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(54) Title: METHODS AND COMPOSITIONS FOR PRODUCING AND SELECTING TRANSGENIC PLANTS

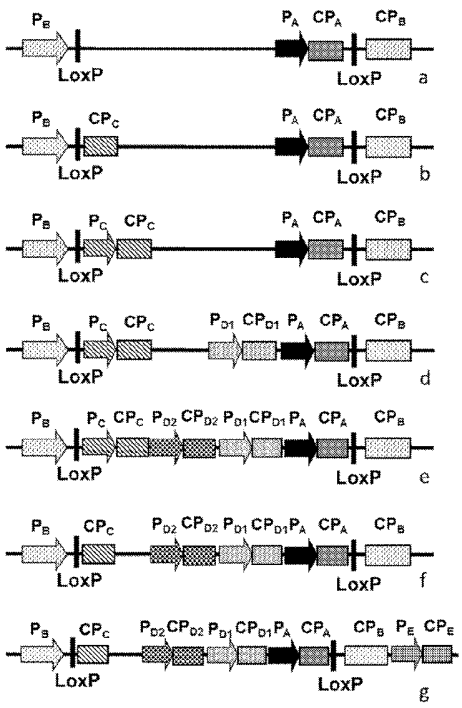

FIG. 9

(57) Abstract: Compositions and methods are provided for the production and selection of transgenic plants and plant parts, for increasing the transformation frequency of a plant or plant part, and for regulating the expression of a transgene, 5 such as a herbicide tolerance polynucleotide. The methods and compositions allow for the delay in the expression of herbicide tolerance polynucleotides until a point in development during which herbicide selection is more efficient. Compositions comprise polynucleotide constructs comprising an excision cassette that separates a transgene, such as a herbicide tolerance 10 polynucleotide, from its promoter and host cells comprising the same. The excision cassette comprises a polynucleotide encoding a site-specific recombinase operably linked to an inducible promoter and expression of the recombinase leads to excision of the excision cassette and expression of the transgene.

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METHODS AND COMPOSITIONS FOR PRODUCING AND SELECTING TRANSGENIC PLANTS

REFERENCE TO A SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing, created on March 12, 2013 and having a size of 308 kilobytes and is filed concurrently with the specification. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the genetic modification of plants. More particularly, the compositions and methods are directed to the production and selection of transgenic plants.

BACKGROUND OF THE INVENTION

Current genetic engineering technology allows for the production of transgenic plants with desired traits. In some instances, it is desirable to delay expression of a transgene until a certain developmental stage is reached or environmental condition is encountered. Such transgenes can confer a desired trait or can serve as a selectable marker to aid in the identification of transgenic plants that have been successfully engineered with a polynucleotide of interest.

For example, herbicide tolerance polynucleotides, which encode polypeptides that confer tolerance to specific herbicides, can be introduced into a plant to generate a herbicide tolerant plant and/or to serve as a selectable marker for the introduction of another polynucleotide of interest. Direct selection with herbicides, such as glyphosate and sulfonylureas, during early stages of transgenic plant production (i.e., tissue proliferation) has been relatively inefficient when transforming maize and sugarcane

(Experimental Example 1 and unpublished data). Larger clusters of maize cells may be less sensitive to herbicides such as glyphosate and some nontransgenic calli may still grow in the presence of the herbicide (Wang *et al.* (2009) Handbook of Maize: Genetics and Genomics, J.L. Bennetzen and S. Hake, eds., pp. 609-639). As observed in wheat, however, selection at the stage of regeneration was more effective and escapes were rarely regenerated (Zhou *et al.* (1995) *Plant Cell Rep* 15:159-163; Hu *et al.* (2003) *Plant Cell Rep* 21:1010-1019).

Thus, methods and compositions are needed that allow for the delayed expression of transgenes to reduce the potential for negative effects on transformed tissues, particularly during development. Such methods and compositions would be especially useful for delaying the expression of herbicide tolerance polynucleotides until a stage at which herbicide selection is more efficient.

BRIEF SUMMARY OF THE INVENTION

Compositions and methods are provided for the production and selection of transgenic plants and plant parts, for increasing the transformation frequency of a plant or plant part, and for regulating the expression of a transgene, such as a herbicide tolerance polynucleotide. The methods and compositions allow for the delay of the expression of a transgene (e.g., herbicide tolerance polynucleotide) by the presence and subsequent excision of an excision cassette that separates the transgene (e.g., herbicide tolerance polynucleotide) from a promoter that drives its expression. Excision of the excision cassette is mediated by a site-specific recombinase, the expression of which is regulated by an inducible promoter, which results in the operable linkage of the transgene (e.g., herbicide tolerance polynucleotide) and its promoter and subsequent expression of the transgene (e.g., herbicide tolerance polynucleotide). These methods and compositions are useful for delaying the expression of transgenes that might otherwise negatively affect the development or growth of a transformed tissue or plant.

The herbicide tolerance polynucleotide can serve as a means for imparting herbicide tolerance to a plant or plant part and/or can function as a selectable marker, aiding in the identification of a transgenic plant or plant part comprising another

polynucleotide of interest or lacking a polynucleotide of interest that has been excised from the excision cassette. In some of these embodiments, the excision of the excision cassette and expression of the herbicide tolerance polynucleotide is delayed until after the tissue proliferation stage of transgenic plant production to allow for more efficient herbicide selection.

In some embodiments, the inducible promoter regulating the expression of the recombinase, excision of the excision cassette, and expression of the herbicide tolerance polynucleotide is one that is induced by stress (e.g., cold temperatures, desiccation) or by a chemical (e.g., antibiotic, herbicide).

Compositions include polynucleotide constructs comprising a promoter that is active in a plant, a herbicide tolerance polynucleotide, and an excision cassette, wherein the excision cassette comprises an inducible promoter operably linked to a site-specific recombinase-encoding polynucleotide, and wherein excision of the excision cassette allows for the operable linkage of the promoter and the herbicide tolerance polynucleotide. Host cells, such as plant cells, and plants and plant parts comprising the polynucleotide constructs are further provided.

The following embodiments are encompassed by the present invention.

1. A polynucleotide construct comprising:

a) an excision cassette comprising an expression cassette A (EC_A) comprising:

i) a promoter A (P_A), wherein said P_A is an inducible promoter; and

ii) a coding polynucleotide A (CP_A) encoding a site-specific recombinase;

wherein said P_A is operably linked to said CP_A; and

wherein said excision cassette is flanked by a first and a second recombination site, wherein said first and said second recombination sites are recombinogenic with respect to one another and are directly repeated, and wherein said site-specific recombinase can recognize and implement recombination at said first and said second recombination sites; thereby excising said excision cassette;

b) a coding polynucleotide B (CP_B) encoding a herbicide tolerance polypeptide; and

c) a promoter B (P_B), wherein said P_B is operably linked to said CP_B after excision of said excision cassette;

wherein said P_A and P_B are active in a plant cell.

