTCGC
 TGTGCTGTGCGCCCATGG [SEQ ID NO: 57], or a nucleotide sequence that shares at least
 10 80% (and at least 81% to at least 99% and all integer percentages in between) sequence
 identity with the sequence set forth in SEQ ID NO: 57 or a complement thereof, or a
 nucleotide sequence that hybridizes to the sequence set forth in SEQ ID NO: 57 or a
 complement thereof, under at least medium or high stringency conditions; and

TCCACCGTACTTGAAGGAGTGTCCATGAGAGCATGTGTGTCCCTAATGTG
 15 TTTTCTTTAAATATAGTTATCCTTTGGAAAGTTGTTATATTAATAAATCAAACAAA
 GATGGAATTTGTTGATGACTCGCGCTACAAACATCGGCCACTTGGATTCATGGAC
 ACCATACTGTTATAAAACGGAAACATGTGTTTGATTTCGCTTTTTCTTTTCATGAAT
 CACAATATAAATATAGACATTCATGTGCGCACTCACACCTTATGGACAACATAATA
 TGTCTATGAGCATCTTCAAAAGACAAAGACGACAGATCTCAAGATTGTTGAGGTC
 20 ATAGCAAGCGTTGCACTCAAACGGGCATGTCGCTTATTAAAGAATAGCGAAGCA
 ATCAAGAATAAATTCAAAAAAATATGAGCAAGCACATCAATCGAGGACTTGACC
 AACTGGGTAGACATGTTCAATCACAAGAGGAGCTCAATTTCTAATCATGGCTAAT
 ATAATCAAACCCACCCAGTATATTACTTAGAGCATCTCCAATAGTTTTTTTTT
 AAAAAATCATCGCAAAAAAAGCTTTCAAAAAAATAATTGGTAAATCAATGAGA
 25 TTTACAAGTCGCTAAAAAAGTTGGAGGCGTAATTGTTGGGGCTTTGACTTTTTTCC
 CCGTTTATAAAATCACGCTCCTAAAGTATAGAAAACAATTTTGCATTAGGAATCT
 TAAAATATTTTCAAATTACCCTAATCATTTTTTATACTTTTTTTCTTTCTTGTATATTT
 GCATTTTGGGAACCCGATTAGAAATCTAATCATGGTTAATCAAACCCAGTTATC
 GGATTTAGAAACAAAAACGGCAAATTGCATACCCAACCTCATCGCTCATATAAAAC
 30 GGCACCAACCCAACATATTTCCACCTAATGCCGTCGCCCCAACTAATTGCCATAAC
 ACTAAGTCGAGCGCCAACCTTGCGGTGCTCTGCTTGCCTCGCTGTTGCTGTTGCCC
 ATGG [SEQ ID NO: 58], or a nucleotide sequence that shares at least 80% (and at least 81%
 to at least 99% and all integer percentages in between) sequence identity with the sequence set
 forth in SEQ ID NO: 58 or a complement thereof, or a nucleotide sequence that hybridizes to

the sequence set forth in SEQ ID NO: 58 or a complement thereof, under at least medium or high stringency conditions.

64. An isolated nucleic acid molecule, which comprises a promoter sequence that is operable in plant cells, wherein the promoter sequence comprises a nucleotide sequence

5 selected from the group consisting of:

(i) a nucleotide sequence set forth in any one of SEQ ID NO: 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 and 58 or a complement thereof;

(ii) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence set forth in any
10 one of SEQ ID NO: 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 and 58 or a complement thereof;
and

(iii) a nucleotide sequence that hybridizes to the sequence set forth in any one of SEQ ID NO: 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 and 58 or a complement thereof, under at least medium or high stringency conditions.

