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(54) Title: NUCLEOTIDE SEQUENCES REGULATING GENE EXPRESSION AND CONSTRUCTS AND METHODS UTILIZING SAME

(57) Abstract: Novel plant derived regulatory sequences and constructs and methods of using such sequences for directing expression of exogenous polynucleotide sequences in plants are provided

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NUCLEOTIDE SEQUENCES REGULATING GENE EXPRESSION AND
CONSTRUCTS AND METHODS UTILIZING SAMEFIELD AND BACKGROUND OF THE INVENTION

5 The present invention relates to isolated polynucleotides which are capable of regulating gene expression in an organism and more specifically, to novel nucleic acid sequences which include constitutive, inducible, tissue-specific and developmental stage-specific promoters which are capable of directing gene expression in plants.

10 A promoter is a nucleic acid sequence approximately 200-1500 base pairs (bp) in length which is typically located upstream of coding sequences. A promoter functions in directing transcription of an adjacent coding sequence and thus acts as a switch for gene expression in an organism. Thus, all cellular processes are ultimately governed by the activity of promoters, making such regulatory elements important research and commercial tools.

15 Promoters are routinely utilized for heterologous gene expression in commercial expression systems, gene therapy and a variety of research applications.

20 The choice of the promoter sequence determines when, where and how strongly the heterologous gene of choice is expressed. Accordingly, when a constitutive expression throughout an organism is desired, a constitutive promoter is preferably utilized. On the other hand, when triggered gene expression is desired, an inductive promoter is preferred. Likewise, when an expression is to be confined to a particular tissue, or a particular physiological or developmental stage, a tissue specific or a stage specific promoter is respectively preferred.

25 Constitutive promoters are active throughout the cell cycle and have been utilized to express heterologous genes in transgenic plants, such that the expression of traits encoded by the heterologous genes is effected throughout the plant at all time. Examples of known constitutive promoters often used for plant transformation include the cauliflower heat shock protein 80 (hsp80) promoter, 35S cauliflower mosaic virus promoter, nopaline synthase (nos) promoter, octopine (ocs) *Agrobacterium* promoter and the mannopine synthase (mas) *Agrobacterium* promoter.

30 Inducible promoters can be switched on by an inducing agent and are typically active as long as they are exposed to the inducing agent. The inducing agent can be a chemical agent, such as a metabolite, growth regulator, herbicide, or phenolic compound, or a physiological stress directly imposed upon the plant such as cold,

heat, salt, toxins, or through the action of a microbial pathogen or an insecticidal pest. Accordingly, inducible promoters can be utilized to regulate expression of desired traits, such as genes that control insect pests or microbial pathogens, whereby the protein is only produced shortly upon infection or first bites of the insect and
5 transiently so as to decrease selective pressure for resistant insects. For example, plants can be transformed to express insecticidal or fungicidal traits such as the *Bacillus thuringiensis* (Bt) toxins, viruses coat proteins, glucanases, chitinases or phytoalexins. In another example, plants can be transformed to tolerate herbicides by overexpressing, upon exposure to a herbicide, the acetohydroxy acid synthase
10 enzyme, which neutralizes multiple types of herbicides [Hattori, J. *et al.*, Mol. General. Genet. 246: 419 (1995)].

Several fruit-specific promoters have been described, including an apple-isolated Thi promoter (U.S. Pat. No. 6,392,122); a strawberry-isolated promoter (U.S. Pat. No. 6,080,914); tomato-isolated E4 and E8 promoters (U.S. Pat. No. 5,859,330);
15 a polygalacturonase promoter (U.S. Pat. No. 4,943,674); and the 2AII tomato gene promoter [Van Haaren *et al.*, Plant Mol. Biol. 21: 625-640 (1993)]. Such fruit specific promoters can be utilized, for example, to modify fruit ripening by regulating expression of ACC deaminase which inhibits biosynthesis of ethylene. Other gene products which may be desired to express in fruit tissue include genes encoding flavor
20 or color traits, such as thaumatin, cyclase or sucrose phosphate synthase.

Seed specific promoters have been described in U.S. Pat. Nos. 6,403,862, 5,608,152 and 5,504,200; and in U.S. Patent Application Ser. Nos. 09/998059 and 10/137964. Such seed specific promoters can be utilized, for example, to alter the
25 levels of saturated or unsaturated fatty acids; to increase levels of lysine- or sulfur-containing amino acids, or to modify the amount of starch contained in seeds.

Several promoters which regulate gene expression specifically during germination stage have been described, including the α -glucuronidase and the cystatin-1 barely-isolated promoters (U.S. Pat. No. 6,359,196), and the hydrolase promoter [Skriver *et al.*, Proc. Natl. Acad. Sci. USA, 88:7266-7270 (1991)].

30 While reducing the present invention to practice, the present inventors have uncovered several regulatory sequences which exhibit a wide range of promoter activities in plants, as is further described hereinunder, such regulatory sequences can be used in a variety of commercial and research applications.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided an isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96,
5 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 211, 210 and 213, wherein the isolated polynucleotide is capable of regulating expression of at least one polynucleotide sequence operably linked thereto.

According to another aspect of the present invention there is provided a
10 nucleic acid construct which includes the isolated polynucleotide comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213.

According to yet another aspect of the present invention there is provided a
15 transgenic cell which includes the isolated polynucleotide comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and
20 213.

According to still another aspect of the present invention there is provided a transgenic cell comprising the nucleic acid construct which includes the isolated polynucleotide comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81,
25 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213.

According to an additional aspect of the present invention there is provided a transgenic organism which includes the isolated polynucleotide comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11,
30 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213.

According to yet an additional aspect of the present invention there is provided a transgenic organism comprising a nucleic acid construct which includes the isolated polynucleotide comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213.

According to still an additional aspect of the present invention there is provided a transgenic plant which includes the isolated polynucleotide comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213.

According to a further aspect of the present invention there is provided a transgenic plant comprising a nucleic acid construct which includes the isolated polynucleotide comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213.

According to yet a further aspect of the present invention there is provided a method of producing a transgenic plant comprising transforming a plant with an isolated polynucleotide which includes a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213.

According to still a further aspect of the present invention there is provided a method of producing a transgenic plant comprising transforming a plant with a nucleic acid construct which includes the isolated polynucleotide comprising the nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213.

According to still a further aspect of the present invention there is provided a method of expressing a polypeptide of interest in a cell comprising transforming the

cell with a nucleic acid construct including a polynucleotide sequence encoding the polypeptide of interest operably linked to a regulatory nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213 thereby expressing the polypeptide of interest in the cell.

According to still a further aspect of the present invention there is provided a method of co-expressing two polypeptides of interest in a cell comprising transforming the cell with a nucleic acid construct including two polynucleotide sequences encoding the two polypeptides of interest operably linked to a regulatory nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213 such that said two polynucleotide sequences flank said regulatory nucleic acid sequence, thereby expressing the two polypeptides of interest in the cell.

According to further features in preferred embodiments of the invention described below, the isolated polynucleotide includes at least one promoter region.

According to still further features in the described preferred embodiments the nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 1, 6, 41, 46, 51, 86, 121, 136, 171, 181 and 202, and whereas the at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in a constitutive manner.

According to still further features in the described preferred embodiments the nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 1, 11, 16, 21, 26, 31, 36, 56, 61, 66, 71, 76, 81, 91, 96, 101, 116, 126, 141, 146, 151, 156, 161, 166, 176, 186, 191, 196, 201, 203, 210 and 213, and whereas the at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in an inductive manner.

According to still further features in the described preferred embodiments the nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 1, 11, 16, 21, 26, 31, 36, 56, 61, 66, 71, 76, 91, 116, 126, 141, 146, 151, 156, 161, 166, 176, 186, 191, 196, 201, 203, 210 and 213, and whereas the at least one promoter region is

capable of directing transcription of said at least one polynucleotide sequence in a tissue specific manner.

According to still further features in the described preferred embodiments the nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 81, 96,
5 101, 106 and 131, and whereas the at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in a developmental stage specific manner.

According to still further features in the described preferred embodiments the nucleic acid construct further includes at least one heterologous polynucleotide
10 operably linked to the isolated polynucleotide.

According to still further features in the described preferred embodiments the at least one heterologous polynucleotide is a reporter gene.

According to still further features in the described preferred embodiments the nucleic acid construct further includes two heterologous polynucleotides each being
15 operably linked to an end of the isolated polynucleotide such that the two heterologous polynucleotides flank the isolated polynucleotide.

The present invention successfully addresses the shortcomings of the presently known configurations by providing a plurality of isolated polynucleotide sequences which exhibit a wide spectrum of promoter function patterns. These polynucleotides
20 can be used to generate nucleic acid constructs, such as expression vectors suitable for transforming an organism. Such nucleic acid constructs can be used to promote expression of desired traits or expression products in transgenic organisms, such as plants, in a constitutive, induced, tissue specific, or a developmental stage specific manner.

25

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of
30 illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the

invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

5 FIGs. 1a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 11 operably linked to a luciferase encoding sequence. Figure 1a shows the transgenic plant under normal light. Figure 1b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression luciferase
10 in flower tissue.

 FIGs. 2a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 21 operably linked to a luciferase encoding sequence. Figure 2a shows the transgenic plant under normal light. Figure 2b is an ultra-low light
15 photograph of the same plant in the dark, illustrating a specific expression of luciferase in root tissue.

 FIGs. 3a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 36 operably linked to a luciferase encoding sequence. Figure 3a
20 shows the transgenic plant under normal light. Figure 3b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in root and flower tissue.

 FIGs. 4a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 61 operably linked to a luciferase encoding sequence. Figure 4a
25 shows the transgenic plant under normal light. Figure 4b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in young tissue.

 FIGs. 5a-b are photographs showing an *Arabidopsis thaliana* seedling transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 66 operably linked to a luciferase encoding sequence. Figure 5a
30 shows the transgenic plant under normal light. Figure 5b is an ultra-low light

photograph of the same plant in the dark, illustrating an expression of luciferase in leaf tissue.

FIGs. 6a-b are photographs showing an *Arabidopsis thaliana* mature plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 66 operably linked to a luciferase encoding sequence. Figure 6a shows the transgenic plant under normal light. Figure 6b is an ultra-low light photograph of the same plant in the dark, illustrating an expression of luciferase in stem tissue.

FIGs. 7a-b are photographs showing an *Arabidopsis thaliana* plant seedlings transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 81 operably linked to a luciferase encoding sequence. Figure 7a shows the transgenic plant under normal light. Figure 7b is an ultra-low light photograph of the same plant in the dark, illustrating an expression of luciferase in above ground tissue.

FIGs. 8a-b are photographs showing an *Arabidopsis thaliana* mature plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 81 operably linked to a luciferase encoding sequence. Figure 8a shows the transgenic plant under normal light. Figure 8b is an ultra-low light photograph of the same plant in the dark, illustrating an expression of luciferase in flower tissue.

FIGs. 9a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 91 operably linked to a luciferase encoding sequence. Figure 9a shows the transgenic plant under normal light. Figure 9b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in root and flower tissue.

FIGs. 10a-b are photographs showing an *Arabidopsis thaliana* seedling transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 96 operably linked to a luciferase encoding sequence. Figure 10a shows the transgenic plant under normal light. Figure 10b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in above ground tissue.

FIGs. 11a-b are photographs showing an *Arabidopsis thaliana* mature plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 96 operably linked to a luciferase encoding sequence. Figure 11a shows the transgenic plant under normal light. Figure 11b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in above ground tissue.

FIGs. 12a-b are photographs showing seeds of an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 111 operably linked to a luciferase encoding sequence. Figure 12a shows the seeds under normal light. Figure 12b is an ultra-low light photograph of the same seeds in the dark, illustrating a specific expression of luciferase in seeds.

FIGs. 13a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 111 operably linked to a luciferase encoding sequence. Figure 13a shows the transgenic plant under normal light. Figure 13b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in roots.

FIGs. 14a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 121 operably linked to a luciferase encoding sequence. Figure 14a shows the transgenic plant under normal light. Figure 14b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in meristematic tissue.

FIGs. 15a-b are photographs showing an *Arabidopsis thaliana* seedling transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 126 operably linked to a luciferase encoding sequence. Figure 15a shows the transgenic plant under normal light. Figure 15b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in root meristematic tissue.

FIGs. 16a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 126 operably linked to a luciferase encoding sequence. Figure 16a shows the transgenic plant under normal light. Figure 16b is an ultra-low light

photograph of the same plant in the dark, illustrating a specific expression of luciferase in flower meristematic tissue.

FIGs. 17a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 131 operably linked to a luciferase encoding sequence. Figure 5 17a shows the transgenic plant under normal light. Figure 17b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in leaf tissue.

FIGs. 18a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 136 operably linked to a luciferase encoding sequence. Figure 10 18a shows the transgenic plant under normal light. Figure 18b is an ultra-low light photograph of the same plant in the dark, illustrating a non-specific constitutive expression of luciferase.

FIGs. 19a-b are photographs showing an *Arabidopsis thaliana* seedling transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 156 operably linked to a luciferase encoding sequence. Figure 15 19a shows the transgenic plant under normal light. Figure 19b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in above ground tissue. 20

FIGs. 20a-b are photographs showing an *Arabidopsis thaliana* mature plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 156 operably linked to a luciferase encoding sequence. Figure 20a shows the transgenic plant under normal light. Figure 20b is an ultra-low light 25 photograph of the same plant in the dark, illustrating a specific expression of luciferase in above ground tissue.

FIGs. 21a-b are photographs showing seeds of an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 161 operably linked to a luciferase encoding sequence. Figure 30 21a shows the seeds under normal light. Figure 21b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in seed tissue.

FIGs. 22a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 186 operably linked to a luciferase encoding sequence. Figure 22a shows the transgenic plant under normal light. Figure 22b is an ultra-low light photograph of the same plant in the dark, illustrating an expression of luciferase in stalk and stem tissue.

FIGs. 23a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 191 operably linked to a luciferase encoding sequence. Figure 23a shows the transgenic plant under normal light. Figure 23b is an ultra-low light photograph of the same plant in the dark, illustrating a weak expression of luciferase in vegetative tissue.

FIGs. 24a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 201 operably linked to a luciferase encoding sequence. Figure 24a shows the transgenic plant under normal light. Figure 24b is an ultra-low light photograph of the same plant in the dark, illustrating an above ground tissue specific expression of luciferase.

FIGs. 25a-b are photographs showing an *Arabidopsis thaliana* plant transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 176 operably linked to a luciferase encoding sequence. Figure 25a shows the transgenic plant under normal light. Figure 25b is an ultra-low light photograph of the same plant in the dark, illustrating a specific expression of luciferase in flower tissue.

FIGs. 26a-b are photographs showing transformed *Arabidopsis thaliana* plants transformed with nucleic acid constructs including partial DREs operably each linked to a GUS encoding sequence. Figure 26a shows a plant transformed with a nucleic acid construct including the nucleic acid sequence set forth in SEQ ID NO: 210 operably linked to a GUS encoding sequence. Figure 26b shows root tips of a plant, transformed with a nucleic acid construct comprising the nucleic acid sequence set forth in SEQ ID NO: 213 operably linked to a GUS encoding sequence.

FIG. 27 is a nucleic acid sequence alignment between DRE 6669 (SEQ ID NO: 61, QUERY) and a prior art sequence (SEQ ID NO: 214, SBJCT), revealing a

different 5' sequence which is important for constitutive expression, as is exemplified in the Examples section hereinbelow.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

5 The present invention provides isolated polynucleotides capable of regulating the expression of operably linked heterologous polynucleotides, and more specifically, novel nucleic acid sequences which are capable of promoting gene expression in a constitutive, inductive, tissue specific and/or developmental stage specific manner. The present invention also provides nucleic acid constructs, as well
10 transgenic organisms which carry the polynucleotides of the present invention and methods of producing thereof.

The principles and operation of the present invention may be better understood with reference to the accompanying descriptions.

15 Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following descriptions or illustrated in the Examples section. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose
20 of description and should not be regarded as limiting.

The term "polynucleotide" or the phrase "nucleic acid sequence" are used herein interchangeably and refer to a polymer of deoxyribonucleotide (DNA) or ribonucleotide (RNA).

25 The phrase "heterologous polynucleotide" refers to a polynucleotide sequence which originates from a heterologous organism or to a polynucleotide sequence which is linked to a regulatory sequence of the same organism which does not normally regulate expression of the polynucleotide sequence in the organism.

30 PCT Publication WO 02/07989 describes a unique approach developed by the present inventors in order to uncover novel regulatory sequences in organisms such as plants. This approach combines molecular and bioinformatics techniques for high throughput isolation of DNA regulating elements (DREs), located within the non-transcribed (non-coding) regions of the genome and which include, for example, promoters, enhancers, suppressors, silencers, locus control regions and the like.

Utilizing this approach, the present inventors have uncovered several novel polynucleotide sequences which, as illustrated in the Examples section which follows, exhibit regulatory activity in plants.

Thus, according to one aspect of the present invention, there is provided
5 isolated polynucleotides which are capable of regulating the expression of at least one polynucleotide operably linked thereto. As is further described in the Examples section which follows, these isolated polynucleotides are as set forth in SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 11, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196,
10 201, 202 and 203, or fragments (e.g., SEQ ID NOS: 210 and 213), variants or derivatives thereof.