2. The polynucleotide construct of embodiment 1, wherein said inducible promoter is selected from the group consisting of a stress-inducible promoter and a chemical-inducible promoter.

3. The polynucleotide construct of embodiment 2, wherein said chemical-inducible promoter comprises a promoter comprising a tet operator.

4. The polynucleotide construct of embodiment 3, wherein said polynucleotide construct further comprises a coding polynucleotide F (CP_F) encoding a sulfonylurea-responsive transcriptional repressor protein, wherein said CP_F is operably linked to a promoter active in a plant cell.

5. The polynucleotide construct of embodiment 2, wherein said stress-inducible promoter can be induced in response to cold, drought, high salinity, desiccation, or a combination thereof.

6. The polynucleotide construct of embodiment 2 or 5, wherein said stress-inducible promoter is a maize *rab17* promoter or an active variant or fragment thereof.

7. The polynucleotide construct of any one of embodiments 2, 5 and 6, wherein said stress-inducible promoter has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence having the sequence set forth in SEQ ID NO: 18;

b) a nucleotide sequence having at least 70% sequence identity to the sequence set forth in SEQ ID NO: 18;

c) a nucleotide sequence comprising at least 50 contiguous nucleotides of the sequence set forth in SEQ ID NO: 18;

d) the nucleotide sequence set forth in nucleotides 291-430 of SEQ ID NO: 18; and

e) a nucleotide sequence having at least 70% sequence identity to the sequence set forth in nucleotides 291-430 of SEQ ID NO: 18.

8. The polynucleotide construct of embodiment 6 or 7, wherein said EC_A further comprises an attachment B (attB) site between said stress-inducible promoter and said CP_A.

9. The polynucleotide construct of embodiment 8, wherein said attB site has a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence having at least 70% sequence identity to the sequence set forth in SEQ ID NO: 20; and

b) the nucleotide sequence set forth in SEQ ID NO: 20.

10. The polynucleotide construct of any one of embodiments 1-9, wherein said site-specific recombinase is selected from the group consisting of FLP, Cre, S-CRE, V-CRE, Dre, SSV1, lambda Int, phi C31 Int, HK022, R, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, and U153.

11. The polynucleotide construct of any one of embodiments 1-10, wherein said CP_A has the nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 33 or 35;

b) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 33 or 35;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 34 or 36; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 34 or 36.

12. The polynucleotide construct of any one of embodiments 1-11, wherein P_B is a constitutive promoter.

13. The polynucleotide construct of embodiment 12, wherein said P_B is selected from the group consisting of a ubiquitin promoter, an oleosin promoter, an actin promoter, and a *Mirabilis* mosaic virus (MMV) promoter.

14. The polynucleotide construct of any one of embodiments 1-13, wherein said excision cassette further comprises a coding polynucleotide C (CP_C) encoding a selectable marker, wherein said CP_C is operably linked to a promoter active in a plant cell.

15. The polynucleotide construct of embodiment 14, wherein said CP_C is operably linked to P_B before excision of the excision cassette.

16. The polynucleotide construct of embodiment 14, wherein said excision cassette further comprises a promoter C (P_C), wherein P_C is operably linked to said CP_C.

17. The polynucleotide construct of embodiment 16, wherein said P_C is a constitutive promoter.

18. The polynucleotide construct of embodiment 17, wherein said P_C is selected from the group consisting of an ubiquitin promoter, an oleosin promoter, an actin promoter, and a *Mirabilis* mosaic virus (MMV) promoter.

19. The polynucleotide construct of any one of embodiments 14-18, wherein said selectable marker is selected from the group consisting of a fluorescent protein, an

antibiotic resistance polypeptide, a herbicide tolerance polypeptide, and a metabolic enzyme.

20. The polynucleotide construct of embodiment 19, wherein said fluorescent protein is selected from the group consisting of a yellow fluorescent protein, a red fluorescent protein, a cyan fluorescent protein, and a green fluorescent protein.

21. The polynucleotide construct of embodiment 19, wherein said fluorescent protein comprises a *Discosoma* red fluorescent protein.

22. The polynucleotide construct of embodiment 19, wherein said antibiotic resistance polypeptide comprises a neomycin phosphotransferase II.

23. The polynucleotide construct of embodiment 19, wherein said herbicide tolerance polypeptide encoded by CP_C comprises a phosphinothricin acetyl transferase.

24. The polynucleotide construct of embodiment 19, wherein said metabolic enzyme comprises a phosphomannose isomerase.

25. The polynucleotide construct of any one of embodiments 14-24, wherein said excision cassette comprises more than one polynucleotide encoding a distinct selectable marker, wherein said polynucleotide encoding a selectable marker is operably linked to a promoter active in a plant cell.

26. The polynucleotide construct of embodiment 25, wherein said excision cassette comprises at least a first and a second polynucleotide encoding a selectable marker, wherein said first polynucleotide encodes a yellow fluorescent protein, and wherein said second polynucleotide encodes a phosphinothricin acetyl transferase or a neomycin phosphotransferase II.

27. The polynucleotide construct of any one of embodiments 1-26, wherein said herbicide tolerance polypeptide encoded by CP_B confers tolerance to a herbicide selected from the group consisting of glyphosate, an ALS inhibitor, an acetyl Co-A carboxylase inhibitor, a synthetic auxin, a protoporphyrinogen oxidase (PPO) inhibitor herbicide, a pigment synthesis inhibitor herbicide, a phosphinothricin acetyltransferase, a phytoene desaturase inhibitor, a glutamine synthase inhibitor, a hydroxyphenylpyruvatedioxygenase inhibitor, and a protoporphyrinogen oxidase inhibitor.

28. The polynucleotide construct of embodiment 27, wherein said ALS inhibitor is selected from the group consisting of a sulfonylurea, a triazolopyrimidine, a pyrimidinyloxy(thio)benzoate, an imidazolinone, and a sulfonylaminocarbonyltriazolinone.

29. The polynucleotide construct of any one of embodiments 1-28, wherein said herbicide tolerance polypeptide encoded by CP_B comprises a glyphosate-N-acetyltransferase (GLYAT) polypeptide or an ALS inhibitor-tolerance polypeptide.

30. The polynucleotide construct of embodiment 29, wherein said polynucleotide encoding said GLYAT polypeptide has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 47 or 49;

b) a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: 47 or 49;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 48 or 50; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 or 50.