15 65. An isolated nucleic acid molecule comprising a chimeric promoter sequence that comprises an heterologous core promoter sequence in operable connection with a nucleotide sequence selected from the group consisting of :

(1) a nucleotide sequence corresponding to at least one segment selected from segments Λ , Φ , Ψ , Z , Δ and Θ of SEQ ID NO: 47-58, as defined herein, or

20 (2) a nucleotide sequence that shares at least 80% (and at least 81% to at least 99% and all integer percentages in between) sequence identity with the sequence of the at least one segment or a complement thereof, or

(3) a nucleotide sequence that hybridizes to the sequence s of the at least one segment or a complement thereof, under at least medium or high stringency conditions.

25 66. An isolated nucleic acid molecule according to any one of claims 1 to 65, comprising plant transcriptional regulatory elements able to confer expression preferentially in a plant sink tissue.

67. An isolated nucleic acid molecule according to claim 66, wherein the plant sink tissue is a mature plant carbohydrate storage tissue.

30 68. An isolated nucleic acid molecule according to claim 67, wherein the mature plant carbohydrate storage tissue is a sugarcane mature stem tissue.

69. A chimeric nucleic acid construct comprising an isolated nucleic acid molecule according to any one of claims 1 to 68 in operable connection with a heterologous nucleic acid sequence to be transcribed.

5 70. A chimeric construct according to claim 69, further comprising a 3' non-translated sequence that is operably connected to the heterologous nucleic acid sequence and that functions in plant cells to terminate transcription and/or to cause addition of a polyadenylated nucleotide sequence to the 3' end of a transcribed RNA sequence.

10 71. A method for expression of a heterologous nucleic acid sequence, the method comprising introducing into a plant cell a chimeric construct according to claim 69 or claim 70.

72. A method for producing a transformed plant cell, the method comprising introducing into a plant cell a chimeric construct according to claim 69 or claim 70.

15 73. A method for producing transformed plant cells, the method comprising introducing into regenerable plant cells a chimeric construct according to claim 69 or claim 70 so as to yield transformed plant cells and identifying or selecting the transformed plant cells.

74. A method for selecting stable genetic transformants from transformed plant cells, the method comprising introducing into regenerable plant cells a chimeric construct according to claim 69 or claim 70 so as to yield transformed plant cells and identifying or selecting a transformed plant cell line from the transformed plant cells.

20 75. A method for producing a differentiated transgenic plant, the method comprising introducing a chimeric construct according to claim 69 or claim 70 into regenerable plant cells so as to yield regenerable transformed cells, identifying or selecting a population of transformed cells, and regenerating a differentiated transgenic plant from the population.

25 76. A method according to claim 75, wherein expression of the chimeric construct renders the differentiated transgenic plant identifiable over the corresponding non-transgenic plant.

77. A differentiated transgenic plant comprising plant cells containing a chimeric construct according to claim 69 or claim 70.

30 78. Cells, tissues, leaves, fruit, flowers, seeds or other plant reproductive material, material used for vegetative propagation, progeny plants including F1 hybrids, male-sterile plants and all other plants and plant products derived from the differentiated transgenic plant of claim 77.

79. An isolated nucleic acid molecule comprising a promoter sequence or biologically active fragment thereof or variant of these, wherein the promoter sequence is located

upstream of a transcribable nucleic acid sequence that hybridizes to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO: 59.