A coding nucleic acid sequence is "operably linked" to a regulatory sequence if it is capable of exerting a regulatory effect on the coding sequence linked thereto. Preferably, the regulatory sequence is positioned 1-500 bp upstream of the ATG
15 codon of the coding nucleic acid sequence, although it will be appreciated that regulatory sequences can also exert their effect when positioned elsewhere with respect to the coding nucleic acid sequence (e.g., within an intron).

As is clearly illustrated in the Examples section which follows, the isolated polynucleotides of the present invention are capable of regulating expression of a
20 coding nucleic acid sequence (e.g., luciferase) operably linked thereto (see Figures 1-25).

The isolated polynucleotides of the present invention range in length from 174 to 3, 348 nucleotides and include one or more sequence regions which are capable of recognizing and binding RNA polymerase II and other proteins (trans-acting
25 transcription factors) involved in transcription.

Although most of the isolated polynucleotides described herein include one promoter region, some include two distinct promoter regions each positioned on a different strand of the same genomic sequence. Such bidirectional DREs are further described in the Examples section which follows (see for example, Tables 3-17).

30 As is further illustrated by the Examples section which follows, the isolated polynucleotides of the present invention exhibit a range of activities and tissue specificities.

Thus for example, the nucleic acid sequences set forth in SEQ ID NOS:1, 6, 41, 46, 51, 86, 121, 136, 171, 181 and 202 or fragment, variants or derivatives thereof, are capable of directing transcription of coding nucleic acid sequences operably linked thereto in a constitutive manner and thus include a constitutive promoter region.

In another example, the nucleic acid sequences set forth in SEQ ID NOS: 1, 11, 16, 21, 26, 31, 36, 56, 61, 66, 71, 76, 81, 91, 96, 101, 116, 126, 141, 146, 151, 156, 161, 166, 176, 186, 191, 196, 201 and 203, or fragments (e.g., SEQ ID NOS: 210 and 213), variants or derivatives thereof, are capable of directing transcription of coding nucleic acid sequences operably linked thereto in an inductive manner and thus include an inductive promoter region.

In yet another example, the nucleic acid sequences set forth in SEQ ID NOS: 1, 11, 16, 21, 26, 31, 36, 56, 61, 66, 71, 76, 91, 116, 126, 141, 146, 151, 156, 161, 166, 176, 186, 191, 196, 201 and 203, or fragments (e.g., SEQ ID NOS: 210 and 213), variants or derivatives thereof, are capable of directing transcription of coding nucleic acid sequences operably linked thereto in a tissue specific manner and thus include a tissue specific promoter region.

In further yet another example, the nucleic acid sequences set forth in SEQ ID NOS: 81, 96, 101, 106 and 131, or fragment, variants or derivatives thereof, are capable of directing transcription of coding nucleic acid sequences operably linked thereto in a developmental stage specific manner and thus include a developmental stage specific promoter region.

Preferably, the polynucleotide of the present invention are modified to create variations in the molecule sequences such as to enhance their promoting activities, using methods known in the art, such as PCR-based DNA modification, or standard DNA mutagenesis techniques, or by chemically synthesizing the modified polynucleotides.

Accordingly, the sequences set forth in SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 11, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202 and 203 may be truncated or deleted and still retain the capacity of directing the transcription of an operably linked DNA sequence (e.g., SEQ ID NOS: 210 and 213). The minimal length of a promoter region can be determined by systematically removing sequences

from the 5' and 3'-ends of the isolated polynucleotide by standard techniques known in the art, including but not limited to removal of restriction enzyme fragments or digestion with nucleases. Consequently, any sequence fragments, portions, or regions of the disclosed polypeptide sequences of the present invention can be used as regulatory sequences. It will be appreciated that modified sequences (mutated, truncated and the like) can acquire different transcriptional properties such as the direction of different pattern of gene expression as compared to the unmodified element (e.g., SEQ ID NO: 61 as compared to SEQ ID NO: 213, see the Examples section which follows).

Optionally, the sequences set forth in SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202 and 203 may be modified, for example for expression in a range of plant systems. In another approach, novel hybrid promoters can be designed or engineered by a number of methods. Many promoters contain upstream sequences which activate, enhance or define the strength and/or specificity of the promoter, such as described, for example, by Atchison [Ann. Rev. Cell Biol. 4:127 (1988)]. T-DNA genes, for example contain "TATA" boxes defining the site of transcription initiation and other upstream elements located upstream of the transcription initiation site modulate transcription levels [Gelvin In: Transgenic Plants (Kung, S.-D. and Us,R., eds, San Diego: Academic Press, pp.49-87, (1988)]. Another chimeric promoter combined a trimer of the octopine synthase (ocs) activator to the mannopine synthase (mas) activator plus promoter and reported an increase in expression of a reporter gene [Min Ni *et al.*, The Plant Journal 7:661 (1995)]. The upstream regulatory sequences of the polynucleotide sequences of present invention can be used for the construction of such chimeric or hybrid promoters. Methods for construction of variant promoters include, but are not limited to, combining control elements of different promoters or duplicating portions or regions of a promoter (see for example, U.S. Pat. Nos. 5,110,732 and 5,097,025). Those of skill in the art are familiar with the specific conditions and procedures for the construction, manipulation and isolation of macromolecules (e.g., DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolation of genes, [see for example Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, (1989); Mailga *et al.*, Methods in

Plant Molecular Biology, Cold Spring Harbor Press, (1995); Birren *et al.*, Genome Analysis: volume 1, Analyzing DNA, (1997); volume 2, Detecting Genes, (1998); volume 3, Cloning Systems, (1999); and volume 4, Mapping Genomes, (1999), Cold Spring Harbor, N.Y].

5 The polynucleotides of the present invention, or fragment, variants or derivatives thereof, can be incorporated into nucleic acid constructs, preferably expression constructs (i.e., expression vectors) which can be introduced and replicate in a host cell.

10 Thus, according to another aspect of the present invention there is provided a nucleic acid construct which includes at least one of the polynucleotides of the present invention, or fragments, variants or derivatives thereof.

 Preferably, the nucleic acid construct of the present invention includes at least one operably linked heterologous polynucleotide. More preferably, at least one operably linked reporter gene.

15 The phrase "reporter gene" used herein refers to a gene encoding a selectable, screenable or detectable phenotype.

 Reporter genes which may be utilized in the present invention may include, but not limited to, LUX or LUC coding for luciferase, GUS coding for β -glucoronidase, GFP coding for green-fluorescent protein, or antibiotic or herbicide tolerance genes. A general review of suitable markers is found in Wilmink and Dons, 20 Plant Mol. Biol. Reprt. 11:165-185 (1993).

 Further preferably, the nucleic acid construct of the present invention includes at least one heterologous polynucleotide encoding a desirable trait or an expression product.

25 A desirable trait which may be utilized in this invention may include, but not limited to, any phenotype associated with organism's morphology, physiology, growth and development, yield, produce quality, nutritional enhancement, disease or pest resistance, or stress tolerance.

30 Alternatively, the heterologous polynucleotide can encode any naturally occurring or man-made recombinant protein, such as pharmaceutical proteins [e.g., growth factors and antibodies Schillberg Naturwissenschaften. (2003) Apr;90(4):145-55] and food additives. It will be appreciated that molecular farming is a well-proven way of producing a range of recombinant proteins, as described in details in Ma Nat

Rev Genet. 2003 Oct;4(10):794-805; Twyman Trends Biotechnol. 2003 Dec;21(12):570-8.

An expression product which may be utilized in this invention may include, but not limited to, pharmaceutical polypeptides, industrial enzymes, oils, dyes, flavors, biofuels, or industrial biopolymers.

In cases of bidirectional DREs, the nucleic acid construct of this invention may include two heterologous polynucleotides each being operably linked to an end of the isolated polynucleotide of this invention, such that the two heterologous polynucleotides flank the isolated polynucleotide of this invention.

The nucleic acid construct can be, for example, a plasmid, a bacmid, a phagemid, a cosmid, a phage, a virus or an artificial chromosome. Preferably, the nucleic acid construct of the present invention is a plasmid vector, more preferably a binary vector.

The phrase "binary vector" refers to an expression vector which carries a modified T-region from Ti plasmid, enable to be multiplied both in *E. coli* and in *Agrobacterium* cells, and usually comprising reporter gene(s) for plant transformation between the two boarder regions. A binary vector suitable for the present invention includes pBI2113, pBI121, pGA482, pGAH, pBIG, pBI101 (Clonetech), or a modification thereof such as pVER1 which is a modified pBI101 plasmid, where the GUS gene was replaced by the LucII gene from pGL3-Basic (Promega).

The nucleic acid construct of the present invention can be utilized to transform a host cell. Thus, according to another aspect of the present invention there is provided a transgenic cell, a transgenic organism or a transgenic plant which is transformed with an isolated polynucleotide of the present invention. Preferably the transgenic cell, the transgenic organism or the transgenic plant is transformed with the nucleic acid construct of the present invention.

As used herein, the terms "transgenic" or "transformed" are used interchangeably referring to a cell or an organism into which cloned genetic material has been transferred.

Methods of introducing nucleic acid constructs into a cell, an organism or a plant are well known in the art. Accordingly, suitable methods for introducing nucleic acid sequences into plants include, but are not limited to, bacterial infection, direct delivery of DNA (e.g., via PEG-mediated transformation,

desiccation/inhibition-mediated DNA uptake, electroporation, agitation with silicon carbide fibers, and acceleration of DNA coated particles, such as described by Potrykus Ann. Rev. Plant Physiol. Plant Mol. Biol. 42:205-225 (1991).

5 Methods for specifically transforming dicots primarily use *Agrobacterium tumefaciens*. For example, transgenic plants reported include but are not limited to cotton (U.S. Pat. Nos. 5,004,863, 5,159,135, 5,518,908; and WO 97/43430), soybean [U.S. Pat. Nos. 5,569,834, 5,416,011; McCabe *et al.*, Bio/Technology, 6:923 (1988); and Christou *et al.*, Plant Physiol., 87:671, (1988)]; Brassica (U.S. Pat. No. 5,463,174), and peanut [Cheng *et al.*, Plant Cell Rep., 15: 653, (1996)].

10 Similar methods have been reported in the transformation of monocots. Transformation and plant regeneration using these methods have been described for a number of crops including but not limited to asparagus [*Asparagus officinalis*; Bytebier *et al.*, Proc. Natl. Acad. Sci. U.S.A., 84: 5345, (1987)]; barley [*Hordeum vulgare*; Wan and Lemaux, Plant Physiol., 104: 37, (1994)]; maize [*Zea mays*; Rhodes, C. A., *et al.*, Science, 240: 204, (1988); Gordon-Kamm, *et al.*, Plant Cell, 2: 603, (1990); Fromm, *et al.*, Bio/Technology, 8: 833, (1990); Koziel, *et al.*, Bio/Technology, 11: 194, (1993)]; oats [*Avena sativa*; Somers, *et al.*, Bio/Technology, 10: 1589, (1992)]; orchardgrass [*Dactylis glomerata*; Horn, *et al.*, Plant Cell Rep., 7: 469, (1988)]; rice [*Oryza sativa*, including indica and japonica varieties, Toriyama, *et al.*, Bio/Technology, 6: 10, (1988); Zhang, *et al.*, Plant Cell Rep., 7: 379, (1988); Luo and Wu, Plant Mol. Biol. Rep., 6: 165, (1988); Zhang and Wu, Theor. Appl. Genet., 76: 835, (1988); Christou, *et al.*, Bio/Technology, 9: 957, (1991)]; sorghum [*Sorghum bicolor*; Casas, A. M., *et al.*, Proc. Natl. Acad. Sci. U.S.A., 90: 11212, (1993)]; sugar cane [*Saccharum* spp.; Bower and Birch, Plant J., 2: 409, (1992)]; tall fescue [*Festuca arundinacea*; Wang, Z. Y. *et al.*, Bio/Technology, 25 10: 691, (1992)]; turfgrass [*Agrostis palustris*; Zhong *et al.*, Plant Cell Rep., 13: 1, (1993)]; wheat [*Triticum aestivum*; Vasil *et al.*, Bio/Technology, 10: 667, (1992); Weeks T., *et al.*, Plant Physiol., 102: 1077, (1993); Becker, *et al.*, Plant, J. 5: 299, (1994)], and alfalfa [Masoud, S. A., *et al.*, Transgen. Res., 5: 313, (1996)]. It is
30 apparent to those of skill in the art that a number of transformation methodologies can be used and modified for production of stable transgenic plants from any number of target crops of interest.

The transformed plants can be analyzed for the expression features conferred by the polynucleotides of the present invention, using methods known in the art for the analysis of transformed plants. A variety of methods are used to assess gene expression and determine if the introduced gene(s) is integrated, functioning properly, and inherited as expected. Preferably, the promoters can be evaluated by determining the expression levels and the expression features of genes to which the promoters are operatively linked. A preliminary assessment of promoter function can be determined by a transient assay method using reporter genes, but a more definitive promoter assessment can be determined from the analysis of stable plants. Methods for plant analysis include but are not limited to Southern blots or northern blots, PCR-based approaches, biochemical analyses, phenotypic screening methods, field evaluations, and immunodiagnostic assays.

Preferably, the capacity of isolated polynucleotides of this invention to promote gene expression in plants is evaluated according to phenotypic expression of reporter genes using procedures as described in the Examples section that follows. Briefly, the expression of luciferase in transgenic *Arabidopsis* is determined and consistently classified by quantitatively scoring certain features of expression, such as the intensity, specificity, development stage and positioning of expression. Accordingly, a luciferase gene that is expressed in a constitutive manner would indicate a putative constitutive promoter activity of the isolated polynucleotide. Likewise, a luciferase gene that is expressed in an inductive, tissue specific or a development-stage specific manner, would respectively indicate a putative inductive, a tissue specific or a stage specific promoter activity.

Hence, the present invention provides a plurality of isolated polynucleotide sequences which exhibit a wide spectrum of promoter function patterns. These polynucleotides can be used to generate nucleic acid constructs, such as expression vectors suitable for transforming an organism. Such nucleic acid constructs can be used to promote expression of desired traits or expression products in transgenic organisms, such as plants, in a constitutive, induced, tissue specific, or a developmental stage specific manner.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the

various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

5

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook *et al.*, (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson *et al.*, "Recombinant DNA", Scientific American Books, New York; Birren *et al.* (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites *et al.* (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications",

Academic Press, San Diego, CA (1990); Marshak *et al.*, "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF DNA REGULATING ELEMENTS (DREs)

Novel DREs were identified by luciferase expression assay driven by bioinformatically identified DNA fragments from *Arabidopsis thaliana* genomic DNA. Positive DREs were fused upstream a reporter gene in a vector which was used to transform *Arabidopsis thaliana* plants. The reporter gene expression driven by these DREs was characterized.

MATERIALS AND EXPERIMENTAL METHODS

Isolation of DREs: A high throughput method of cloning DNA regulating elements (DREs) using a single reaction tube, referred to herein as the "one-tube" method, was utilized in order to enable large scale production of DRE transformed plants. Accordingly, genomic DNA (gDNA) was extracted from leaves of *Arabidopsis thaliana* Col1 using DNAeasy Plant Mini Kit (Qiagen, Germany). Primers for PCR amplification of DREs were designed using PRIMER3[®] software and modified to contain restriction sites absent from the DRE sequence, for PCR product insertion into pVER1 binary plasmid, which is a pBI101 (clontech) modified plasmid, where the GUS reporter gene was replaced by LucII gene from pGL3-Basic (promega). Briefly, GUS gene was cut out of pBI101 using the blunt restriction enzymes Ecl136II and SmaI. The pGL-Basic plasmid [after eliminating the HindIII and BamHI sites, by digestion, fill-in using klenow fragment (Roche) and self ligating the plasmid, using T4 DNA ligase (Roche)] was cut SacI and XbaI and the LucII gene insert was inserted into pBluescript, digested with the same enzymes. The new plasmid was digested SmaI, as a result a blunt ends LucII gene was cut out. The LucII gene was inserted into The pBI plasmid instead of the GUS gene. To eliminate all possible read-through of the Nos-promoter, which regulates Kanamycin resistance

gene on pBI101, a poly-A signal was added between the Nos-terminator and the LucII gene. Poly-A signal was amplified from pGL3-Basic using proof reading Taq polymerase PFU (Promega) and using primers 5'-aggacttgagcggccgca-3' and 5'-tagagaaatgttctggcacctg-3'. The Product was inserted into HindIII site on pVerI after filling the overhang 5' ends, using Klenow fragment (Roche).

Polymerase chain reaction analyses were performed using Taq Expand Long Template PCR kit (Roche), according to the manufacturer's instructions, using as thermal cycle: 92 °C/2 min → 10 × [94 °C/10 min → 55 °C/30 sec → 68 °C/5 min] → 18 × [94 °C/10 min → 55 °C/30 sec → 68 °C/5 min (+ 20 sec each cycle)] → 68 °C/7 min. PCR products were double-digested with restriction endonucleases according to the protocols described in Table 1.