31. The polynucleotide construct of embodiment 29, wherein said ALS inhibitor-tolerance polypeptide comprises the highly resistant ALS (HRA) mutation of acetolactate synthase.

32. The polynucleotide constructs of any one of embodiments 1-31, wherein said polynucleotide construct comprises more than one polynucleotide encoding a distinct herbicide tolerance polypeptide, wherein the polynucleotide encoding a herbicide tolerance polypeptide is operably linked to a promoter active in a plant cell.

33. The polynucleotide construct of embodiment 32, wherein said polynucleotide construct comprises at least a first and a second polynucleotide encoding a herbicide tolerance polypeptide, wherein said first polynucleotide encodes an ALS inhibitor-tolerance polypeptide and wherein said second polynucleotide encodes a GLYAT polypeptide.

34. The polynucleotide construct of any one of embodiments 1-33, wherein said excision cassette further comprises a coding polynucleotide D (CP_D) encoding a cell proliferation factor, wherein said CP_D is operably linked to a promoter active in a plant cell.

35. The polynucleotide construct of embodiment 34, wherein said cell proliferation factor is selected from the group consisting of a Lec1 polypeptide, a Kn1 polypeptide, a WUSCHEL polypeptide, a Zwille polypeptide, a babyboom polypeptide, an Aintegumenta polypeptide (ANT), a FUS3 polypeptide, a Kn1polypeptide, a STM polypeptide, an OSH1 polypeptide, and a SbH1 polypeptide.

36. The polynucleotide construct of embodiment 35, wherein said cell proliferation factor is selected from the group consisting of a WUSCHEL polypeptide and a babyboom polypeptide.

37. The polynucleotide construct of any one of embodiments 34-36, wherein said babyboom polypeptide comprises at least two AP2 domains and at least one of the following amino acid sequences:

a) the amino acid sequence set forth in SEQ ID NO: 67 or an amino acid sequence that differs from the amino acid sequence set forth in SEQ ID NO: 67 by one amino acid; and

b) the amino acid sequence set forth in SEQ ID NO: 68 or an amino acid sequence that differs from the amino acid sequence set forth in SEQ ID NO: 68 by one amino acid.

38. The polynucleotide construct of any one of embodiments 34-36, wherein said CP_D has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 55, 57, 58, 60, 74, 76, 78, 80, 82, 84, 86, 87, 88, 90, 92, 94, 96, 98, 99, or 101;

b) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 55, 57, 58, 60, 74, 76, 78, 80, 82, 84, 86, 87, 88, 90, 92, 94, 96, 98, 99, or 101;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 56, 59, 75, 77, 79, 81, 83, 85, 89, 91, 93, 95, 97, 100, or 102; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 70% sequence identity to the amino acid sequence set forth in SEQ ID NO: 56, 59, 75, 77, 79, 81, 83, 85, 89, 91, 93, 95, 97, 100, or 102.

39. The polynucleotide construct of any one of embodiments 34-38, wherein said excision cassette further comprises a promoter D (P_D) operably linked to said CP_D.

40. The polynucleotide construct of embodiment 39, wherein said P_D is a constitutive promoter.

41. The polynucleotide construct of embodiment 40, wherein said P_D is a ubiquitin promoter or an oleosin promoter.

42. The polynucleotide construct of any one of embodiments 36-41, wherein said excision cassette comprises more than one coding polynucleotide D (CP_D) encoding a distinct cell proliferation factor, wherein the CP_D is operably linked to a promoter active in a plant cell.

43. The polynucleotide construct of embodiment 42, wherein said excision cassette comprises at least a first coding polynucleotide D (CP_{D1}) encoding a babyboom polypeptide and a second coding polynucleotide D (CP_{D2}) encoding a WUSCHEL polypeptide.

44. The polynucleotide construct of any one of embodiments 35, 36, 42, and 43, wherein said polynucleotide encoding a WUSCHEL polypeptide has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 103, 105, 107, or 109; and

b) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 103, 105, 107, or 109;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 104, 106, 108, or 110; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 104, 106, 108, or 110.

45. The polynucleotide construct of any one of embodiments 35, 36, 42, 43, and 44, wherein said polynucleotide encoding a WUSCHEL polypeptide is operably linked to a maize In2-2 promoter or a nopaline synthase promoter.

46. The polynucleotide construct of any one of embodiments 1-45, wherein said polynucleotide construct further comprises a coding polynucleotide E (CP_E) encoding a polypeptide of interest, wherein said CP_E is operably linked to a promoter active in a plant cell.

47. The polynucleotide construct of embodiment 46, wherein said excision cassette comprises said CP_E.

48. The polynucleotide construct of embodiment 46, wherein said CP_E is outside of the excision cassette.

49. The polynucleotide construct of any one of embodiments 46-48, wherein said polynucleotide construct further comprises a promoter E (P_E) operably linked to said CP_E.

50. The polynucleotide construct of embodiment 1, wherein said polynucleotide construct comprises:

a) a first ubiquitin promoter;

b) an excision cassette flanked by loxP recombination sites that are are recombinogenic with respect to one another and are directly repeated, wherein said excision cassette comprises:

i) a polynucleotide encoding a phosphinothricin acetyl transferase (PAT) or a neomycin phosphotransferase II (NPTII);

ii) a second ubiquitin promoter;

iii) a polynucleotide encoding a yellow fluorescent protein;

iv) a promoter comprising a maize *rab17* promoter and an attachment B (attB) site;

v) a polynucleotide encoding a CRE recombinase;

vi) a nopaline synthase promoter;

vii) a polynucleotide encoding a maize Wuschel 2 polypeptide;

viii) a third ubiquitin promoter; and

ix) a babyboom polynucleotide; and

c) a GLYAT polynucleotide;

wherein said first ubiquitin promoter is operably linked to said polynucleotide encoding said PAT or NPTII and wherein said first ubiquitin promoter is operably linked to said GLYAT polynucleotide upon excision of said excision cassette;

wherein said second ubiquitin promoter is operably linked to said polynucleotide encoding said yellow fluorescent protein;

wherein said promoter comprising said maize *rab17* promoter and said attB site is operably linked to said polynucleotide encoding said CRE recombinase;

wherein said nopaline synthase promoter is operably linked to said polynucleotide encoding said maize Wuschel 2 polypeptide;

and wherein said third ubiquitin promoter is operably linked to said babyboom polynucleotide.