1/5

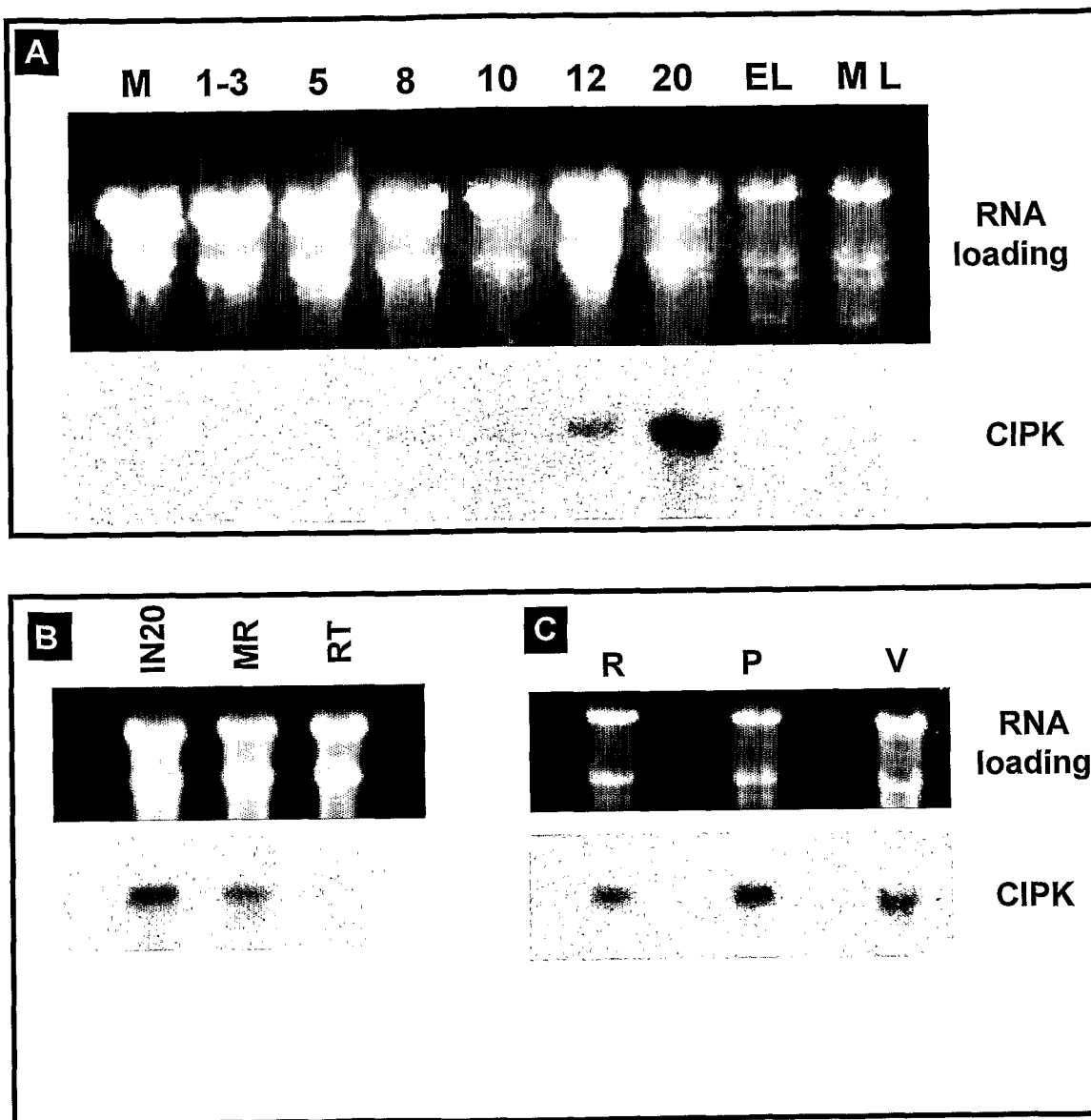