Table 1: DRE double digestion protocols

Enzyme combination	First digest	Buffer (Roche)	Digest time (min)	Heat inactivation conditions	Second digest	Buffer	Digest time (min)	Heat inactivation conditions
HindIII, Sall	HindIII	M	90	20 min, 70 °C	Sall	M + NaCl + Tris	60	20 min, 70 °C
HindIII, BamHI	HindIII	B	30	No	BamHI	B	60	20 min, 70 °C
Sall, BamHI	BamHI	M	60	20 min, 80 °C	Sall	M + NaCl + Tris	60	20 min, 70 °C
HindIII, EcoRV	HindIII	B	30	No	EcoRV	B	60	20 min, 70 °C
Sall, ScaI	Sall, ScaI	H	60	20 min, 80 °C				
BamHI, SmaI	SmaI	A	60 (30 °C)	20 min, 70 °C	BamHI	A	60	20 min, 80 °C
Sall, PvuII	PvuII	M	60	20 min, 80 °C	Sall	M + NaCl + Tris	60	20 min
HindIII, PvuII	HindIII	M	30	No	PvuII	M	60	20 min, 80 °C
HindIII, StuI	HindIII, StuI	B	90	20 min, 80 °C				
BamHI, StuI	StuI	B	30	No	BamHI	B	60	20 min, 80 °C

15 **Cloning of DREs in luciferase reporter gene expression:** PCR amplified DREs were cloned into a luciferase reporter gene expression vector pVER1, derived from the binary vector pBI101 (Clontech), was double-digested using the same restriction endonucleases used to excise cloned DREs from vector, purified using

PCR Purification Kit (Qiagen, Germany), treated with alkaline-phosphatase (Roche) according to the manufacturer's instructions and re-purified using PCR Purification Kit (Qiagen, Germany). Insertion of DRE into vector pVER1 was performed by adding to DRE digests: 500 ng of double digested pVer1 plasmid, 1 μ l of T4 DNA
5 ligase (40 U/ μ l; Roche) and 6 μ l of T4 buffer (Roche). Following overnight incubation of ligation mixes at 4 °C, *Agrobacterium tumefaciens* GV303 competent cells were transformed using 1–2 μ l of ligation reaction mixture by electroporation, using a MicroPulser electroporator (Biorad), 0.2 cm cuvettes (Biorad) and EC-2 electroporation program (Biorad). *Agrobacterium* cells were grown on LB at 28 °C
10 for 3 h and plated on LB-agar plates supplemented with the antibiotics gentamycin 50 mg/L (Sigma) and kanamycin 50 mg/L (Sigma). Plates were then incubated at 28 °C for 48 h. Cloned DREs were identified by PCR analysis of bacterial colony DNA using the vector specific, insert flanking upstream and downstream primers 5'-AGGTA CTTGGAGCGGCCGCA-3' and 5'-CGAACACCACGGTAGGCTG-3',
15 respectively and the thermal cycle: 92 °C/3 min \rightarrow 31 \times [94 °C/30 sec \rightarrow 54 °C/30 sec \rightarrow 72 °C/*X* min (*X* = length (kb) of longest PCR product expected)] \rightarrow 72 °C/10 min. Positive *Agrobacterium* colonies were subsequently used for *Arabidopsis* plant transformation.

Plant transformation and cultivation: *Arabidopsis thaliana* Columbia (T₀
20 plants) were transformed using the Floral Dip procedure described by Clough SJ and Bent AF [The Plant J. 16:735-743 (1998)] and by Desfeux *et al.* [Plant Physiology 123:895-904 (2000)] with minor modifications. Briefly, T₀ Plants were sown in 250 ml pots filled with wet peat-based growth mix. The pots were covered with aluminum foil and a plastic dome, kept at 4 °C for 3 – 4 days, then uncovered and
25 incubated in a growth chamber at 18 – 24 °C under 16/8 hr light/dark cycle. The T₀ plants were ready for transformation six days before anthesis.

Single colonies of *Agrobacterium* carrying plant DREs were cultured in LB medium supplemented with kanamycin (50 mg/L) and gentamycin (50 mg/L). The cultures were incubated at 28⁰C for 48 hours under vigorous shaking and centrifuged
30 at 4000 rpm for 5 minutes. The pellets comprising *Agrobacterium* cells were resuspended in a transformation medium which contained half-strength (2.15 g/L) Murashig-Skoog (Duchefa); 0.044 μ M benzylamino purine (Sigma); 112 μ g/L B5

Gambourg vitamins (Sigma); 5% sucrose; and 0.2 ml/L Silwet L-77 (OSI Specialists, CT) in double-distilled water, at pH of 5.7.

Transformation of T_0 plants was effected by inverting each plant into an *Agrobacterium* suspension such that the above ground plant tissue was submerged for 3-5 seconds. Each inoculated T_0 plant was immediately placed in a plastic tray, then covered with clear plastic dome to maintain humidity and kept in the dark at room temperature for eighteen hours to facilitate infection and transformation. Transformed (transgenic) plants were then uncovered and transferred to a greenhouse for recovery and maturation. The transgenic T_0 plants were grown in the greenhouse for 3-5 weeks until siliques were brown and dry then seeds were harvested from plants and kept at room temperature until sowing

Generating T_1 and T_2 transgenic plants harboring DREs: Seeds collected from transgenic T_0 plants were surface-sterilized by soaking in 70% ethanol for 1 minute, followed by soaking in 5% sodium hypochloride and 0.05% triton for 5 minutes. The surface-sterilized seeds were thoroughly washed in sterile distilled water then placed on culture plates containing half-strength Murashig-Skoog (Duchefa); 2% sucrose; 0.8% plant agar; 50 mM kanamycin; and 200 mM carbenicylin (Duchefa). The culture plates were incubated at 4⁰C for 48 hours then transferred to a growth room at 25⁰C for an additional week of incubation. Vital T_1 *Arabidopsis* plants were transferred to a fresh culture plates for another week of incubation. Following incubation the T_1 plants were removed from culture plates and planted in growth mix contained in 250 ml pots. The transgenic were allowed to grow in a greenhouse to maturity. Seeds harvested from T_1 plants were cultured and grown to maturity as T_2 plants under the same conditions as used for culturing and growing the T_1 plants.

Evaluating DRE gene-promoting activity in transgenic plants: The ability of DREs to promote gene expression in plants was determined based on the expression of luciferase reporter gene. Accordingly, transgenic *Arabidopsis* plantlets at a development stage of 2-3 true leaves were subjected to luminescence assays using the procedure described by Messinner R. [Plant. J. 22:265 (2000)]. The imaging of luciferase was performed in a darkroom using ultra-low light detection camera (Princeton Instruments Inc., USA). Using the procedure described by Messinner R. [Plant. J. 22:265 (2000)].

Scoring promoter activity in transgenic plants: DREs promoting gene expression was characterized based luciferase expression in transgenic plants using quantitative values such as to enable consistent evaluations of a large volume of transgenic plants, as follows:

5 **Scoring distribution and intensity of expression:** The distribution of reporter genes' expression in transgenic plants was presented in a three variables functions, as follows: (i) plant ID (X axis), (ii) plant organ (Y axis), and (iii) development stage (Z axis). The intensity of expression, relevant to any of these three variables, was measured by a distribution function value (DF), referred hereinbelow as
 10 $f_{x,y,z}(\text{Promoter})$. The DF received a value ranging from 0 to 5, representing no expression and the highest expression intensity, respectively.

Scoring specificity of expression: The specificity of reporter genes' expression in transgenic plants was calculated by summing two independent addends: (a) the zero value/nonzero values ratio, as described in table 2 below and which
 15 further referred to as the Binary Function $B(\)$; and (b) the variance of the nonzero values only.

Table 2

No. of non zero values	No. of zero values				
	0	1	2	3	4
0		0	0	0	0
1	0	0.7	1.5	2	
2	0	0.6	1		
3	0	0.5			
4	0				

20 The Organ Specificity expression value (SpOr) was calculated according to the following equation:

$$SpOr(\text{promoter}) = Var_y(Av_{x,z}(f_{x,y,z}(\text{promoter}))|_{y>0}) + B(Av_{x,z}(f_{x,y,z}(\text{promoter})))$$

Whereas Var is the variance, Av is the average and B is the Binary Function.

 The development Stage Specificity expression value (SpDs) was calculated according to the following equation:

25 $SpDS(\text{promoter}) = Var_z(Av_{x,y}(f_{x,y,z}(\text{promoter}))|_{z>0}) + B(Av_{x,y}(f_{x,y,z}(\text{promoter})))$

Whereas Var is the variance, Av is the average and B is the Binary Function.

Scoring position effect: Similarly to the Binary Factor approach described above, position values were also classified as either zero or nonzero values.

Accordingly, the reporter genes' expression in a given organ in a given development stage was measured by a Local Position Effect value (LoPoEf). The Position Effect value (PoEf) was the average of all the Local Position Effects, calculate in three steps as follows:

$$5 \quad 1) h_{x,y,z}(promoter) = \begin{cases} 0 & f_{x,y,z}(promoter) = 0 \\ 1 & f_{x,y,z}(promoter) = 1,2,3,4,5 \end{cases}$$

2)

$$LoPoEf(promoter, organ, development_stage) = \min \left(\frac{no_of_0s_in(h_{x,y,z}(promoter))}{no_of_non_0s_in(h_{x,y,z}(promoter))}, \frac{no_of_non_0s_in(h_{x,y,z}(promoter))}{no_of_0s_in(h_{x,y,z}(promoter))} \right)$$

$$3) PoEf(promoter) = Av(LoPoEf(promoter, Y, Z)).$$

10 **Scoring expression level:** The average expression level value (ExLe) and the ExLe variance (VrExLe) were calculated per each DRE promoter x plant organ x plant development stage combination, according the following equations:

$$ExLe(promoter, organ, development_stage) = Av_x(f_{x,y,z}(promoter))$$

$$VrExLe(promoter, organ, development_stage) = var_x(f_{x,y,z}(promoter)).$$

15 **Scoring evaluation reliability:** The General Reliability value (Grel) was the number of independent plants that were used for evaluating a specific DRE promoter activity. Hence, $GRel(promoter) = Count_x(f_{x,y,z}(promoter))$. The Development Stage Reliability value (Rel(DS)) was the number of independent plants that were used for evaluating a specific DRE promoter activity in any given plant developing stage. $Rel(promoter, development_stage) = Count_{x|z=development_stage}(f_{x,y,z}(promoter))$.

20 **Creation of partial fragments from vDREs 4209 and 6669:** Genomic DNA derived from *Arabidopsis thaliana* var Col0 was extracted and PCR-amplified using oligonucleotide primers complementary to sequences within vDRE 4209 (SEQ ID NO:36) [sense primer 5'- GTTGGTTCGTCGACTAGAGAAGGT -3' (SEQ ID NO: 208), antisense primer 5'- TTGGATCCGGGAGGCAATGATGCTTTAG - 3' (SEQ ID NO: 209)], and vDRE 6669 (SEQ ID NO:61) [sense primer 5'- TTGTAAGCTTGCAGGGATACGGATGGGTAG -3' (SEQ ID NO: 211), antisense primer 5'- AAATATTGGATCCTTTGGGGTTCTC - 3' (SEQ ID NO: 212)].

The above PCR amplifications resulted in a 470 bp fragment, containing bp 76-548 of the original vDRE 4209 (SEQ ID NO:210) and a 1569 bp fragment, containing bp 748-2316 of the original vDRE 6669 (SEQ ID NO:213), respectively.

5 PCR products were digested with HindIII and BamHI and ligated into the binary vector, pBI121 (Clontech, accession number: AF485783) upstream to the GUS gene, generating plasmids p4209short-GUS , and p6669short-GUS, respectively. Arabidopsis plants (var col0) were transformed with the binary constructs generated (p4209short-GUS and p6669short-GUS), and GUS activity was analyzed on 10 independent T1 transformed plants using standard GUS staining protocol [Jefferson
10 RA, Kavanagh TA, Bevan MW. 1987. GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. 6(13): 3901-7]. Genomic DNA extraction, PCR amplification, DNA restriction, ligation and transformation of Arabidopsis plant were preformed according to the protocols described above.

15

EXPERIMENTAL RESULTS

Characterization of DREs :

Various features of the isolated DREs of the present invention are described in Tables 3-17 which follow. As is clearly evident from the Table provided data, the DREs of the present invention exhibit a wide range of gene-promoting activities
20 including: constitutive, inductive, tissue specific, and stage specific activities.

Table 3

DRE number ¹	1345	1495	2176
Cluster reference ²	Z18125	Z17428	ATBIBBI
Cluster position ²	Upstream	Upstream	Upstream
DRE regulatory direction ³	Bidirectional	Unidirectional	Unidirectional
DRE length (bp)	1611	901	2192
DRE sequence	SEQ ID NO: 1	SEQ ID NO: 6	SEQ ID NO: 11
Internal forward primer sequence ⁴	SEQ ID NO: 2	SEQ ID NO: 7	SEQ ID NO: 12
External forward primer sequence ⁴	SEQ ID NO: 3	SEQ ID NO: 8	SEQ ID NO: 13
Internal reverse primer sequence ⁴	SEQ ID NO: 4	SEQ ID NO: 9	SEQ ID NO: 14
External reverse primer sequence ⁴	SEQ ID NO: 5	SEQ ID NO: 10	SEQ ID NO: 15
Position Effect Value ⁵	0.37	0.21	8.33
Development Stage Specificity Value ⁵	1.09	0.32	0.62
Organ Specificity Value ⁵	1.56	0.38	2.60
Number of transgenic plants	11	10	7
Young roots score (No., Ave., Var) ⁶	10, 12, 3.36	6, 2.333, 1.555	4, 0, 0
Mature roots score (No., Ave., Var) ⁶	7, 1.571, 3.387	7, 3, 2.285	5, 0, 0
Young above-ground Tissue (No., Ave., Var) ⁶	10, 3.3, 2.21	6, 4.16, 0.13	4, 0, 0
Mature above-ground tissue (No., Ave., Var) ⁶	7, 3, 2	7, 3.28, 1.06	5, 0, 0
Siliques/Seed (No., Ave., Var) ⁶	3, 4.33, 0.88	3, 2, 2	3, 1.67, 5.56
Flowers (No., Ave., Var) ⁶	7, 1.42, 3.10	7, 3.14, 4.40	5, 4.2, 2.56
Description	Constitutive. Strong in seeds.	Constitutive.	Specific to flower tissue. Strong in flower buds. Lower expression in open flowers.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 4

DRE number ¹	2524	3560	3583
Cluster reference ²	Z17778	Z17937	av558751
Cluster position ²	Upstream	Upstream	Upstream
DRE regulatory direction	Bidirectional	Bidirectional	Bidirectional
DRE length (bp)	1975	3126	2501
DRE sequence	SEQ ID NO:16	SEQ ID NO:21	SEQ ID NO:26
Internal forward primer sequence ⁴	SEQ ID NO:17	SEQ ID NO:22	SEQ ID NO:27
External forward primer sequence ⁴	SEQ ID NO:18	SEQ ID NO:23	SEQ ID NO:28
Internal reverse primer sequence ⁴	SEQ ID NO:19	SEQ ID NO:24	SEQ ID NO:29
External reverse primer sequence ⁴	SEQ ID NO:20	SEQ ID NO:25	SEQ ID NO:30
Position Effect Value ⁵	0.15	0.3	5.555
Development Stage Specificity Value ⁵	0.69	0.77	1.5
Organ Specificity Value ⁵	1.16	1.14	2
Number of transgenic plants	8	11	6
Young roots score (No., Ave., Var) ⁶	5, 0, 0	6, 3.5, 1.92	5, 0, 0
Mature roots score (No., Ave., Var) ⁶	5, 0, 0	7, 3.71, 1.63	4, 0, 0
Young above-ground Tissue (No., Ave., Var) ⁶	5, 0.4, 0.24	6, 1.83, 2.14	5, 0, 0
Mature above-ground tissue (No., Ave., Var) ⁶	5, 2, 0.8	7, 1.43, 1.10	4, 0.25, 0.19
Siliques/Seed (No., Ave., Var) ⁶	3, 0, 0	3, 0.67, 0.89	3, 0, 0
Flowers (No., Ave., Var) ⁶	5, 0.4, 0.64	7, 1.86, 1.55	4, 0, 0
Description	Specific to above ground tissue.	Specific to root tissue. Strong expression, mainly in root meristems. Weak expression in above ground tissues.	Weak in above ground tissue.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 5

DRE number ¹	3714	4209	5095
Cluster reference ²	Z25961	Z29176	AI996150
Cluster position ²	Upstream	Downstream	Downstream
DRE regulatory direction	Unidirectional	Bidirectional	Bidirectional
DRE length (bp)	513	1022	1056
DRE sequence	SEQ ID NO:31	SEQ ID NO:36	SEQ ID NO:41
Internal forward primer sequence ⁴	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:42
External forward primer sequence ⁴	SEQ ID NO:33	SEQ ID NO:38	SEQ ID NO:43
Internal reverse primer sequence ⁴	SEQ ID NO:34	SEQ ID NO:39	SEQ ID NO:44
External reverse primer sequence ⁴	SEQ ID NO:35	SEQ ID NO:40	SEQ ID NO:45
Position Effect Value ⁵	0.3625	0.40	0.6
Development Stage Specificity Value ⁵	0.11241	0.57	0
Organ Specificity Value ⁵	0.377	0.40	0.85
Number of transgenic plants	11	18	3
Young roots score (No., Ave., Var) ⁶	9, 0.611, 0.987	14, 3.46, 2.87	2, 0.5, 0.25
Mature roots score (No., Ave., Var) ⁶	3, 0, 0	9, 2.11, 3.65	2, 1, 1
Young above-ground Tissue (No., Ave., Var) ⁶	9, 2.38, 1.20	14, 2.89, 2.36	2, 1.5, 1.25
Mature above-ground tissue (No., Ave., Var) ⁶	3, 1, 2	9, 2.44, 1.80	2, 2, 0
Siliques/Seed (No., Ave., Var) ⁶	3, 1.66, 0.22	3, 1.33, 1.56	Not available
Flowers (No., Ave., Var) ⁶	3, 1.33, 3.55	9, 2, 3.78	2, 0, 0
Description	Weak in above ground tissue	Strong in roots, mainly root tips, and flower buds. Lower expression in veins. Very low expression in seeds.	Constitutive, weak.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 6