51. The polynucleotide construct of embodiment 1, wherein said polynucleotide construct comprises:

a) a ubiquitin promoter;

b) an excision cassette flanked by loxP recombination sites that are are recombinogenic with respect to one another and are directly repeated, wherein said excision cassette comprises:

i) a polynucleotide encoding a *Discosoma* red fluorescent protein;

ii) a promoter comprising a maize *rab17* promoter and an attachment B (attB) site; and

iii) a polynucleotide encoding a CRE recombinase; and

c) a GLYAT polynucleotide;

wherein said ubiquitin promoter is operably linked to said polynucleotide encoding said *Discosoma* red fluorescent protein and wherein said ubiquitin promoter is

operably linked to said GLYAT polynucleotide upon excision of said excision cassette; and

wherein said promoter comprising said maize *rab17* promoter and said attB site is operably linked to said polynucleotide encoding said CRE recombinase.

52. The polynucleotide construct of embodiment 1, wherein said polynucleotide construct comprises:

a) a ubiquitin promoter;

b) an excision cassette flanked by loxP recombination sites that are are recombinogenic with respect to one another and are directly repeated, wherein said excision cassette comprises:

i) an actin promoter;

ii) a polynucleotide encoding a *Discosoma* red fluorescent protein;

iii) a promoter comprising a maize *rab17* promoter and an attachment B (attB) site; and

iv) a polynucleotide encoding a CRE recombinase; and

c) a GLYAT polynucleotide;

wherein said ubiquitin promoter is operably linked to said GLYAT polynucleotide upon excision of said excision cassette;

wherein said actin promoter is operably linked to said polynucleotide encoding said *Discosoma* red fluorescent protein; and

wherein said promoter comprising said maize *rab17* promoter and said attB site is operably linked to said polynucleotide encoding said CRE recombinase.

53. A host cell comprising the polynucleotide construct of any one of embodiments 1-52.

54. A plant cell comprising the polynucleotide construct of any one of embodiments 1-52.

55. A plant or plant part comprising said plant cell of embodiment 54.

56. The plant or plant part of embodiment 55, wherein said plant or plant part is a dicot.

57. The plant or plant part of embodiment 55, wherein said plant or plant part is a monocot.

58. The plant or plant part of embodiment 57, wherein said monocot is selected from the group consisting of maize, rice, sorghum, barley, wheat, millet, oat, rye, triticale, sugarcane, switchgrass, and turf/forage grass.

59. The plant or plant part of any one of embodiments 55-58, wherein said plant or plant part is recalcitrant.

60. The plant or plant part of embodiment 59, wherein said plant or plant part is a sugarcane cultivar selected from the group consisting of CP96-1252, CP01-1372, CPCL97-2730, HoCP85-845, CP89-2143, and KQ228.

61. The plant or plant part of any one of embodiments 55-60, wherein said plant part is a seed.

62. A method for producing a transgenic plant or plant part, said method comprising introducing said polynucleotide construct of any one of embodiments 1-52 into a plant or plant part.

63. A method for regulating the expression of a herbicide tolerance polynucleotide, wherein said method comprises:

a) providing the host cell of embodiment 53, the plant cell of embodiment 54, or the plant or plant part of any one of embodiments 55-61; and,

b) inducing the expression of said site-specific recombinase, thereby excising said excision cassette from said polynucleotide construct and expressing said herbicide tolerance polynucleotide.

64. A method for selecting a herbicide tolerant plant cell, said method comprising the steps of:

A) providing a population of plant cells, wherein at least one plant cell in the population comprises a polynucleotide construct comprising:

a) an excision cassette comprising an expression cassette A (EC_A) comprising:

i) a promoter A (P_A), wherein said P_A is an inducible promoter; and

ii) a coding polynucleotide A (CP_A) encoding a site-specific recombinase;

wherein said P_A is operably linked to said CP_A;

b) a coding polynucleotide B (CP_B) encoding a herbicide tolerance polypeptide; and

c) a promoter B (P_B), wherein said P_B is operably linked to said CP_B after excision of said excision cassette;

wherein said P_A and P_B are active in a plant cell; and

wherein said excision cassette is flanked by a first and a second recombination site, wherein said first and said second recombination sites are recombinogenic with respect to one another and are directly repeated, and wherein said site-specific recombinase can recognize and implement recombination at said first and said second recombination sites; thereby excising said excision cassette;

B) inducing the expression of said site-specific recombinase; and

C) contacting said population of plant cells with a herbicide to which said herbicide tolerance polypeptide confers tolerance, thereby selecting for a plant cell having tolerance to said herbicide.

65. The method of embodiment 64, wherein said provided population of plant cells is cultured into a population of plant tissues or plants prior to, during, or after said step B), and wherein said step C) comprises contacting said population of plant tissues or plants with said herbicide.

66. The method of embodiment 65, wherein said step C) occurs during or after regeneration of said provided population of plant cells into a population of plants.

67. The method of embodiment 64, wherein said provided population of plant cells is a population of immature or mature seeds, wherein at least one immature or mature seed within said population of immature or mature seeds comprises said polynucleotide construct.

68. The method of embodiment 67, wherein said provided population of seeds is planted prior to, during, or after said step B) to produce a population of plants, and wherein said step C) comprises contacting said population of plants with said herbicide.

69. The method of embodiment 75, wherein said provided population of plant cells is a population of plant tissues, wherein at least one plant tissue within said population of plant tissues comprises said polynucleotide construct.

70. The method of embodiment 69, wherein said provided population of plant tissues is cultured into a population of plants prior to, during, or after said step B), and wherein said step C) comprises contacting said population of plants with said herbicide.

71. The method of embodiment 64, wherein said provided population of plant cells is a population of plants, wherein at least one plant within said population of plants comprises said polynucleotide construct.

72. The method of any one of embodiments 64-71, wherein said method further comprises introducing said polynucleotide construct into said at least one plant cell before step A).

73. The method of any one of embodiments 64-72, wherein said inducible promoter P_A is selected from the group consisting of a stress-inducible promoter and a chemical-inducible promoter.

74. The method of embodiment 73, wherein said chemical-inducible promoter comprises a promoter comprising a tet operator.

75. The method of embodiment 74, wherein said polynucleotide construct or said at least one plant cell further comprises a coding polynucleotide F (CP_F) encoding a sulfonylurea-responsive transcriptional repressor protein, wherein said CP_F is operably linked to a promoter active in a plant cell, and wherein said inducing comprises contacting said population of plant cells with a sulfonylurea compound.

76. The method of embodiment 73, wherein said stress-inducible promoter is induced in response to cold, drought, desiccation, high salinity, or a combination thereof.

77. The method of embodiment 73 or 76, wherein said stress-inducible promoter comprises a drought-inducible promoter, and wherein said inducing comprises desiccating said population of plant cells.

78. The method of embodiment 77, wherein said desiccating occurs during the maturation of an immature seed.

79. The method of embodiment 73, wherein said stress-inducible promoter is a maize *rab17* promoter or an active variant or fragment thereof.