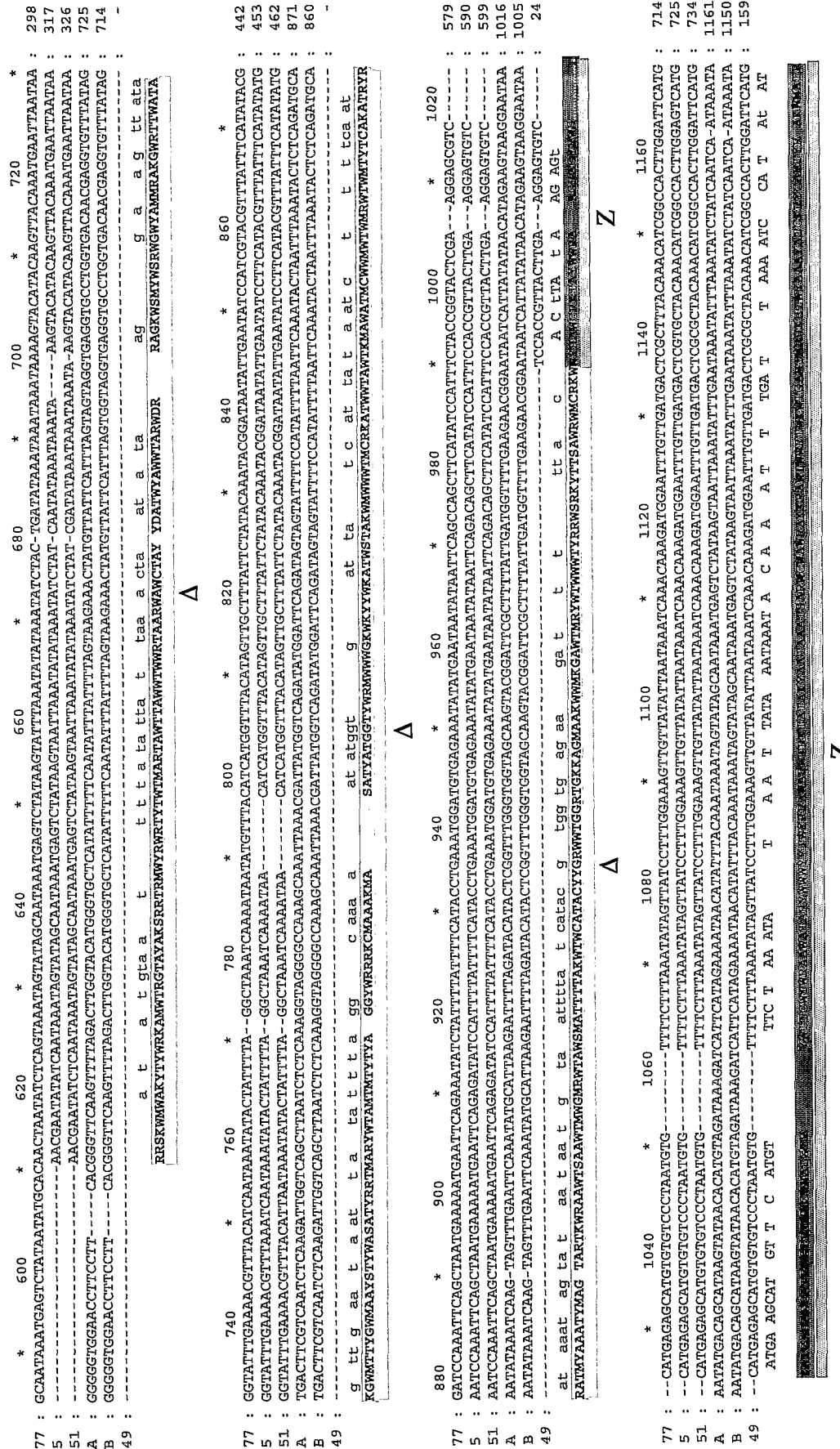