DRE number ¹	5311	5532	5587
Cluster reference ²	ATHCOL2A	ATASCO	Z26363
Cluster position ²	Upstream	Upstream	Upstream
DRE regulatory direction	Bidirectional	Unidirectional	Unidirectional
DRE length (bp)	435	3348	1331
DRE sequence	SEQ ID NO:46	SEQ ID NO:51	SEQ ID NO:56
Internal forward primer sequence ⁴	SEQ ID NO:47	SEQ ID NO:52	SEQ ID NO:57
External forward primer sequence ⁴	SEQ ID NO: (none)	SEQ ID NO:53	SEQ ID NO:58
Internal reverse primer sequence ⁴	SEQ ID NO:49	SEQ ID NO:54	SEQ ID NO:59
External reverse primer sequence ⁴	SEQ ID NO: (none)	SEQ ID NO:55	SEQ ID NO:60
Position Effect Value ⁵	0.36	0.25	8.33
Development Stage Specificity Value ⁵	1.15	1.30932	1.5
Organ Specificity Value ⁵	0.332	1.246	2
Number of transgenic plants	8	6	4
Young roots score (No., Ave., Var) ⁶	7, 0.36, 0.48	5, 2.2, 1.36	4, 0, 0
Mature roots score (No., Ave., Var) ⁶	4, 0.5, 0.75	4, 4.25, 0.187	3, 0, 0
Young above-ground Tissue (No., Ave., Var) ⁶	7, 1.57, 1.74	5, 3.6, 1.84	4, 0, 0
Mature above-ground tissue (No., Ave., Var) ⁶	4, 1.5, 2.25	4, 3.5, 0.25	3, 0, 0
Siliques/Seed (No., Ave., Var) ⁶	Not available	3, 0.67, 0.22	3, 1.33, 3.55
Flowers (No., Ave., Var) ⁶	4, 0.25, 0.18	4, 2, 4.5	3, 0, 0
Description	Constitutive, weak.	Constitutive, mainly in vegetative tissue.	Siliques specific. High position effect.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 7

DRE number ¹	6669	6762	7357
Cluster reference ²	Z26440	Z17588	F13952
Cluster position ²	Upstream	Upstream	Upstream
DRE regulatory direction	Unidirectional	Bidirectional	Unidirectional
DRE length (bp)	2316	379	979
DRE sequence	SEQ ID NO:61	SEQ ID NO:66	SEQ ID NO:71
Internal forward primer sequence ⁴	SEQ ID NO:62	SEQ ID NO:67	SEQ ID NO:72
External forward primer sequence ⁴	SEQ ID NO:63	SEQ ID NO:68	SEQ ID NO:73
Internal reverse primer sequence ⁴	SEQ ID NO:64	SEQ ID NO:69	SEQ ID NO:74
External reverse primer sequence ⁴	SEQ ID NO:65	SEQ ID NO:70	SEQ ID NO:75
Position Effect Value ⁵	0.28	9.72	0.32
Development Stage Specificity Value ⁵	1.18	0.16	0.6
Organ Specificity Value ⁵	1.32	1.42	0.64
Number of transgenic plants	4	11	7
Young roots score (No., Ave., Var) ⁶	3, 2.67, 0.22	8, 1.25, 4.69	6, 0.5, 0.58
Mature roots score (No., Ave., Var) ⁶	4, 4.75, 0.19	9, 0.33, 0.89	5, 0, 0
Young above-ground Tissue (No., Ave., Var) ⁶	3, 3.67, 3.56	8, 4.19, 0.87	6, 0.43, 0.47
Mature above-ground tissue (No., Ave., Var) ⁶	4, 1, 3	9, 3.61, 1.10	5, 0.8, 0.16
Siliques/Seed (No., Ave., Var) ⁶	4, 0.75, 0.69	3, 3.33, 1.56	3, 0, 0
Flowers (No., Ave., Var) ⁶	4, 3, 4.5	9, 3.11, 2.57	5, 1.2, 1.36
Description	Specific to young and meristematic tissue.	Strong in above ground tissue.	Weak in above ground tissue.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 8

DRE number ¹	7617	8463	9136
Cluster reference ²	Z17636	Z26728	F15462
Cluster position ²	Upstream	Downstream	Downstream
DRE regulatory direction	Bidirectional	Unidirectional	Unidirectional
DRE length (bp)	665	2834	486
DRE sequence	SEQ ID NO:76	SEQ ID NO:81	SEQ ID NO:86
Internal forward primer sequence ⁴	SEQ ID NO:77	SEQ ID NO:82	SEQ ID NO:87
External forward primer sequence ⁴	SEQ ID NO:78	SEQ ID NO:83	SEQ ID NO:88
Internal reverse primer sequence ⁴	SEQ ID NO:79	SEQ ID NO:84	SEQ ID NO:89
External reverse primer sequence ⁴	SEQ ID NO:80	SEQ ID NO:85	SEQ ID NO:90
Position Effect Value ⁵	0.42	0.16	0.48
Development Stage Specificity Value ⁵	0.16	0.68	0.60
Organ Specificity Value ⁵	0.41	2.02	0.53
Number of transgenic plants	3	12	13
Young roots score (No., Ave., Var) ⁶	3, 0, 0	6, 0, 0	9, 0.778, 2.617
Mature roots score (No., Ave., Var) ⁶	3, 0, 0	7, 1.14, 3.55	11, 0.73, 1.107
Young above-ground Tissue (No., Ave., Var) ⁶	3, 0.17, 5.56	6, 3.33, 3.32	9, 0.778, 0.84
Mature above-ground tissue (No., Ave., Var) ⁶	3, 0.33, 0.22	7, 2, 2.57	11, 1.18, 2.51
Siliques/Seed (No., Ave., Var) ⁶	2, 1, 1	4, 0, 0	3, 0, 0
Flowers (No., Ave., Var) ⁶	3, 0.33, 0.22	7, 3.57, 3.96	11, 0.55, 0.98
Description	Very weak in above ground tissue.	Strong in above ground tissue of seedlings. Strong in flower tissue of mature plants.	Constitutive, weak.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 9

DRE number ¹	10826	12582	13257
Cluster reference ²	Z30896	Z33953	Z17541
Cluster position ²	Upstream	Downstream	Upstream
DRE regulatory direction	Bidirectional	Unidirectional	Bidirectional
DRE length (bp)	1840	1665	807
DRE sequence	SEQ ID NO:91	SEQ ID NO:96	SEQ ID NO:101
Internal forward primer sequence ⁴	SEQ ID NO:92	SEQ ID NO:97	SEQ ID NO:102
External forward primer sequence ⁴	SEQ ID NO:93	SEQ ID NO:98	SEQ ID NO:103
Internal reverse primer sequence ⁴	SEQ ID NO:94	SEQ ID NO:99	SEQ ID NO:104
External reverse primer sequence ⁴	SEQ ID NO:95	SEQ ID NO:100	SEQ ID NO:105
Position Effect Value ⁵	0.27	0.19	0
Development Stage Specificity Value ⁵	0.50	0.32	1.5
Organ Specificity Value ⁵	8.19	1.38	1.14
Number of transgenic plants	5	20	2
Young roots score (No., Ave., Var) ⁶	3, 1.67, 2.89	18, 0.56, 1.36	2, 0, 0
Mature roots score (No., Ave., Var) ⁶	4, 3.38, 4.17	10, 0.5, 1.05	2, 0, 0
Young above-ground Tissue (No., Ave., Var) ⁶	3, 2, 2.67	18, 2.39, 3.90	2, 0, 0
Mature above-ground tissue (No., Ave., Var) ⁶	4, 3.12, 1.55	10, 3.2, 0.36	2, 2.5, 0.25
Siliques/Seed (No., Ave., Var) ⁶	Not available	3, 1, 0	2, 0, 0
Flowers (No., Ave., Var) ⁶	4, 3.25, 3.06	10, 4.4, 1.84	2, 2, 1
Description	Strong in root and flower tissue.	Strong in above ground tissue of seedlings. Lower expression in mature plants.	Specific to above ground tissue of mature plants.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 10

DRE number ¹	13277	15980	16665
Cluster reference ²	Z18392	BE522497	T04806
Cluster position ²	Upstream	Downstream	Downstream
DRE regulatory direction	Bidirectional	Unidirectional	Bidirectional
DRE length (bp)	3297	2183	1358
DRE sequence	SEQ ID NO:106	SEQ ID NO:111	SEQ ID NO:116
Internal forward primer sequence ⁴	SEQ ID NO:107	SEQ ID NO:112	SEQ ID NO:117
External forward primer sequence ⁴	SEQ ID NO:108	SEQ ID NO:113	SEQ ID NO:118
Internal reverse primer sequence ⁴	SEQ ID NO:109	SEQ ID NO:114	SEQ ID NO:119
External reverse primer sequence ⁴	SEQ ID NO:110	SEQ ID NO:115	SEQ ID NO:120
Position Effect Value ⁵	0.22	0.38	0.33
Development Stage Specificity Value ⁵	1.5	1.18	4.44
Organ Specificity Value ⁵	1	1.45	1.5
Number of transgenic plants	5	16	5
Young roots score (No., Ave., Var) ⁶	5, 0.6, 0.24	10, 2.1, 1.49	5, 0, 0
Mature roots score (No., Ave., Var) ⁶	3, 0, 0	13, 2.46, 0.86	2, 0, 0
Young above-ground Tissue (No., Ave., Var) ⁶	5, 0.4, 0.24	10, 12, 1.76	5, 0.6, 0.34
Mature above-ground tissue (No., Ave., Var) ⁶	3, 0, 0	13, 0.46, 0.86	2, 0.5, 0.25
Siliques/Seed (No., Ave., Var) ⁶	3, 0, 0	4, 3.75, 1.69	Not available
Flowers (No., Ave., Var) ⁶	3, 0, 0	13, 0.92, 1.76	2, 0, 0
Description	Weak in seedlings.	Root tissue, mainly root tips; and seeds.	Above ground vegetative tissue of mature plants.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 11

DRE number ¹	16900	17109	17809
Cluster reference ²	Z25996	Z17897	Z18103
Cluster position ²	Upstream	Upstream	Upstream
DRE regulatory direction	Bidirectional	Bidirectional	Bidirectional
DRE length (bp)	824	2927	3165
DRE sequence	SEQ ID NO:121	SEQ ID NO:126	SEQ ID NO:131
Internal forward primer sequence ⁴	SEQ ID NO:122	SEQ ID NO:127	SEQ ID NO:132
External forward primer sequence ⁴	SEQ ID NO:123	SEQ ID NO:128	SEQ ID NO:133
Internal reverse primer sequence ⁴	SEQ ID NO:124	SEQ ID NO:129	SEQ ID NO:134
External reverse primer sequence ⁴	SEQ ID NO:125	SEQ ID NO:130	SEQ ID NO:135
Position Effect Value ⁵	4.17	0.26	0.21
Development Stage Specificity Value ⁵	0.21	0.63	0.60
Organ Specificity Value ⁵	0.22	1.85	1.38
Number of transgenic plants	5	10	10
Young roots score (No., Ave., Var) ⁶	4, 3.5, 0.75	6, 4, 1.25	5, 0.8, 0.56
Mature roots score (No., Ave., Var) ⁶	5, 3.2, 0.56	7, 3.07, 2.60	7, 1.5, 1.5
Young above-ground Tissue (No., Ave., Var) ⁶	4, 4, 0	6, 0.42, 0.37	5, 4, 0.8
Mature above-ground tissue (No., Ave., Var) ⁶	5, 3.8, 0.56	7, 1.05, 1.07	7, 3.07, 1.03
Siliques/Seed (No., Ave., Var) ⁶	3, 4.67, 0.22	3, 0, 0	3, 0, 0
Flowers (No., Ave., Var) ⁶	5, 4, 4	7, 1.07, 2.89	7, 2.71, 2.99
Description	Constitutive pattern. Strong in meristematic tissue and seeds.	Strong in root, flower and meristematic tissue.	Strong in leaf tissue of seedlings. Variable in mature plants.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 12

DRE number ¹	19672	19678	19827
Cluster reference ²	Z25683	BE523552	Z17577
Cluster position ²	Upstream	Upstream	Upstream
DRE regulatory direction	Unidirectional	Bidirectional	Bidirectional
DRE length (bp)	1155	2877	578
DRE sequence	SEQ ID NO:136	SEQ ID NO:141	SEQ ID NO:146
Internal forward primer sequence ⁴	SEQ ID NO:137	SEQ ID NO:142	SEQ ID NO:147
External forward primer sequence ⁴	SEQ ID NO:138	SEQ ID NO:143	SEQ ID NO:148
Internal reverse primer sequence ⁴	SEQ ID NO:139	SEQ ID NO:144	SEQ ID NO:149
External reverse primer sequence ⁴	SEQ ID NO:140	SEQ ID NO:145	SEQ ID NO:150
Position Effect Value ⁵	0.03	5.55	0.37
Development Stage Specificity Value ⁵	9.78	1.5	0.60
Organ Specificity Value ⁵	3.99	2	0.64
Number of transgenic plants	17	5	12
Young roots score (No., Ave., Var) ⁶	15, 4.33, 0.76	5, 0, 0	10, 0, 0
Mature roots score (No., Ave., Var) ⁶	17, 4, 1.76	4, 0, 0	5, 0.1, 0.04
Young above-ground Tissue (No., Ave., Var) ⁶	15, 4.53, 0.38	5, 0, 0	10, 2.9, 2.29
Mature above-ground tissue (No., Ave., Var) ⁶	17, 3.12, 2.22	4, 0.25, 0.18	5, 0.8, 1.36
Siliques/Seed (No., Ave., Var) ⁶	3, 4.33, 0.22	3, 0, 0	3, 0, 0
Flowers (No., Ave., Var) ⁶	17, 4.06, 2.17	4, 0, 0	5, 0.8, 1.36
Description	Strong, constitutive. Lower expression in mature leaf tissue.	Very weak. High position effect.	Above ground tissue. Weak.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 13

DRE number ¹	20607	22397	22604
Cluster reference ²	AI998130	ATHD12A	ATHFEDAA
Cluster position ²	Upstream	Upstream	Downstream
DRE regulatory direction	Bidirectional	Unidirectional	Bidirectional
DRE length (bp)	2819	1313	2080
DRE sequence	SEQ ID NO:151	SEQ ID NO:156	SEQ ID NO:161
Internal forward primer sequence ⁴	SEQ ID NO:152	SEQ ID NO:157	SEQ ID NO:162
External forward primer sequence ⁴	SEQ ID NO:153	SEQ ID NO:158	SEQ ID NO:163
Internal reverse primer sequence ⁴	SEQ ID NO:154	SEQ ID NO:159	SEQ ID NO:164
External reverse primer sequence ⁴	SEQ ID NO:155	SEQ ID NO:160	SEQ ID NO:165
Position Effect Value ⁵	0.25	0.38	9.72
Development Stage Specificity Value ⁵	2.50	0.89	0.71
Organ Specificity Value ⁵	0.916	1.33	1.10
Number of transgenic plants	5	12	17
Young roots score (No., Ave., Var) ⁶	5, 0, 0	12, 1.13, 2.09	15, 0, 0
Mature roots score (No., Ave., Var) ⁶	3, 0, 0	12, 1.67, 4.22	13, 0, 0
Young above-ground Tissue (No., Ave., Var) ⁶	5, 2.2, 2.16	12, 3.33, 0.89	15, 0.2, 0.16
Mature above-ground tissue (No., Ave., Var) ⁶	3, 2, 2	12, 2.63, 2.69	13, 0, 0
Siliques/Seed (No., Ave., Var) ⁶	Not available	3, 4, 0.67	4, 0.75, 1.69
Flowers (No., Ave., Var) ⁶	3, 1, 2	12, 1.21, 3.06	13, 0, 0
Description	Above ground tissue.	Above ground tissue and seed.	Above ground tissue and seed. High position effect. Very weak.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 14