80. The method of embodiment 73, wherein said stress-inducible promoter has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence having the sequence set forth in SEQ ID NO: 18;

b) a nucleotide sequence having at least 70% sequence identity to the sequence set forth in SEQ ID NO: 18;

c) a nucleotide sequence comprising at least 50 contiguous nucleotides of the sequence set forth in SEQ ID NO: 18;

d) the nucleotide sequence set forth in nucleotides 291-430 of SEQ ID NO: 18; and

e) a nucleotide sequence having at least 70% sequence identity to the sequence set forth in nucleotides 291-430 of SEQ ID NO: 18.

81. The method of embodiment 79 or 80, wherein said EC_A further comprises an attachment B (attB) site between said stress-inducible promoter and said CP_A.

82. The method of embodiment 81, wherein said attB site has a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence having at least 70% sequence identity to the sequence set forth in SEQ ID NO: 20; and

b) the nucleotide sequence set forth in SEQ ID NO: 20.

83. The method of any one of embodiments 64-82, wherein said site-specific recombinase is selected from the group consisting of FLP, Cre, S-CRE, V-CRE, Dre, SSV1, lambda Int, phi C31 Int, HK022, R, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, and U153.

84. The method of any one of embodiments 64-83, wherein said CP_A has the nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 33 or 35;

b) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 33 or 35;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 34 or 36; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 34 or 36.

85. The method of any one of embodiments 64-84, wherein P_B is a constitutive promoter.

86. The method of embodiment 85, wherein said P_B is selected from the group consisting of a ubiquitin promoter, an oleosin promoter, an actin promoter, and a *Mirabilis* mosaic virus promoter.

87. The method of any one of embodiments 64-86, wherein said excision cassette further comprises a coding polynucleotide C (CP_C), wherein said CP_C encodes a selectable marker, wherein said CP_C is operably linked to a promoter active in a plant cell, and wherein said method further comprises a selection step prior to step B), wherein those plant cells within said population of plant cells that comprise said selectable marker are identified and wherein these selected plant cells comprise the population of plant cells that are induced in step B).

88. The method of embodiment 87, wherein said CP_C is operably linked to P_B.

89. The method of embodiment 87, wherein said excision cassette further comprises a promoter C (P_C), wherein P_C is operably linked to said CP_C.

90. The method of embodiment 89, wherein P_C is a constitutive promoter.

91. The method of embodiment 90, wherein said P_C is selected from the group consisting of a ubiquitin promoter, an oleosin promoter, an actin promoter, and a *Mirabilis* mosaic virus promoter.

92. The method of any one of embodiments 87-91, wherein said selectable marker is selected from the group consisting of a fluorescent protein, an antibiotic resistance polypeptide, a herbicide tolerance polypeptide, and a metabolic enzyme.

93. The method of embodiment 92, wherein said fluorescent protein is selected from the group consisting of a yellow fluorescent protein, a red fluorescent protein, a cyan fluorescent protein, and a green fluorescent protein.

94. The method of embodiment 92, wherein said fluorescent protein comprises a *Discosoma* red fluorescent protein.

95. The method of embodiment 92, wherein said antibiotic resistance polypeptide comprises a neomycin phosphotransferase II.

96. The method of embodiment 92, wherein said herbicide tolerance polypeptide encoded by CP_C comprises a phosphinothricin acetyl transferase.

97. The method of embodiment 92, wherein said metabolic enzyme comprises a phosphomannose isomerase.

98. The method of any one of embodiments 87-97, wherein said excision cassette comprises more than one polynucleotide encoding a distinct selectable marker, wherein said polynucleotide encoding a selectable marker is operably linked to a promoter active in a plant cell.

99. The method of embodiment 98, wherein said excision cassette comprises at least a first and a second polynucleotide encoding a selectable marker, wherein said first polynucleotide encodes a yellow fluorescent protein, and wherein said second polynucleotide encodes a phosphinothricin acetyl transferase or a neomycin phosphotransferase II.

100. The method of any one of embodiments 64-99, wherein said herbicide tolerance polypeptide encoded by CP_B confers tolerance to a herbicide selected from the group consisting of glyphosate, an ALS inhibitor, an acetyl Co-A carboxylase inhibitor, a synthetic auxin, a protoporphyrinogen oxidase (PPO) inhibitor herbicide, a pigment synthesis inhibitor herbicide, a phosphinothricin acetyltransferase, a phytoene desaturase inhibitor, a glutamine synthase inhibitor, a hydroxyphenylpyruvatedioxygenase inhibitor, and a protoporphyrinogen oxidase inhibitor.

101. The method of embodiment 100, wherein said ALS inhibitor is selected from the group consisting of a sulfonylurea, a triazolopyrimidine, a pyrimidinyloxy(thio)benzoate, an imidazolinone, and a sulfonylaminocarbonyltriazolinone.

102. The method of any one of embodiments 64-101, wherein said herbicide tolerance polypeptide encoded by CP_B comprises a glyphosate-N-acetyltransferase (GLYAT) polypeptide or an ALS inhibitor-tolerance polypeptide.

103. The method of embodiment 102, wherein said polynucleotide encoding said GLYAT polypeptide has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 47 or 49;

b) a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: 47 or 49;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 48 or 50; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 or 50.

104. The method of embodiment 102, wherein said ALS inhibitor-tolerance polypeptide comprises the highly resistant ALS (HRA) mutation of acetolactate synthase.

105. The method of any one of embodiments 64-104, wherein said polynucleotide construct comprises more than one polynucleotide encoding a distinct herbicide tolerance polypeptide, wherein said polynucleotide encoding a herbicide tolerance polypeptide is operably linked to a promoter active in a plant cell.

106. The method of embodiment 105, wherein said polynucleotide construct comprises at least a first and a second polynucleotide encoding a herbicide tolerance polypeptide, wherein said first polynucleotide encodes an ALS inhibitor-tolerance polypeptide, and wherein said second polynucleotide encodes a GLYAT polypeptide.

107. The method of any one of embodiments 64-106, wherein said excision cassette further comprises a coding polynucleotide D (CP_D), wherein said CP_D encodes a cell proliferation factor, and wherein said CP_D is operably linked to a promoter active in a plant cell.

108. The method of embodiment 107, wherein said cell proliferation factor is selected from the group consisting of a Lec1 polypeptide, a Kn1 polypeptide, a WUSCHEL polypeptide, a Zwille polypeptide, a babyboom polypeptide, an Aintegumenta polypeptide (ANT), a FUS3 polypeptide, a Kn1polypeptide, a STM polypeptide, an OSH1 polypeptide, and a SbH1 polypeptide.

109. The method of embodiment 108, wherein said cell proliferation factor is selected from the group consisting of a WUSCHEL polypeptide and a babyboom polypeptide.