FIGURE 1

[illegible]

FIGURE 2



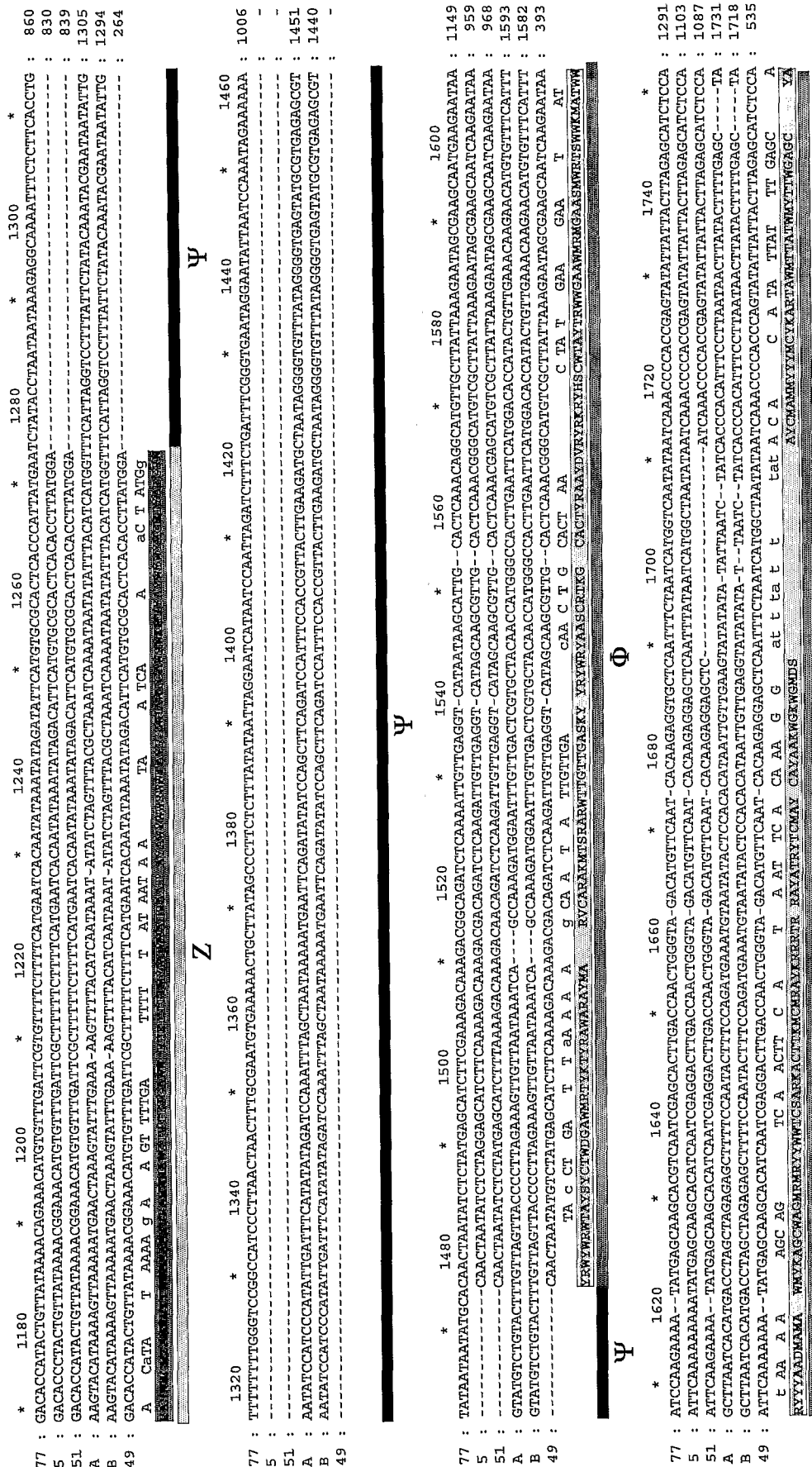




FIGURE 2 cont'd

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2009/001407

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C12N 15/11 (2006.01) A01H 5/00 (2006.01) C12N 15/82 (2006.01)

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)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

MEDLINE, HCA, BIOSIS, AGRICOLA (CIPK-1, CBL-interacting protein kinase-1, promoter)

GENOMEQUEST (SEQ ID No's 2 - 7, 14, 18, 21, 24, 28, 31, 39, 42)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	XIANG, Y. <i>et al.</i> Characterization of Stress-Responsive CIPK Genes in Rice for Stress Tolerance Improvement. Plant Physiology. 2007, Vol. 144, No. 3, pages 1416 – 1428. See whole document.	
A	GenBank Accession No. CG066298. 19 August 2003. Residues 274 – 579 share 65.27% identity with SEQ ID No. 3 and 65.59% identity with SEQ ID No. 7.	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:			
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
26 November 2009

Date of mailing of the international search report

01 DEC 2009

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