DRE number ¹	24136	24291	24728
Cluster reference ²	Z34788	Z17960	AV530349
Cluster position ²	Downstream	Upstream	Upstream
DRE regulatory direction	Unidirectional	Bidirectional	Unidirectional
DRE length (bp)	174	2096	1617
DRE sequence	SEQ ID NO:166	SEQ ID NO:171	SEQ ID NO:176
Internal forward primer sequence ⁴	SEQ ID NO:167	SEQ ID NO:172	SEQ ID NO:177
External forward primer sequence ⁴	SEQ ID NO: none	SEQ ID NO:173	SEQ ID NO:178
Internal reverse primer sequence ⁴	SEQ ID NO:169	SEQ ID NO:174	SEQ ID NO:179
External reverse primer sequence ⁴	SEQ ID NO: none	SEQ ID NO:175	SEQ ID NO:180
Position Effect Value ⁵	0.17	0.56	5.71
Development Stage Specificity Value ⁵	1.5	7.76	0.17
Organ Specificity Value ⁵	2	6.93	1.75
Number of transgenic plants	5	12	9
Young roots score (No., Ave., Var) ⁶	1, 0, 0	9, 1.56, 3.14	8, 0, 0
Mature roots score (No., Ave., Var) ⁶	2, 0, 0	8, 2.37, 1.48	8, 0.5, 1.75
Young above-ground Tissue (No., Ave., Var) ⁶	1, 0, 0	9, 2.33, 3.11	8, 2.63, 0.48
Mature above-ground tissue (No., Ave., Var) ⁶	2, 0.25, 0.06	8, 1.62, 2.33	8, 2.5, 1.5
Siliques/Seed (No., Ave., Var) ⁶	3, 0, 0	4, 2, 1.5	Not available
Flowers (No., Ave., Var) ⁶	2, 0, 0	8, 1.37, 1.98	8, 3.38, 2.73
Description	Above ground tissue. Very weak.	Constitutive.	Strong in flower tissue. Low expression in veins.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 15

DRE number ¹	24811	4209	5095
Cluster reference ²	H36200	H36237	Z29720
Cluster position ²	Upstream	Upstream	Upstream
DRE regulatory direction	Bidirectional	Bidirectional	Bidirectional
DRE length (bp)	428	1022	1056
DRE sequence	SEQ ID NO:181	SEQ ID NO:186	SEQ ID NO:191
Internal forward primer sequence ⁴	SEQ ID NO:182	SEQ ID NO:187	SEQ ID NO:192
External forward primer sequence ⁴	SEQ ID NO: none	SEQ ID NO:188	SEQ ID NO:193
Internal reverse primer sequence ⁴	SEQ ID NO:184	SEQ ID NO:189	SEQ ID NO:194
External reverse primer sequence ⁴	SEQ ID NO: none	SEQ ID NO:190	SEQ ID NO:195
Position Effect Value ⁵	0.53	0.60	0.33
Development Stage Specificity Value ⁵	8.11	0.28	0.61
Organ Specificity Value ⁵	0.23	0.49	1.35
Number of transgenic plants	4	5	3
Young roots score (No., Ave., Var) ⁶	4, 0.75, 1.69	5, 0.4, 0.34	3, 0.33, 0.22
Mature roots score (No., Ave., Var) ⁶	3, 1, 2	4, 1.2, 2.16	2, 1, 1
Young above-ground Tissue (No., Ave., Var) ⁶	4, 1, 3	5, 2.4, 1.84	3, 1.33, 1.56
Mature above-ground tissue (No., Ave., Var) ⁶	3, 1.33, 3.56	5, 2, 2.8	2, 2, 0
Siliques/Seed (No., Ave., Var) ⁶	Not available	5, 0.4, 0.24	2, 0, 0
Flowers (No., Ave., Var) ⁶	3, 2, 4	5, 1.6, 3.44	2, 0, 0
Description	Constitutive, weak.	Leaf-stalk and stem tissue.	Vegetative tissue, weak.

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 16

DRE number ¹	17109	20607	24811
Cluster reference ²	R29912	R90407	T22055
Cluster position ²	Downstream	Downstream	Downstream
DRE regulatory direction	Bidirectional	Bidirectional	Bidirectional
DRE length (bp)	2027	2834	428
DRE sequence	SEQ ID NO:196	SEQ ID NO:201	SEQ ID NO:202
Internal forward primer sequence ⁴	SEQ ID NO:197	SEQ ID NO:168	SEQ ID NO:48
External forward primer sequence ⁴	SEQ ID NO:198	SEQ ID NO:170	SEQ ID NO: none
Internal reverse primer sequence ⁴	SEQ ID NO:199	SEQ ID NO:183	SEQ ID NO:50
External reverse primer sequence ⁴	SEQ ID NO:200	SEQ ID NO:185	SEQ ID NO:none
Position Effect Value ⁵	0.46	0.26	0.24
Development Stage Specificity Value ⁵	0	0.60	0.61
Organ Specificity Value ⁵	0.49	1.16	0.51
Number of transgenic plants	5	5	5
Young roots score (No., Ave., Var) ⁶	5, 0.6, 0.64	5, 0, 0	4, 0.75, 1.69
Mature roots score (No., Ave., Var) ⁶	Not available	5, 0, 0	5, 0.6, 1.44
Young above-ground Tissue (No., Ave., Var) ⁶	5, 2, 2	5, 2.2, 2.16	4, 1, 3
Mature above-ground tissue (No., Ave., Var) ⁶	Not available	5, 1.8, 2.16	5, 0.8, 2.56
Siliques/Seed (No., Ave., Var) ⁶	Not available	4, 0, 0	3, 0, 0
Flowers (No., Ave., Var) ⁶	Not available	5, 1.2, 1.36	5, 0.8, 2.56
Description	Above ground tissue, weak	Above ground tissue, mainly in leaves.	Constitutive, weak

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Table 17

DRE number ¹	16665		
Cluster reference ²	Z26101		
Cluster position ²	Upstream		
DRE regulatory direction	Bidirectional		
DRE length (bp)	1358		
DRE sequence	SEQ ID NO:203		
Internal forward primer sequence ⁴	SEQ ID NO:204		
External forward primer sequence ⁴	SEQ ID NO:206		
Internal reverse primer sequence ⁴	SEQ ID NO:206		
External reverse primer sequence ⁴	SEQ ID NO:207		
Position Effect Value ⁵	0.51		
Development Stage Specificity Value ⁵	8.82		
Organ Specificity Value ⁵	0.403		
Number of transgenic plants	12		
Young roots score (No., Ave., Var) ⁶	10, 0, 0		
Mature roots score (No., Ave., Var) ⁶	5, 0.6, 0.64		
Young above-ground Tissue (No., Ave., Var) ⁶	10, 1.5, 3.05		
Mature above-ground tissue (No., Ave., Var) ⁶	5, 2, 2.08		
Siliques/Seed (No., Ave., Var) ⁶	3, 1, 0.66		
Flowers (No., Ave., Var) ⁶	5, 1.8, 3.76		
Description	Above ground tissue		

¹ ID number of the DRE as assigned by the present inventors.

² Internal reference assigned by the present inventors to a cluster of *Arabidopsis* genes (contig) downstream or upstream of the DRE.

³ Unidirectional implies that only the sense strand of the DRE is capable of regulating gene expression ; Bidirectional implies that both the sense and antisense strands of the DRE are capable of regulating gene expression.

⁴ A PCR primer for isolating the DRE from *Arabidopsis* genomic DNA.

⁵ Position Effect Values (PoEf), Development Stage-Specificity Values (SpDs) and Organ Specificity Values (SpOr) were calculated as described in the materials and methods section hereinabove.

⁶ No. = number; Ave. = average; Var. = variance.

Deletion analysis of DREs 4209 and 6669:

The ability of partial DRE sequences to modify *in vivo* gene expression pattern, was tested by comparing reporter gene expression driven by unmodified DREs (SEQ ID NO:36 and 61) with that of deletion mutants thereof (SEQ ID NO:210 and 213, respectively).

GUS expression pattern in p4209short-GUS (including the DRE 4209 partial sequence set forth in SEQ ID NO:210) transformed plants was similar to that driven by the full length promoter sequence, DRE 4209 (SEQ ID NO:36). As is shown in Figure 26a, expression was strong in roots, mainly root tips, as well as in flower buds. Interestingly, p4209short-GUS transformed plants exhibited lower reporter gene expression in veins, while leaves exhibited higher expression. Note, expression in seeds was not examined.

GUS expression pattern in the p6669short-GUS (comprising the DRE 6669 partial sequence set forth in SEQ ID NO:213) transformed plants was restricted to the root tips (Figure 26b) while expression in other young or meristematic tissues, as was obtained by the full length DRE 6669 promoter (SEQ ID NO:61), was lost.

These results demonstrate that the 5' nucleic acid sequence of SEQ ID NO: 61 (e.g., nucleotide coordinates 1-747), is important for constitutive gene expression. Indeed, a DNA sequence (SEQ ID NO: 214, see Figure 27 WO 02/16655) which does not include the 5' first 400 nucleotides of SEQ ID NO: 61 has been implicated in stress regulated gene expression.

These results indicate that the promoters of the present invention may be modified by partial deletions, to generate inductive or tissue specific expression pattern as demonstrated for DRE 6669 (SEQ ID NO:61).

As is clearly illustrated by Tables 3-17 and Figures 1-26, the DREs isolated according to the teachings of the present invention exhibit a wide range of activities as well as a wide range of tissue and developmental stage specificities. The DREs of the present invention were classified according to function as determined using the *Arabidopsis* assay described hereinabove.

The luciferase gene was expressed in a constitutive manner in *Arabidopsis* when functionally linked to SEQ ID NOS: 1, 6, 41, 46, 51, 86, 121, 136, 171, 181 and 202 (illustrated in Figure 18), thus the promoters of these DREs are putatively classified herein as constitutive promoters.

The luciferase gene was expressed in an inductive manner in *Arabidopsis* when functionally linked to SEQ ID NOS: 1, 11, 16, 21, 26, 31, 36, 56, 61, 66, 71, 76, 81, 91, 96, 101, 116, 126, 141, 146, 151, 156, 161, 166, 176 and 203, thus the promoters of these DREs are putatively classified herein as inductive promoters.

5 The luciferase gene was expressed in a young or meristematic, tissue-specific manner in *Arabidopsis* when functionally linked to SEQ ID NOS: 61, 121, 126, 213 (illustrated in Figures 4, 14, 15, 16 and 26b), thus the promoters of these DREs are putatively classified herein as young or meristematic, tissue-specific promoters.

10 The luciferase gene was expressed in root tissue specific manner in *Arabidopsis* when functionally linked to SEQ ID NOS: 21, 36, 91, 111, and 126 (illustrated in Figures 2, 3, 9, and 13), thus the promoters of these DREs are putatively classified herein as root tissue-specific promoters

15 The luciferase gene was expressed in an above ground tissue-specific manner in *Arabidopsis* when functionally linked to SEQ ID NOS: 16, 26, 31, 66, 71, 76, 81, 96, 106, 101, 116, 131, 146, 151, 156, 161, 166, 196, 201 and 203 (illustrate in Figures 10, 11, 17, 19 and 20), thus the promoters of these DREs are putatively classified herein as above ground tissue-specific promoters.

20 The luciferase gene was expressed in a stem tissue specific manner in *Arabidopsis* when functionally linked to SEQ ID NO: 186 (illustrated in Figure 22), thus the promoter(s) of this DRE are putatively classified herein as stem tissue specific promoter(s).

25 The luciferase gene was expressed in a flower tissue specific manner in *Arabidopsis* when functionally linked to SEQ ID NOS: 11, 36, 81, 91, 126, 176 and 210 (illustrated in Figures 1, 3, 9 and 26a), thus the promoters of these DREs are putatively classified herein as flower tissue-specific promoters.

The luciferase gene was expressed in a fruit (silique) tissue specific manner in *Arabidopsis* when functionally linked to SEQ ID NO: 56, thus the promoter(s) of this DRE are putatively classified herein as fruit (silique) tissue specific promoter(s).

30 The luciferase gene was expressed in a seed tissue specific manner in *Arabidopsis* when functionally linked to SEQ ID NOS: 1, 156, and 161 (illustrated in figures 12 and 21), thus the promoters of these DREs are putatively classified herein as seed tissue specific promoters.

The luciferase gene was expressed in a developmental stage specific manner in *Arabidopsis* when functionally linked to SEQ ID NOS: 81, 96, 101, 106, and 131 (illustrated comparatively in Figures 5-6, 7-8, 10-11 and 15-16), thus the promoters of these DREs are putatively classified herein as developmental stage specific promoters.

The GUS gene was expressed in an inductive manner in *Arabidopsis* when functionally linked to SEQ ID NOS: 210 and 213 (illustrated in Figure 26b), thus the promoters of these partial DREs sequences are putatively classified herein as inductive promoters.

The GUS gene was expressed in a root, as well as in a flower bud tissue specific manner in *Arabidopsis* when functionally linked to SEQ ID NO: 210 (illustrated in Figure 26a), thus the promoter of this partial DRE sequence is putatively classified herein as a root as well as a flower tissue-specific promoter.

The GUS gene was expressed in a root-tip tissue specific manner in *Arabidopsis* when functionally linked to SEQ ID NO: 213 (illustrated in Figure 26b), thus this promoter is putatively classified herein as a root tissue-specific promoter.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents, patent applications and sequences identified by their accession numbers mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent, patent application or sequence identified by their accession number was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213 wherein the isolated polynucleotide is capable of regulating expression of at least one polynucleotide sequence operably linked thereto.
2. The isolated polynucleotide of claim 1, wherein said isolated polynucleotide includes at least one promoter region.
3. The isolated polynucleotide of claim 2, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 1, 6, 41, 46, 51, 86, 121, 136, 171, 181 and 202, and whereas said at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in a constitutive manner.
4. The isolated polynucleotide of claim 2, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 1, 11, 16, 21, 26, 31, 36, 56, 61, 66, 71, 76, 81, 91, 96, 101, 116, 126, 141, 146, 151, 156, 161, 166, 176, 186, 191, 196, 201, 203, 210 and 213, and whereas said at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in an inductive manner.
5. The isolated polynucleotide of claim 2, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 1, 11, 16, 21, 26, 31, 36, 56, 61, 66, 71, 76, 91, 116, 126, 141, 146, 151, 156, 161, 166, 176, 186, 191, 196, 201, 203, 210 and 213, and whereas said at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in a tissue specific manner.

6. The isolated polynucleotide of claim 2, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 81, 96, 101, 106 and 131, and whereas said at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in a developmental stage specific manner.

7. A nucleic acid construct, comprising the isolated polynucleotide of claim 1.

8. The nucleic acid construct of claim 7, further comprising at least one heterologous polynucleotide operably linked to the isolated polynucleotide.

9. The nucleic acid construct of claim 8, wherein said at least one heterologous polynucleotide is a reporter gene.

10. The nucleic acid contrast of claim 8, further comprising two heterologous polynucleotides each being operably linked to an end of the isolated polynucleotide such that said two heterologous polynucleotides flank the isolated polynucleotide.

11. The nucleic acid construct of claim 7, wherein said isolated polynucleotide includes at least one promoter region.

12. The nucleic acid construct of claim 11, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 1, 6, 41, 46, 51, 86, 121, 136, 171, 181 and 202, and whereas said at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in a constitutive manner.

13. The nucleic acid construct of claim 11, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 1, 11, 16, 21, 26, 31, 36, 56, 61, 66, 71, 76, 81, 91, 96, 101, 116, 126, 141, 146, 151, 156, 161, 166, 176, 186, 191, 196, 201, 203, 210 and 213 and whereas said at least one promoter region is

capable of directing transcription of said at least one polynucleotide sequence in an inductive manner.

14. The nucleic acid construct of claim 11, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 1, 11, 16, 21, 26, 31, 36, 56, 61, 66, 71, 76, 91, 116, 126, 141, 146, 151, 156, 161, 166, 176, 186, 191, 196, 201, 203, 210 and 213 and whereas said at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in a tissue specific manner.

15. The nucleic acid construct of claim 11, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOS: 81, 96, 101, 106 and 131, and whereas said at least one promoter region is capable of directing transcription of said at least one polynucleotide sequence in a developmental stage specific manner.

16. A transgenic cell comprising the isolated polynucleotide of claim 1.
17. A transgenic cell comprising the nucleic acid construct of claim 7.
18. A transgenic organism comprising the isolated polynucleotide of claim 1.
19. A transgenic organism comprising the nucleic acid construct of claim 7.
20. A transgenic plant comprising the isolated polynucleotide of claim 1.
21. A transgenic plant comprising the nucleic acid construct of claim 7.
22. A method of producing a transgenic plant, comprising transforming a plant with the polynucleotide of claim 1.

23. A method of producing a transgenic plant, comprising transforming a plant with the nucleic acid construct of claim 7.

24. A method of expressing a polypeptide of interest in a cell comprising transforming the cell with a nucleic acid construct including a polynucleotide sequence encoding the polypeptide of interest operably linked to a regulatory nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 11, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213 thereby expressing the polypeptide of interest in the cell.

25. A method of co-expressing two polypeptides of interest in a cell comprising transforming the cell with a nucleic acid construct including two polynucleotide sequences encoding the two polypeptides of interest operably linked to a regulatory nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 26, 31, 36, 41, 46, 56, 61, 66, 71, 76, 81, 86, 91, 96, 101, 106, 11, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 202, 203, 210 and 213 such that said two polynucleotide sequences flank said regulatory nucleic acid sequence, thereby expressing the two polypeptides of interest in the cell.