110. The method of any one of embodiments 107-109, wherein said babyboom polypeptide comprises at least two AP2 domains and at least one of the following amino acid sequences:

a) the amino acid sequence set forth in SEQ ID NO: 67 or an amino acid sequence that differs from the amino acid sequence set forth in SEQ ID NO: 67 by one amino acid; and

b) the amino acid sequence set forth in SEQ ID NO: 68 or an amino acid sequence that differs from the amino acid sequence set forth in SEQ ID NO: 68 by one amino acid.

111. The method of any one of embodiments 107-109, wherein said CP_D has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 55, 57, 58, 60, 74, 76, 78, 80, 82, 84, 86, 87, 88, 90, 92, 94, 96, 98, 99, or 101;

b) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 55, 57, 58, 60, 74, 76, 78, 80, 82, 84, 86, 87, 88, 90, 92, 94, 96, 98, 99, or 101;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 56, 59, 75, 77, 79, 81, 83, 85, 89, 91, 93, 95, 97, 100, or 102; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 70% sequence identity to the amino acid sequence set forth in SEQ ID NO: 56, 59, 75, 77, 79, 81, 83, 85, 89, 91, 93, 95, 97, 100, or 102.

112. The method of any one of embodiments 107-111, wherein said excision cassette further comprises a promoter D (P_D), wherein said P_D is operably linked to said CP_D.

113. The method of embodiment 112, wherein said P_D is a constitutive promoter.

114. The method of embodiment 112 or 113, wherein said P_D is an ubiquitin promoter or an oleosin promoter.

115. The method of any one of embodiments 107-114, wherein said excision cassette comprises more than one polynucleotide encoding a distinct cell proliferation factor, wherein the polynucleotide encoding a cell proliferation factor is operably linked to a promoter active in a plant cell.

116. The method of embodiment 115, wherein said excision cassette comprises at least a first coding polynucleotide D (CP_{D1}) encoding a babyboom polypeptide and a second coding polynucleotide D (CP_{D2}) encoding a WUSCHEL polypeptide.

117. The method of any one of embodiments 108, 109, and 116, wherein said polynucleotide encoding a WUSCHEL polypeptide has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 103, 105, 107, or 109; and

b) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 103, 105, 107, or 109;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 104, 106, 108, or 110; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 104, 106, 108, or 110.

118. The method of any one of embodiments 108, 109, 116, and 117, wherein said polynucleotide encoding a WUSCHEL polypeptide is operably linked to a maize In2-2 promoter or a nopaline synthase promoter.

119. The method of any one of embodiments 64-118, wherein said polynucleotide construct further comprises a coding polynucleotide E (CP_E) encoding a polypeptide of interest, whrein the CP_E is operably linked to a promoter active in a plant cell.

120. The method of embodiment 119, wherein said excision cassette comprises said CP_E, and wherein said selected herbicide tolerant plant cell lacks said CP_E.

121. The method of embodiment 119, wherein said CP_E is outside of the excision cassette, and wherein said selected herbicide tolerant plant cell comprises said CP_E.

122. The method of any one of embodiments 119-121, wherein said polynucleotide construct further comprises a promoter E (P_E) operably linked to said CP_E.

123. The method of embodiment 64, wherein said polynucleotide construct comprises:

a) a first ubiquitin promoter;

b) an excision cassette flanked by loxP recombination sites that are are recombinogenic with respect to one another and are directly repeated, wherein said excision cassette comprises:

i) a polynucleotide encoding a phosphinothricin acetyl transferase (PAT) or a neomycin phosphotransferase II (NPTII);

ii) a second ubiquitin promoter;

iii) a polynucleotide encoding a yellow fluorescent protein;

iv) a promoter comprising a maize *rab17* promoter and an attachment B (attB) site;

v) a polynucleotide encoding a CRE recombinase;

vi) a nopaline synthase promoter;

vii) a polynucleotide encoding a maize Wuschel 2 polypeptide;

viii) a third ubiquitin promoter; and

ix) a babyboom polynucleotide; and

c) a GLYAT polynucleotide;

wherein said first ubiquitin promoter is operably linked to said polynucleotide encoding said PAT or NPTII and wherein said first ubiquitin promoter is operably linked to said GLYAT polynucleotide upon excision of said excision cassette;

wherein said second ubiquitin promoter is operably linked to said polynucleotide encoding said yellow fluorescent protein;

wherein said promoter comprising said maize *rab17* promoter and said attB site is operably linked to said polynucleotide encoding said CRE recombinase;

wherein said nopaline synthase promoter is operably linked to said polynucleotide encoding said maize Wuschel 2 polypeptide;

and wherein said third ubiquitin promoter is operably linked to said babyboom polynucleotide.

124. The method of embodiment 64, wherein said polynucleotide construct comprises:

a) a ubiquitin promoter;

b) an excision cassette flanked by loxP recombination sites that are are recombinogenic with respect to one another and are directly repeated, wherein said excision cassette comprises:

i) a polynucleotide encoding a *Discosoma* red fluorescent protein;

ii) a promoter comprising a maize *rab17* promoter and an attachment B (attB) site; and

iii) a polynucleotide encoding a CRE recombinase; and

c) a GLYAT polynucleotide;

wherein said ubiquitin promoter is operably linked to said polynucleotide encoding said *Discosoma* red fluorescent protein and wherein said ubiquitin promoter is operably linked to said GLYAT polynucleotide upon excision of said excision cassette; and

wherein said promoter comprising said maize *rab17* promoter and said attB site is operably linked to said polynucleotide encoding said CRE recombinase.

125. The method of embodiment 64, wherein said polynucleotide construct comprises:

a) a ubiquitin promoter;

b) an excision cassette flanked by loxP recombination sites that are are recombinogenic with respect to one another and are directly repeated, wherein said excision cassette comprises:

i) an actin promoter;

ii) a polynucleotide encoding a *Discosoma* red fluorescent protein;

iii) a promoter comprising a maize *rab17* promoter and an attachment B (attB) site; and

iv) a polynucleotide encoding a CRE recombinase; and

c) a GLYAT polynucleotide;

wherein said ubiquitin promoter is operably linked to said GLYAT polynucleotide upon excision of said excision cassette;

wherein said actin promoter is operably linked to said polynucleotide encoding said *Discosoma* red fluorescent protein; and

wherein said promoter comprising said maize *rab17* promoter and said attB site is operably linked to said polynucleotide encoding said CRE recombinase.

126. The method of any one of embodiments 64-125, wherein said plant cells are dicotyledonous.

127. The method of any one of embodiments 64-125, wherein said plant cells are monocotyledonous.

128. The method of embodiment 127, wherein said monocotyledonous plant cell is selected from the group consisting of maize, rice, sorghum, barley, wheat, millet, oat, rye, triticale, sugarcane, switchgrass, and turf/forage grass.