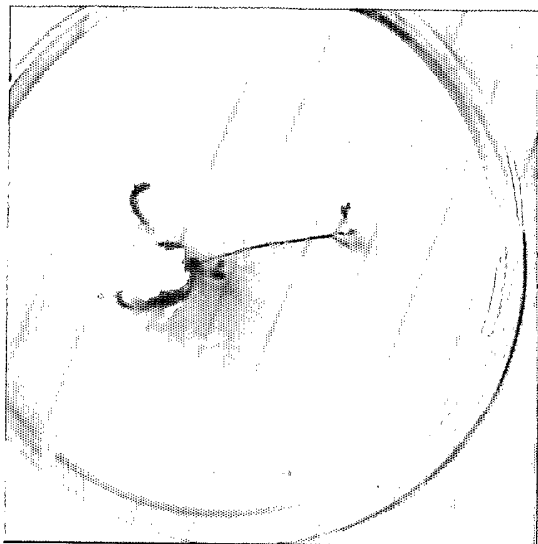


Fig. 1a

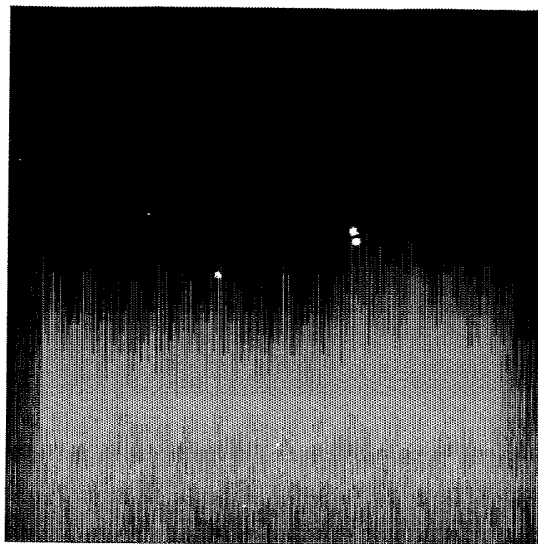


Fig. 1b



Fig. 2a

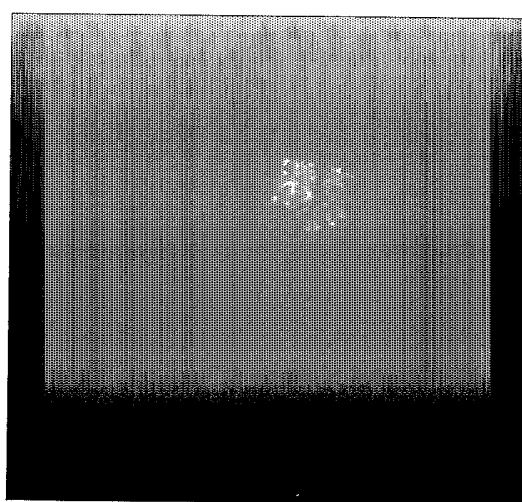


Fig. 2b

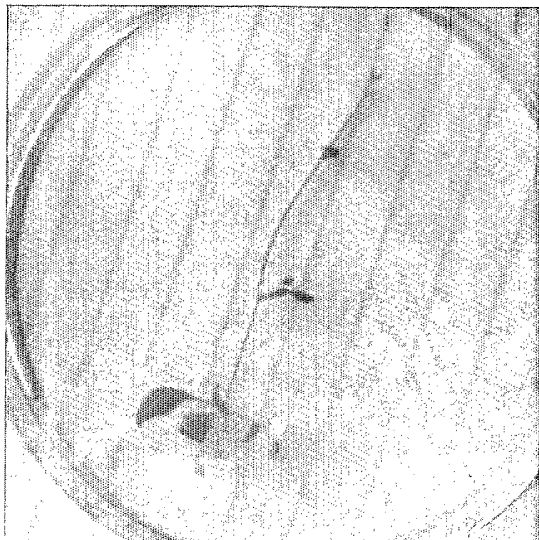


Fig. 3a

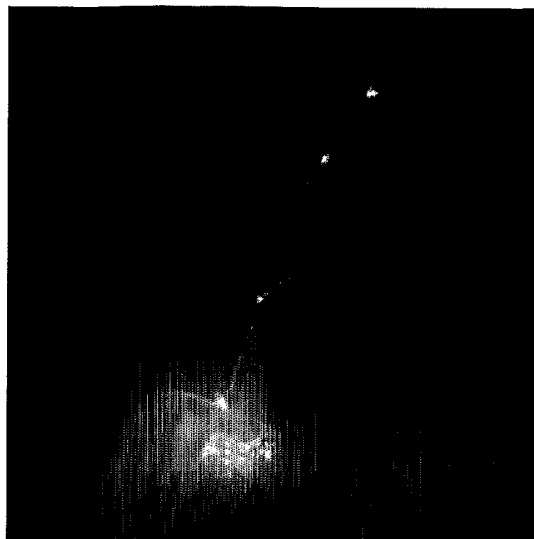


Fig. 3b

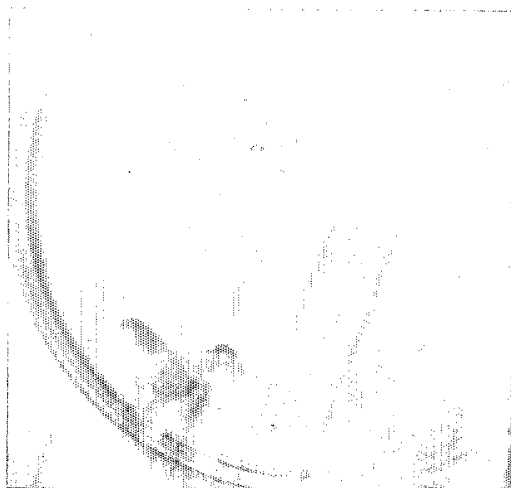


Fig. 4a

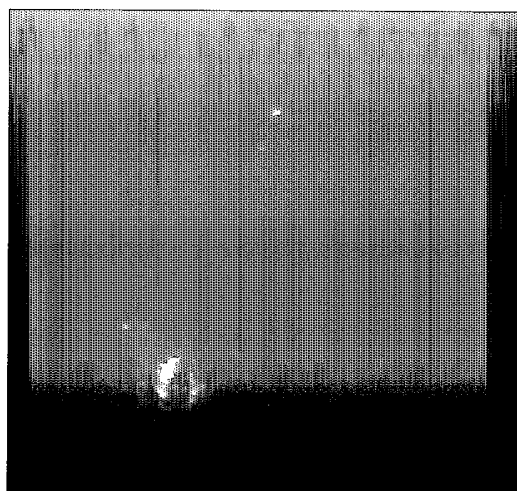


Fig. 4b

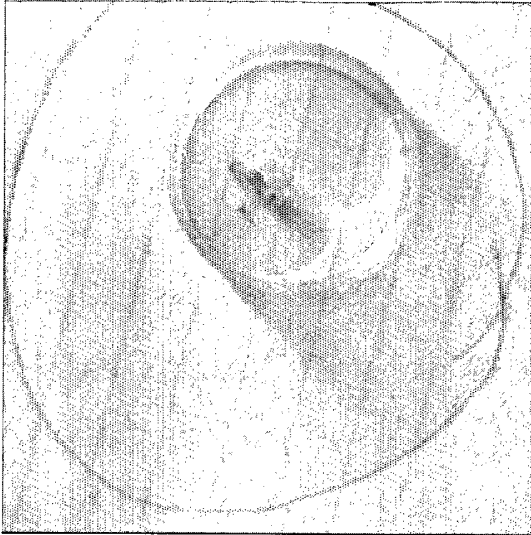


Fig. 5a

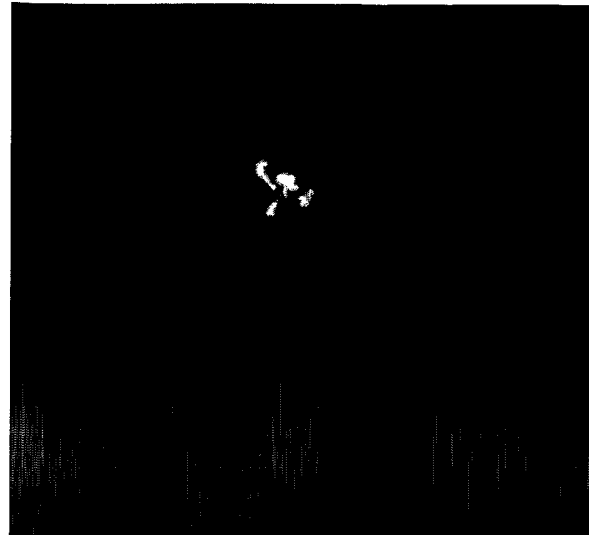


Fig. 5b

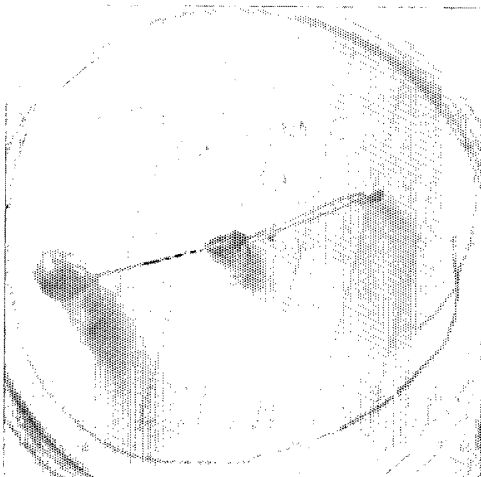


Fig. 6a

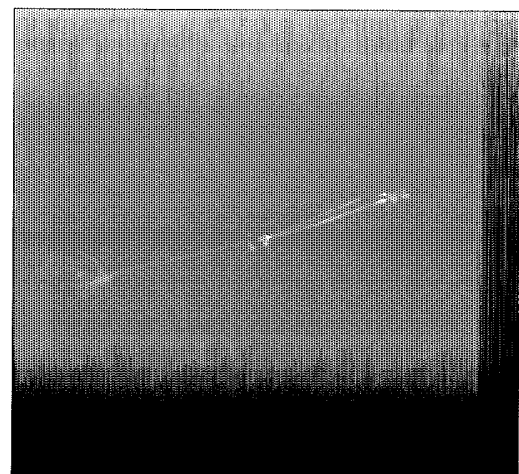


Fig. 6b

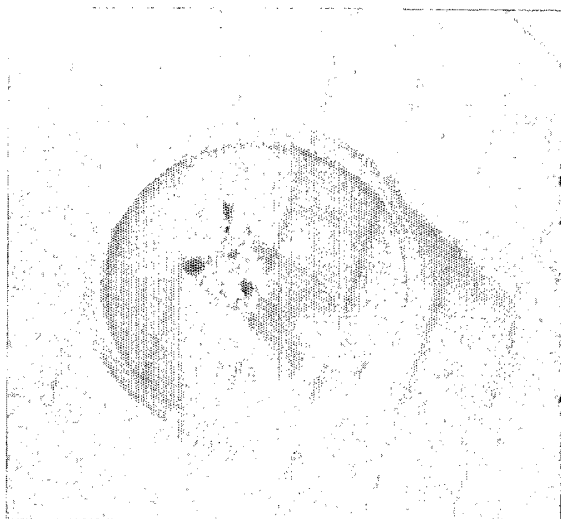


Fig. 7a

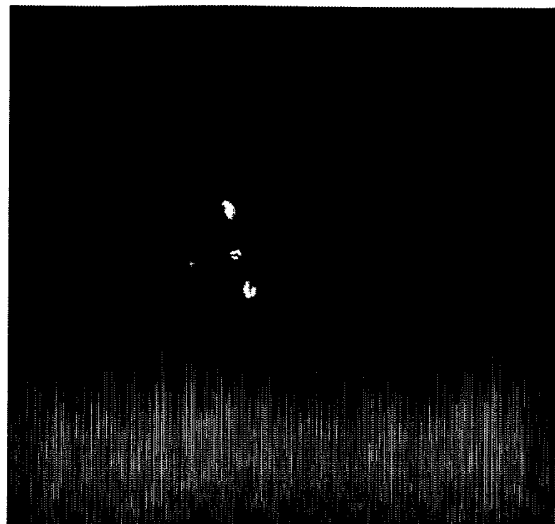


Fig. 7b

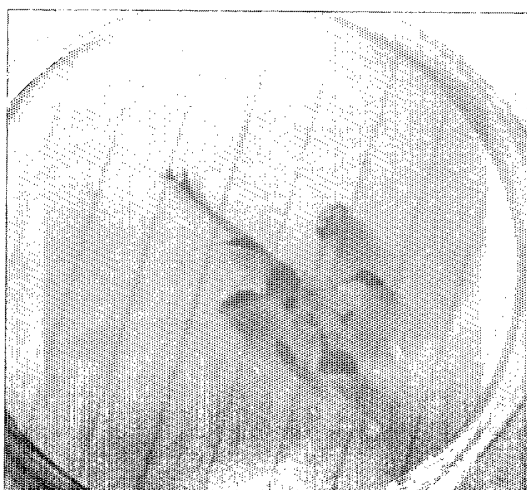


Fig. 8a

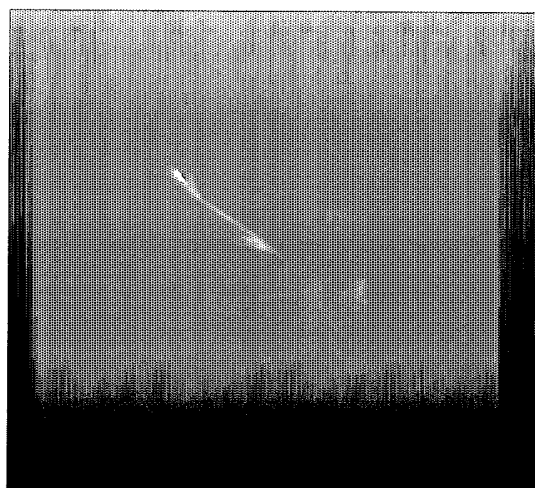


Fig. 8b

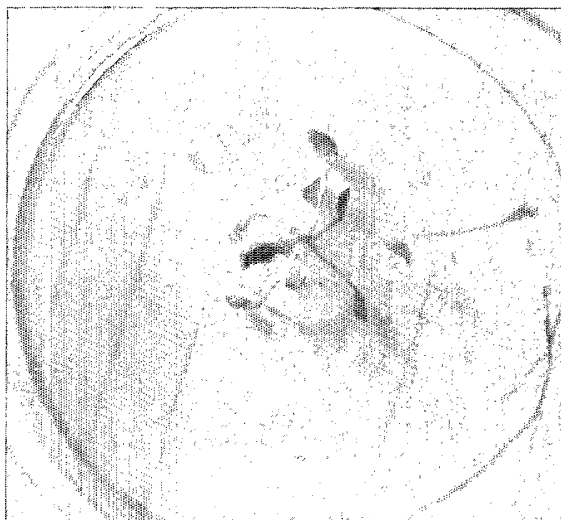


Fig. 9a

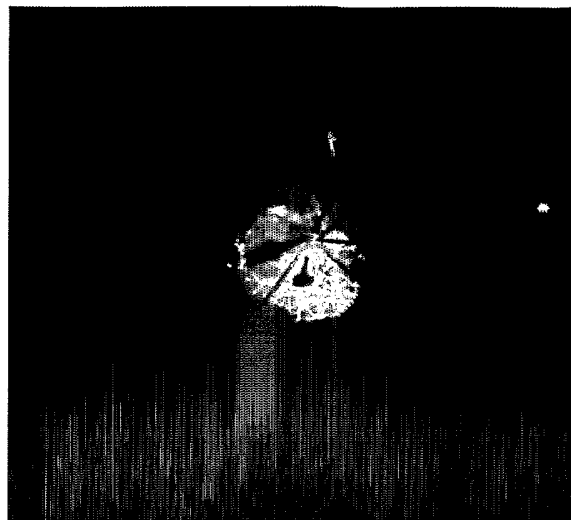


Fig. 9b

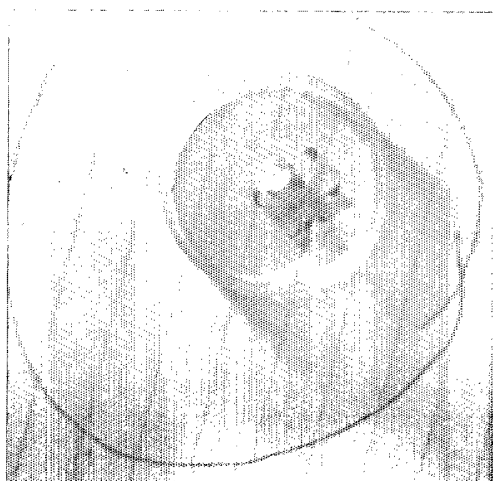


Fig. 10a

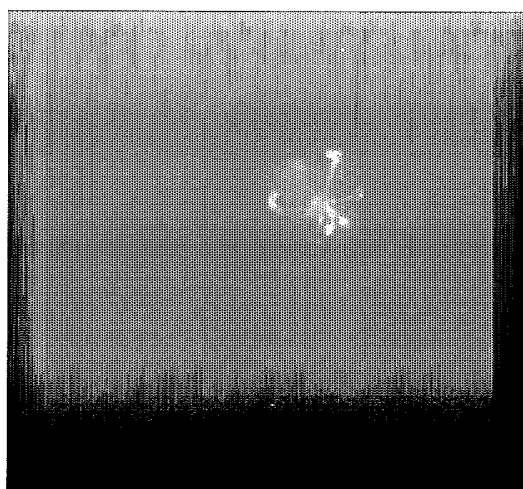


Fig. 10b



Fig. 11a

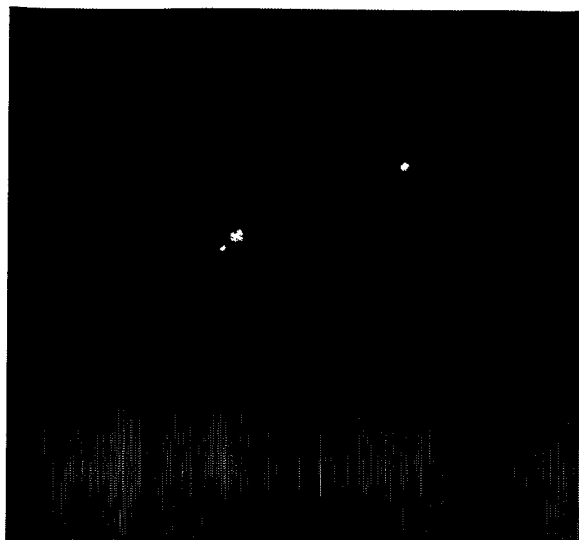


Fig. 11b

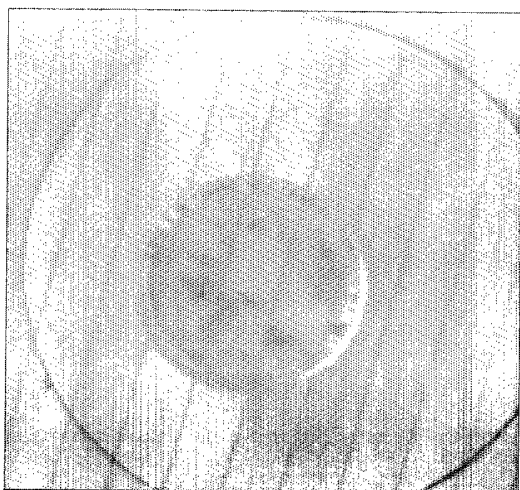


Fig. 12a

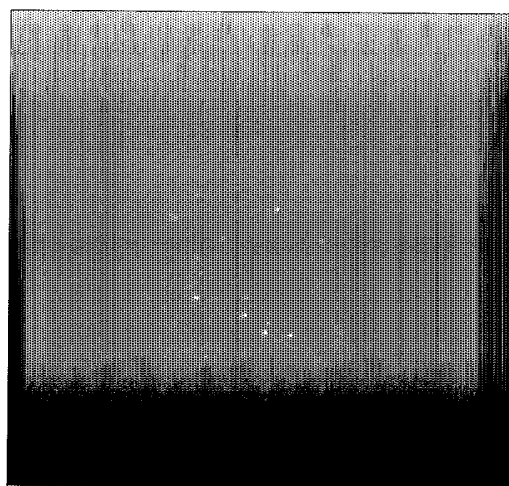


Fig. 12b

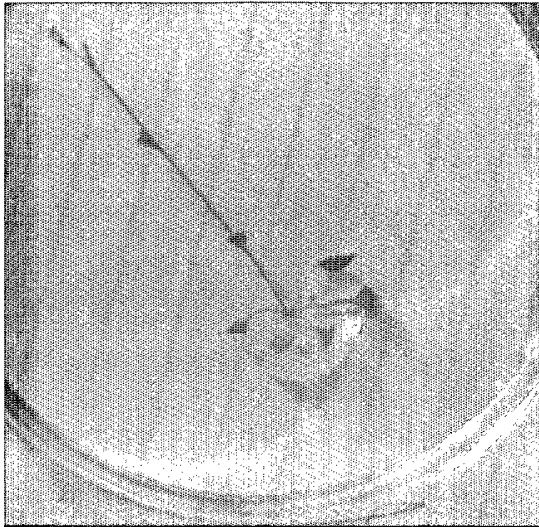


Fig. 13a

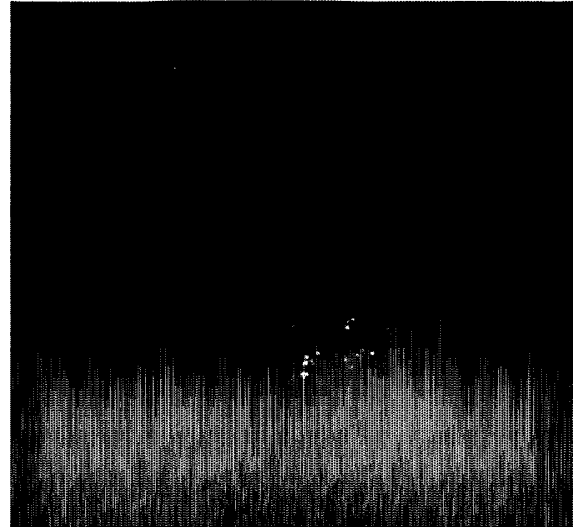


Fig. 13b



Fig. 14a

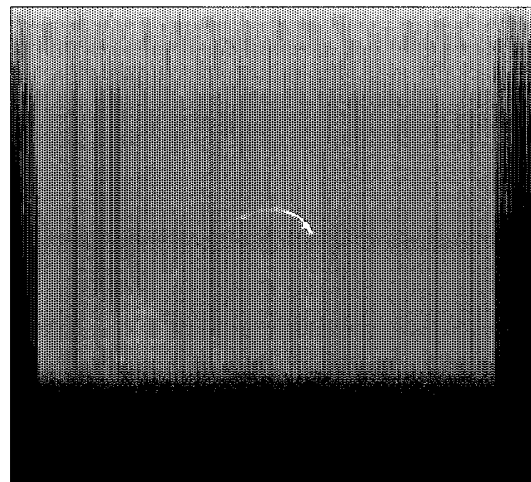


Fig. 14b

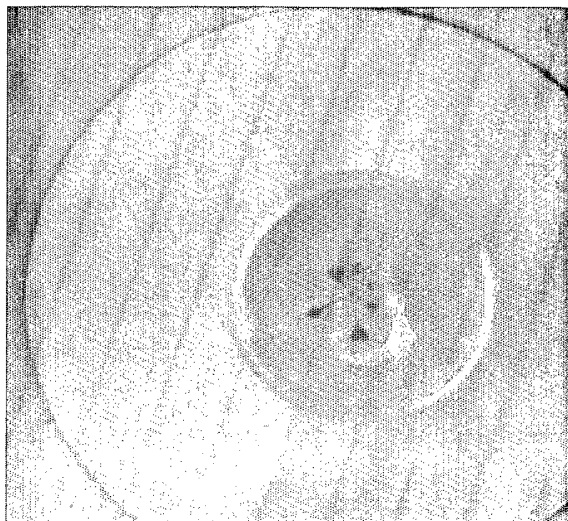


Fig. 15a

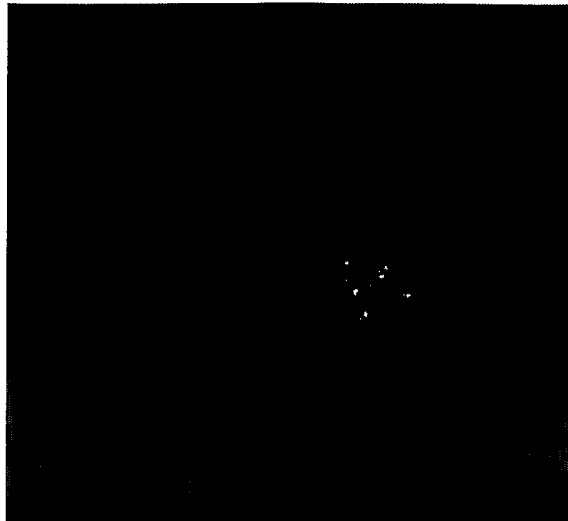


Fig. 15b



Fig. 16a

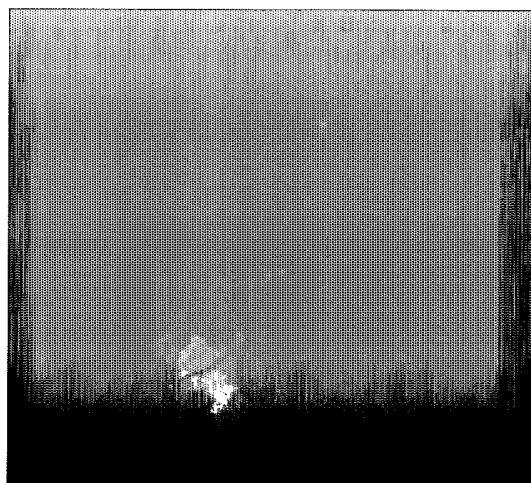


Fig. 16b

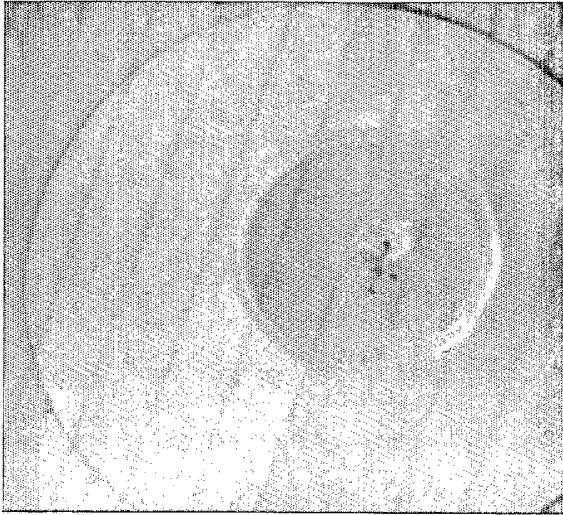


Fig. 17a

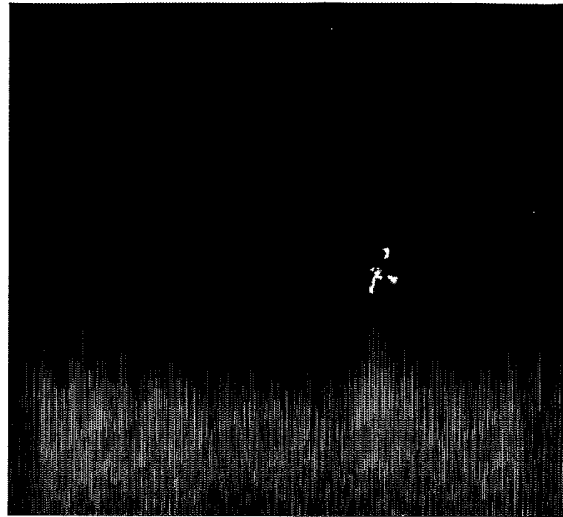


Fig. 17b

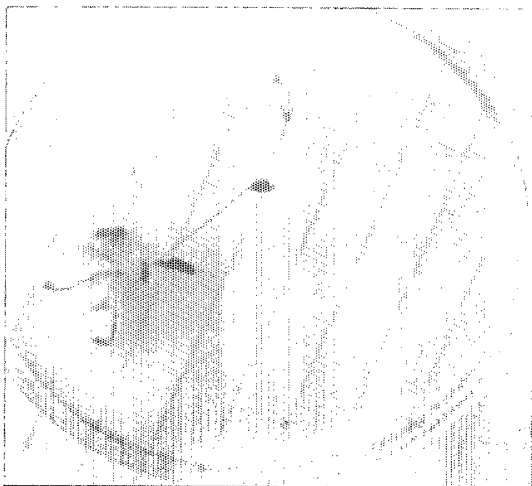


Fig. 18a

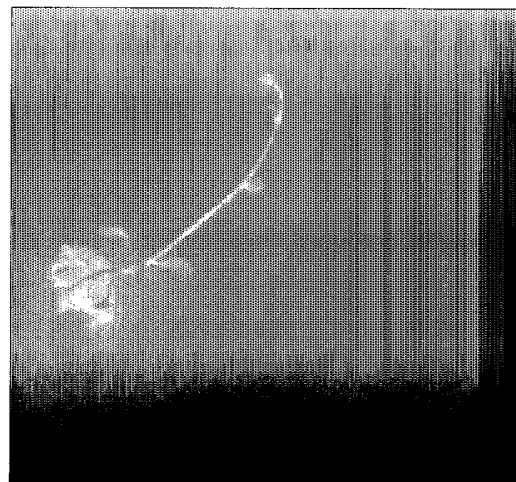


Fig. 18b

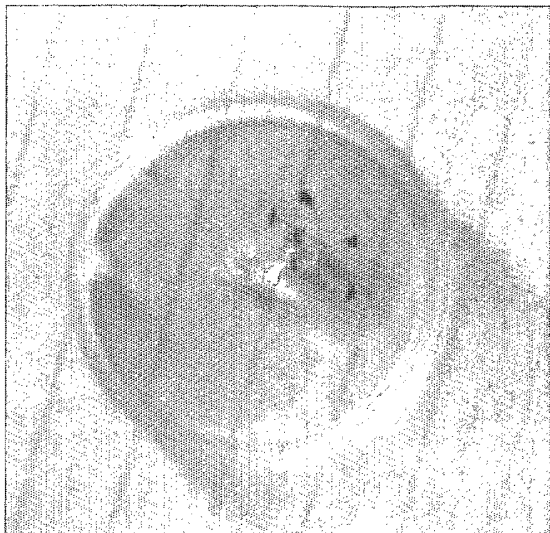


Fig. 19a

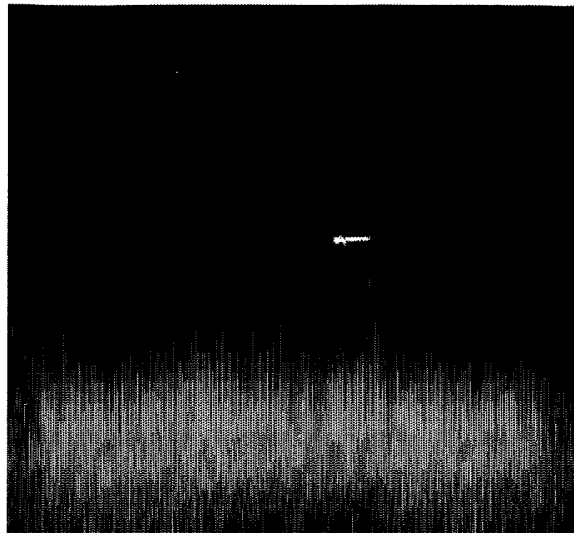


Fig. 19b



Fig. 20a

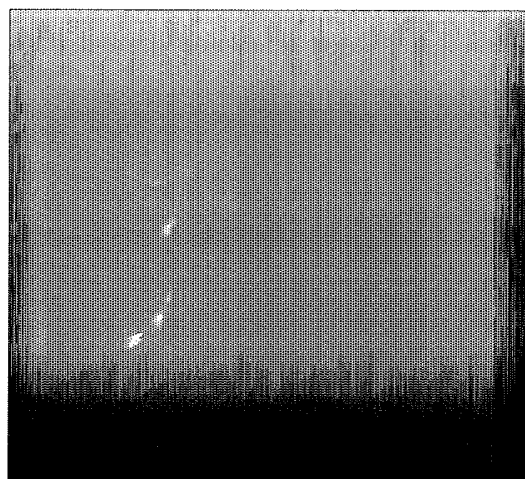


Fig. 20b

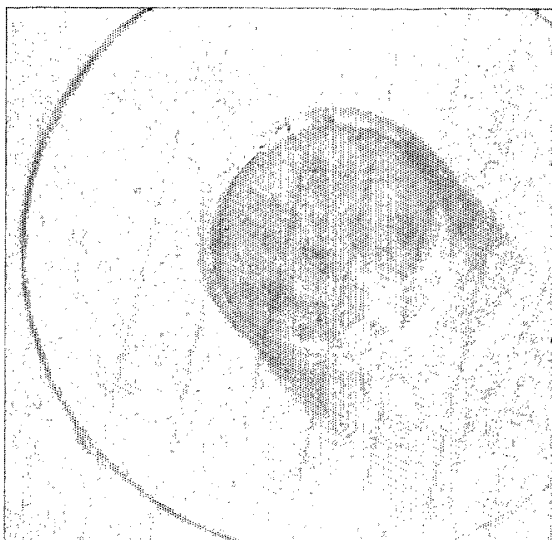


Fig. 21a

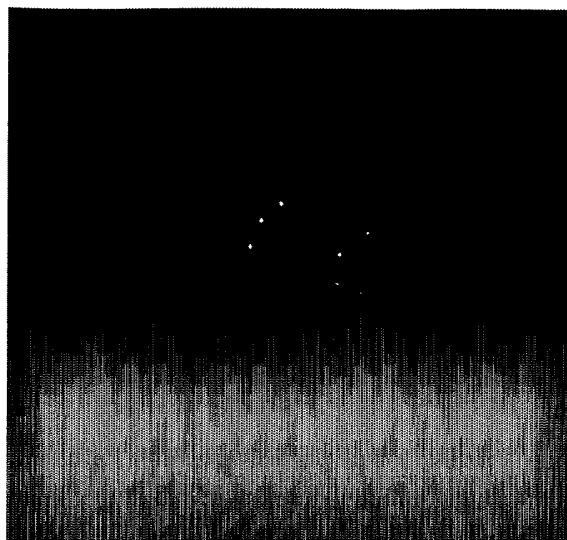


Fig. 21b

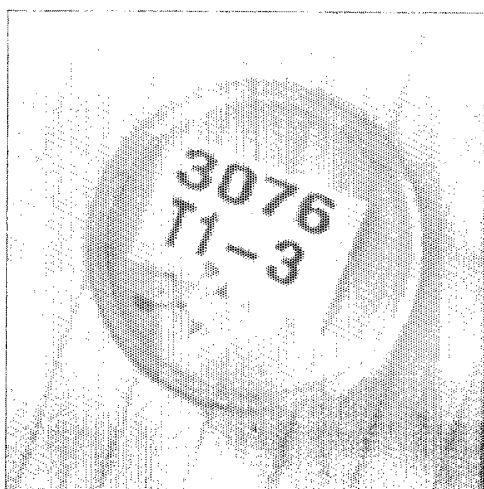


Fig. 22a

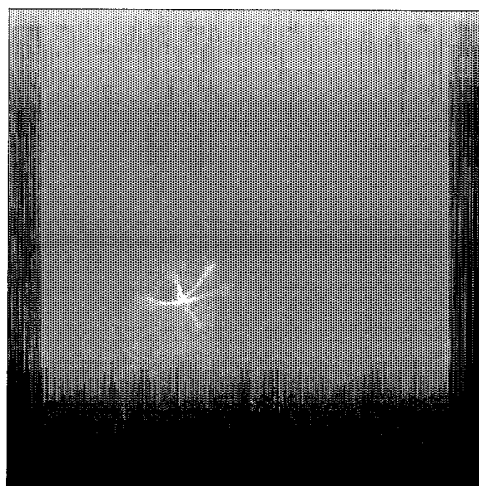


Fig. 22b

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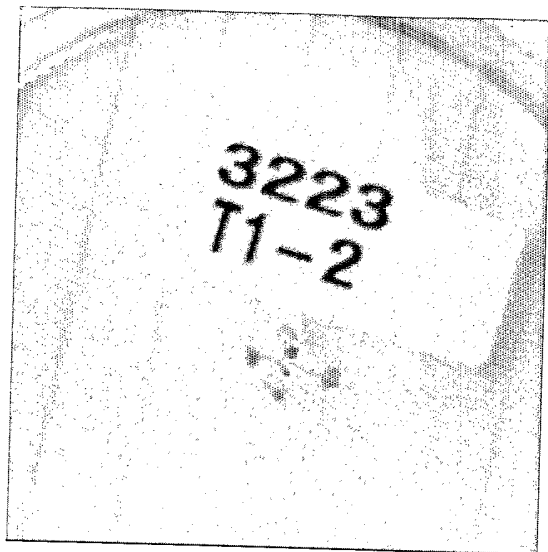