129. The method of any one of embodiments 64-128, wherein said plant cells are recalcitrant.

130. The method of embodiment 129, wherein said recalcitrant plant cells are cells of a sugarcane cultivar selected from the group consisting of CP96-1252, CP01-1372, CPCL97-2730, HoCP85-845, CP89-2143, and KQ228.

131. A method for increasing the transformation frequency of a plant tissue, the method comprising the steps of:

a) providing a population of plant cells, wherein at least one plant cell in the population comprises the polynucleotide construct of any one of claims 1-52;

b) culturing the population of plant cells in the absence of a herbicide to which the herbicide tolerance polypeptide confers herbicide resistance for a period of time sufficient for the population of plant cells to proliferate;

c) inducing the expression of the site-specific recombinase, thereby excising the excision cassette;

d) contacting the population of plant cells from c) with the herbicide to which the herbicide tolerance polypeptide confers tolerance; and

e) selecting for a plant cell having tolerance to the herbicide, wherein the transformation frequency is increased compared to a comparable plant cell not comprising the excision cassette and selected directly by herbicide selection.

132. The method of embodiment 131, wherein the inducing comprises desiccating the population of plant cells.

133. The method of embodiment 131 or 132, wherein the population of plant cells is cultured in the absence of the herbicide to which the herbicide tolerance polypeptide confers herbicide resistance for about 1 hour to about 6 weeks prior to excision.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 provides a depiction of vector PHP35648. The vector comprises a coding sequence for the cyan fluorescent protein (CFP), the expression of which is regulated by the ubiquitin promoter (Ubi Pro; comprising the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113)). The PHP35648 vector comprises the maize *rab17* promoter with an attachment B site (Rab17 Pro) that drives the expression of the CRE site-specific recombinase. The vector further comprises expression cassettes for the maize Wuschel 2 (WUS2) protein (the expression of which is regulated by the nopaline synthase (Nos) promoter), the maize babyboom (BBM) protein and the maize optimized phosphinothricin acetyl transferase (moPAT) (both of which are regulated by the ubiquitin promoter; comprising the maize ubiquitin promoter (Ubi Pro; comprising the UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113)). The yellow fluorescent protein (YFP) is expressed when a fragment of the vector that is

flanked by LoxP recombination sites (the excision cassette) is excised by the CRE recombinase.

Figure 2 provides a depiction of vector PHP54561. The vector comprises a coding sequence for moPAT or neomycin phosphotransferase II (nptII), the expression of which is regulated by the ubiquitin promoter (Ubi Pro; comprising the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113)). An ubiquitin promoter (Ubi Pro) also regulates the expression of yellow fluorescent protein (YFP) and the maize BBM protein. The PHP54561 vector further comprises the maize *rab17* promoter with an attachment B site (Rab17 Pro) that drives the expression of the CRE recombinase and an expression cassette for WUS2 under the regulation of the Nos promoter. The ubiquitin promoter (Ubi Pro) regulates the expression of the glyphosate-N-acetyltransferase (GLYAT) gene when an excision cassette flanked by LoxP sites is excised by the CRE recombinase.

Figure 3 provides an image of glyphosate selection on tissue proliferation/regeneration medium of tissues of sugarcane cultivars CP01-1372 (top) and CP88-1762 (bottom) that had been transformed with the PHP54561 vector and desiccated.

Figure 4 provides images of glyphosate selection on regeneration/rooting medium of sugarcane cultivars CP01-1372 (left) and CP88-1762 (right) that had been transformed with the PHP54561 vector and desiccated.

Figure 5 provides images of a second round of glyphosate selection on rooting medium containing 30 μM glyphosate of sugarcane that had been transformed with the PHP54561 vector and desiccated.

Figure 6 provides a depiction of vector PHP54353. The vector comprises a coding sequence for the red fluorescent protein from *Discosoma* (dsRED), the expression of which is regulated by the ubiquitin promoter (Ubi Pro; comprising the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113)). The PHP54353 vector comprises the maize *rab17* promoter with an attachment B site (Rab17

Pro) that drives the expression of the CRE site-specific recombinase. The ubiquitin promoter (Ubi Pro) regulates the expression of the glyphosate-N-acetyltransferase (GLYAT) gene when an excision cassette flanked by LoxP sites is excised by the CRE recombinase.

Figure 7 provides a depiction of another polynucleotide construct embodiment. The vector comprises a coding sequence for the red fluorescent protein from *Discosoma* (dsRED), the expression of which is regulated by the actin promoter (Actin Pro). The vector further comprises the maize *rab17* promoter with an attachment B site (Rab17 Pro) that drives the expression of the CRE site-specific recombinase. The ubiquitin promoter (Ubi Pro; comprising the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113) regulates the expression of the glyphosate-N-acetyltransferase (GLYAT) gene when an excision cassette flanked by LoxP sites is excised by the CRE recombinase.

Figure 8 provides a depiction of vector PHP55062. The vector comprises a coding sequence for the red fluorescent protein from *Discosoma* (dsRED), the expression of which is regulated by the enhanced *Mirabilis* mosaic virus (dMMV) promoter. The vector further comprises the maize *rab17* promoter with an attachment B site (Rab17 Pro) that drives the expression of the CRE site-specific recombinase. A separate dMMV promoter regulates the expression of a hygromycin phosphotransferase (Hyg (hpt)) gene and also regulates the expression of the glyphosate-N-acetyltransferase (GLYAT) gene when an excision cassette flanked by LoxP sites is excised by the CRE recombinase.

Figure 9 provides depictions of various embodiments of the presently disclosed polynucleotide constructs. The constructs all comprise an excision cassette (flanked by LoxP sites) comprising a polynucleotide encoding a site-specific recombinase (CP_A), the expression of which is regulated by an inducible promoter A (P_A). Upon activation of P_A and excision of the excision cassette, promoter B (P_B) is operably linked to the polynucleotide encoding a herbicide tolerance polypeptide (CP_B) and the herbicide tolerance polypeptide is produced. The excision cassette of the constructs of Figures 9b-9g further comprise a polynucleotide encoding a selectable marker (CP_C) in the excision

cassette that is either operably linked to P_B or to another promoter (P_C). The excision cassettes of the constructs of Figures 9d-9g further comprises at least one polynucleotide encoding a cell proliferation factor (CP_{D1} and CP_{D2}), each of which are operably linked to a promoter (P_{D1} or P_{D2}, respectively). The polynucleotide construct of Figure 9g further comprises (outside of the excision cassette) a polynucleotide encoding a polypeptide of interest (CP_E) that is operably linked to a promoter E (P_E).