Fig. 23a

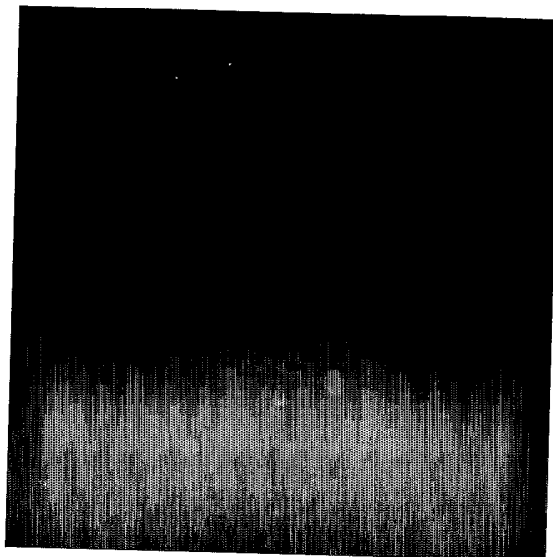


Fig. 23b

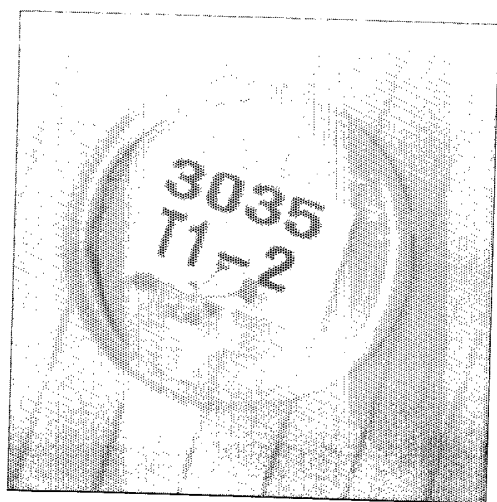


Fig. 24a

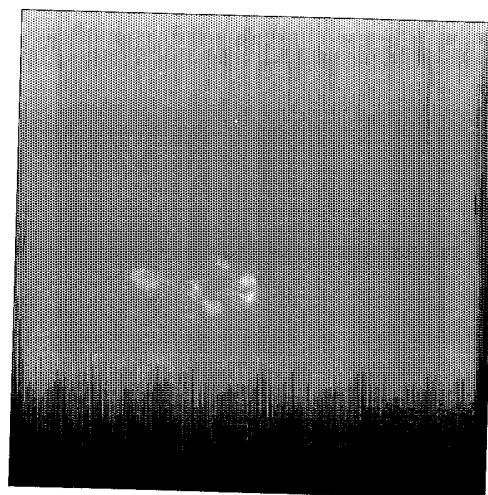


Fig. 24b



Fig. 25a



Fig. 25b

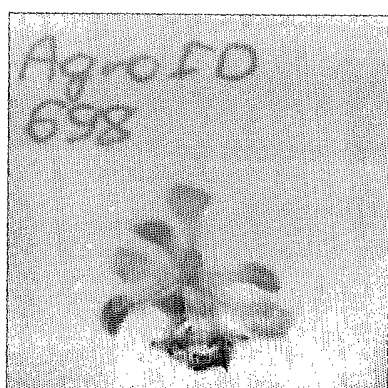


Fig. 26a

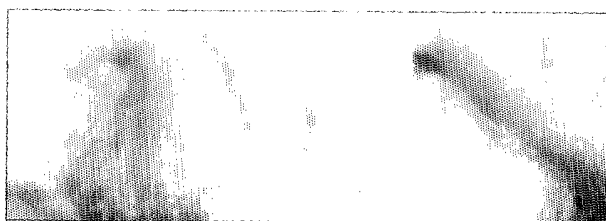


Fig. 26b

14/17

Score = 1388 bits (722), Expect = 0.0
Identities = 722/722 (100%)
Strand = Plus / Plus

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Query: 1630 cactaaaagggttcacaacggacgattgtattggacttccagatcataaacatgcaaaac 1689
|
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Query: 1690 tgaaaaccacaagaataaattagttctaactttagaacgttcgtacgtgtttcatgttcaa 1749
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Sbjct: 1288 tgaaaaccacaagaataaattagttctaactttagaacgttcgtacgtgtttcatgttcaa 1347

Query: 1750 aaagcgtcaattataaaagttgggaaataacttttgagtttgacatttctaaggacagt 1809
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Sbjct: 1348 aaagcgtcaattataaaagttgggaaataacttttgagtttgacatttctaaggacagt 1407

Query: 1810 caaatatgacaacattgggatgcaacttaccttgtattaacttattttgttataaaacca 1869
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Sbjct: 1408 caaatatgacaacattgggatgcaacttaccttgtattaacttattttgttataaaacca 1467

Query: 1870 tatattacatatttttaagggttgataaataatcaaataacaaaacatagcttttcaa 1929
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Query: 2170 gcttaaacacataacagccattacgagtcacaggtaagggtataatagtaaagaatcaat 2229
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Sbjct: 1768 gcttaaacacataacagccattacgagtcacaggtaagggtataatagtaaagaatcaat 1827

Fig. 27

15/17

Query: 2230 ctatataatatacgcaccaccctttctcattctttctggagagtaacatcgagacaaaaga 2289
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 ||
 Sbjct: 1888 ag 1889
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 Strand = Plus / Plus

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 Sbjct: 601 atatgatttcgaaaaaactatgaagattaacgggtatcttaaacatgttaagatacaccg 660

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 Sbjct: 841 agattcaatgaactccgtgtaacacaaacacgcccatagatgagctcatttttggtattt 900

Query: 1303 ccaatattgccactccatgataatatcatctagggatgggggttcatttattttgaaatct 1362
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 Strand = Plus / Plus

Fig. 27 (Cont.)

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Sbjct: 181 gcagatcacctactaccattcaaaaatgaacaaaataatttcatttacattttcctagca 240

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Query: 703 ttaaattttgcttgtctatactctacagctcatttaataacggaaacaaaaataattgca 762
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Sbjct: 301 ttaaattttgcttgtctatactctacagctcatttaataacggaaacaaaaataattgca 360

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Fig. 27 (Cont.)

17/17

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Gapped

Lambda	K	H
1.33	0.621	1.12

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Gap Penalties: Existence: 5, Extension: 2
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Number of Sequences: 0
Number of extensions: 13
Number of successful extensions: 6
Number of sequences better than 10.0: 1
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length of database: 2,385,885,539
effective HSP length: 24
effective length of query: 2292
effective length of database: 2,385,885,515
effective search space: 5468449600380
effective search space used: 5468449600380
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A: 30
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X2: 26 (50.0 bits)
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S2: 20 (39.1 bits)

Fig. 27 (Cont.)

1

SEQUENCE LISTING

<110> Karchi, Hagai
 Meissner, Rafi
 Ronen, Gil
 Golan, Ezekiel
 Livneh, Erez
 Bar-Even, Arren
 Kirschbaum, Natanja
 Rabinovich, Larisa
 Zeligler, Naama
 Savir, Noa
 Weiss, Udi

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<400> 19
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<210> 23
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 <212> DNA
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<220>

<223> Single strand DNA oligonucleotide

<400> 23
agagactaac ctagccgtaa c 21

<210> 24
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<212> DNA
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<220>
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<400> 24
gagagagga tccaagagaa gagc 24

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<220>
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cttcaccttc aactgtgata gc 22

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<210> 28
 <211> 25
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<220>
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<400> 28
 ataccatcgg ttaaacatc tcaag 25

<210> 29
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 <400> 29
 ggtacgttgg atccggggta ttttg 25

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 <400> 30
 tcttcataga agaagagagc cac 23

 <210> 31
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 <210> 33
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 <400> 33
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 <210> 34
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ctcacaggat aagtcgacga gaca 24

<210> 35
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caagagaaag cagatagtga gta 23

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<210> 38
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<213> Artificial sequence

<220>
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<400> 38
gcaatgaaga tgatgatgtg c 21

<210> 39
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<220>
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<400> 39
cttactcggg atccacatct tcac 24

<210> 40
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<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 40
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<210> 41
<211> 1056
<212> DNA
<213> Arabidopsis thaliana

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 cccttatctc tcagtcgacc ctc 23

 <210> 43
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 <210> 44
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 <400> 44
 ttcaaaataa gcttctagac cagac 25

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 <400> 45
 aagagatfff caaagtgtgg 20

 <210> 46
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 <213> Arabidopsis thaliana

 <400> 46
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 tccaactact tttaacaacc actaactctg cgccacgtgt atatctccaa agattctatc 360
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gcaatgattg tcgacaaaag g 21

<210> 48
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<220>
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<400> 48
ccggagaggg atcctacgaa agt 23

<210> 49
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ttggtcaciaa gcttattact tacca 25

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gggaggagga tccagccac 19

<210> 51
<211> 3333
<212> DNA
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<220>
 <223> Single strand DNA oligonucleotide

<400> 52
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<210> 53
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 53
 aaaacagctt catacgagtg g 21

<210> 54
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 54
 cattaagcac ggatccagcc aagc 24

<210> 55
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 55
 tcactattcc ccacagctta g 21

<210> 56
 <211> 1331
 <212> DNA
 <213> Arabidopsis thaliana

<400> 56
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 tataagcgat cgttgactct gcacatgtaa ctctttctctg aataaaaaat ctttaagtct 180
 ttaatttcta catcttttag gattatataa acgttactat ataaataaaa aagaaaaaaa 240

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<212> DNA
<213> Artificial sequence

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<220>
<223> Single strand DNA oligonucleotide

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<400> 57
tctaaaggtc gacttggtag gact 24

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<210> 58
<211> 22
<212> DNA
<213> Artificial sequence

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<220>
<223> Single strand DNA oligonucleotide

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<400> 58
ttaggtcttg atcaaaaagc gt 22

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<210> 59
<211> 23
<212> DNA
<213> Artificial sequence

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<220>
<223> Single strand DNA oligonucleotide

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<400> 59
agaaatgaga gggatccgac gga 23

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<210> 60
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 <212> DNA
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<220>
 <223> Single strand DNA oligonucleotide

<400> 60
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<210> 61
 <211> 2316
 <212> DNA
 <213> Arabidopsis thaliana

<400> 61
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 aacttcattt tttttagttt taatagagag tttatatgac ttttaaaaat tgatttaaaa 180
 ctgtgtcaaa aattaaaaagg acaataaaaa atttgcatac aaccgaaaat acttatattt 240
 agacaagaaa aaataaact tgtgatgctg attttatttt attatataatc atgaatcatg 300
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20

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 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 62
 tagtttggtc agatgggaaa cg 22

<210> 63
 <211> 21
 <212> DNA
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<220>
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<400> 63
 tataaccagtg gagacgaaaag c 21

<210> 64
 <211> 25
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 64
 aaatattgga tcctttgggg ttctc 25

<210> 65
 <211> 21
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 65
 taaactaccc gtcgttctct g 21

<210> 66
 <211> 379
 <212> DNA
 <213> Arabidopsis thaliana

<400> 66
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 taatcgtatc ctcacaaaca aaatcttctt ctgcttcttc tcttgcttgc cagaatccaa 300
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 tagagaagaa gaagaagaa 379

<210> 67
 <211> 23
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 67
 gtcgggaatt attaagctta ctt 23

<210> 68
 <211> 20
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 68
 actgacgtac tccttagcac 20

<210> 69
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 69
 ggccattttg cgtcgacttc ttct 24

<210> 70
 <211> 22
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Single strand DNA oligonucleotide

<400> 70
 gaattatgga ctcttattgg ct 22

<210> 71
 <211> 979
 <212> DNA
 <213> Arabidopsis thaliana

<400> 71
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22

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<213> Artificial sequence

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<220>
<223> Single strand DNA oligonucleotide

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<400> 72
acactgcttt ccaactcaatc ac 22

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<210> 73
<211> 22
<212> DNA
<213> Artificial sequence

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<220>
<223> Single strand DNA oligonucleotide

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<400> 73
taggaaattc aacgaaacga gc 22

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<210> 74
<211> 23
<212> DNA
<213> Artificial sequence

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<220>
<223> Single strand DNA oligonucleotide

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<400> 74
gatctcacgg atccgaagag aga 23

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<210> 75
<211> 22
<212> DNA
<213> Artificial sequence

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<220>
<223> Single strand DNA oligonucleotide

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<400> 75
tgaagaatca gagaagaacc gg 22

<210> 76
<211> 665
<212> DNA
<213> Arabidopsis thaliana

<400> 76
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ctttccaaaa tgttataaatt agcttcattc tttaaacgat ttcttgtgta aatctttggt 180
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gatcc 665

<210> 77
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 77
gacaagtaca agctttactg gaac 24

<210> 78
<211> 21
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<213> Artificial sequence

<220>
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<400> 78
acttcttgtt gattcaccac c 21

<210> 79
<211> 23
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<220>
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<400> 79
ccaagaacag gatccgagat ggc 23

<210> 80
<211> 21
<212> DNA
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<220>
 <223> Single strand DNA oligonucleotide

 <400> 80
 gtggcttatg tccgtcaata g 21

 <210> 81
 <211> 2834
 <212> DNA
 <213> Arabidopsis thaliana

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1840

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<400> 115
 ataggctctt caatgtttcc tc 22

<210> 116
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<210> 118
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 tcgcagaagt tgttgtaagt g 21

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 <220>
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 gtgaatggag gatccacaag cga 23

 <210> 120
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 <212> DNA
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 <220>
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 ttacatactg agggaagctc g 21

 <210> 121
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 <213> Arabidopsis thaliana

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<210> 124
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<220>
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 gatcaaccag gatcctaatac ccca 24

<210> 125
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<220>
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<210> 126
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 <212> DNA
 <213> Arabidopsis thaliana

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<210> 128
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<220>
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<400> 128
tctcaaactg aacctatgaa ga 22

<210> 129
<211> 25
<212> DNA
<213> Artificial sequence

<220>
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 <400> 129
 cagagagaag aggatccgga gagaa 25

 <210> 130
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 <220>
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 <400> 130
 caacagagaa tgacaaagaa ga 22

 <210> 131
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 <213> Arabidopsis thaliana

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<400> 132

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21

<210> 133

<211> 22

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gtattattac accatcagct cc 22

<210> 134
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<210> 136
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<210> 139
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<210> 141
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 <213> Arabidopsis thaliana

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<210> 146
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<210> 151
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 <213> Arabidopsis thaliana

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 <210> 153
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 <220>
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 <210> 154
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 <220>
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 gtacgatgac ggatccactt gcg 23

 <210> 155
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<220>
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<400> 157
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<210> 158
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<220>
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<400> 158
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<210> 159
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<210> 161

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 <220>
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 <400> 164
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<211> 23
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52

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<220>
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<212> DNA
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<210> 186
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<400> 188
 gcaatgaaga tgatgatgtg c 21

<210> 189
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<400> 189
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<210> 191
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<210> 193
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<400> 193
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<210> 194
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<400> 194
 ttcaaaataa gcttctagac caga 24

<210> 195
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<220>
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<210> 196
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<213> Arabidopsis thaliana

<400> 196

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<220>
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 <213> Arabidopsis thaliana

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