DETAILED DESCRIPTION OF THE INVENTION

Compositions and methods are provided for regulating the expression of a transgene, such as a herbicide tolerance polynucleotide, for producing and selecting transgenic plants and plant parts, and for increasing the transformation frequency of a plant or plant part. Compositions include polynucleotide constructs comprising an excision cassette, a transgene (e.g., herbicide tolerance polynucleotide) and a promoter that becomes operably linked to the transgene (e.g., herbicide tolerance polynucleotide) upon excision of the excision cassette from the polynucleotide construct. The excision cassette comprises an inducible promoter operably linked to a polynucleotide that encodes a site-specific recombinase and the excision cassette is flanked by a first and a second recombination site, wherein the first and second recombination sites are recombinogenic with respect to one another and are directly repeated, and wherein the site-specific recombinase can recognize and implement recombination at the first and second recombination sites, thereby excising the excision cassette and allowing for the operable linkage of the transgene (e.g., herbicide tolerance polynucleotide) with its promoter. In some embodiments, the polynucleotide construct further comprises a polynucleotide of interest, either within or outside of the excision cassette. In certain embodiments, the excision cassette further comprises at least one coding polynucleotide for a cell proliferation factor, such as a babyboom polypeptide or a Wuschel polypeptide.

In some embodiments, the polynucleotide construct further comprises at least one selectable marker. In some embodiments, the selectable marker is selected from the group consisting of a fluorescent protein, an antibiotic resistance polypeptide, a herbicide tolerance polypeptide, and a metabolic enzyme. In some embodiments, the plant or plant

part is recalcitrant to transformation. In some embodiments, the plant or plant part is a monocotyledonous. In some embodiments the plant or plant part is maize, rice, wheat, barley, sorghum, oats, rye, triticale and sugarcane.

It is intended that the excision cassette is not limited by the number and or order of the coding polynucleotides within the excision cassette. It is envisioned that the excision cassette can be constructed with any number of coding polynucleotides in any order. It is also intended that the polynucleotide construct may also include, beyond the promoter and polynucleotide encoding the herbicide tolerance polypeptide flanking the recombination sites, one or more polynucleotide encoding polypeptide(s) of interest.

The use of the term "polynucleotide" is not intended to limit compositions to polynucleotides comprising DNA. Polynucleotides can comprise ribonucleotides and combinations of ribonucleotides and deoxyribonucleotides. Such deoxyribonucleotides and ribonucleotides include both naturally occurring molecules and synthetic analogues. The polynucleotides also encompass all forms of sequences including, but not limited to, single-, double-, or multi-stranded forms, hairpins, stem-and-loop structures, circular plasmids, and the like.

An "isolated" or "purified" polynucleotide or protein, or biologically active portion thereof, is substantially or essentially free from components that normally accompany or interact with the polynucleotide or protein as found in its naturally occurring environment. Thus, an isolated or purified polynucleotide or protein is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Optimally, an "isolated" polynucleotide is free of sequences (optimally protein encoding sequences) that naturally flank the polynucleotide (*i.e.*, sequences located at the 5' and 3' ends of the polynucleotide) in the genomic DNA of the organism from which the polynucleotide is derived. For example, in various embodiments, the isolated polynucleotide can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequence that naturally flank the polynucleotide in genomic DNA of the cell from which the polynucleotide is derived. A protein that is substantially free of cellular material includes preparations of protein having less than

about 30%, 20%, 10%, 5%, or 1% (by dry weight) of contaminating protein. When the protein or biologically active portion thereof is recombinantly produced, optimally culture medium represents less than about 30%, 20%, 10%, 5%, or 1% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

As used herein, a "polynucleotide construct" refers to a polynucleotide molecule comprised of various types of nucleotide sequences having different functions and/or activities. For example, a polynucleotide construct may comprise one or more of any of the following: expression cassettes, coding polynucleotides, regulatory sequences (e.g., enhancers, promoters, termination sequences), origins of replication, restriction sites, recombination sites, and excision cassettes.

The presently disclosed polynucleotide constructs can comprise one or more expression cassettes, wherein a coding polynucleotide is operably linked to a regulatory sequence.

As used herein, a "coding polynucleotide" refers to a polynucleotide that encodes a polypeptide and therefore comprises the requisite information to direct translation of the nucleotide sequence into a specified polypeptide. Alternatively, a "coding polynucleotide" can refer to a polynucleotide that encodes a silencing polynucleotide that reduces the expression of target genes. Non-limiting examples of a silencing polynucleotide include a small interfering RNA, micro RNA, antisense RNA, a hairpin structure, and the like.

As used herein, an "expression cassette" refers to a polynucleotide that comprises at least one coding polynucleotide operably linked to regulatory sequences sufficient for the expression of the coding polynucleotide. "Operably linked" is intended to mean a functional linkage between two or more elements. For example, an operable linkage between a coding polynucleotide and a regulatory sequence (i.e., a promoter) is a functional link that allows for expression of the coding polynucleotide. Operably linked elements may be contiguous or non-contiguous. When used to refer to the joining of two protein coding regions, by operably linked is intended that the coding regions are in the same reading frame.

An expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a coding polynucleotide, and a transcriptional and translational termination region (i.e., termination region) functional in plants. The regulatory regions (i.e., promoters, transcriptional regulatory regions, and translational termination regions) and/or the coding polynucleotide may be native/analogous to a host cell comprising the presently disclosed polynucleotide constructs or to each other. Alternatively, the regulatory regions and/or the coding polynucleotide may be heterologous to the host cell or to each other. As used herein, "heterologous" in reference to a sequence is a sequence that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. A heterologous polynucleotide is also referred to herein as a "transgene". For example, a promoter operably linked to a heterologous polynucleotide is from a species different from the species from which the polynucleotide was derived, or, if from the same/analogous species, one or both are substantially modified from their original form and/or genomic locus, or the promoter is not the native promoter for the operably linked polynucleotide. While it may be optimal to express the sequences using heterologous promoters, the native promoter sequences may be used.

The termination region may be native with the transcriptional initiation region, may be native with the operably linked coding polynucleotide, may be native with the host cell, or may be derived from another source (i.e., foreign or heterologous) to the promoter, the coding polynucleotide, the host cell, or any combination thereof. Convenient termination regions are available from the potato proteinase inhibitor (PinII) gene or the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau *et al.* (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon *et al.* (1991) *Genes Dev.* 5:141-149; Mogen *et al.* (1990) *Plant Cell* 2:1261-1272; Munroe *et al.* (1990) *Gene* 91:151-158; Ballas *et al.* (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi *et al.* (1987) *Nucleic Acid Res.* 15:9627-9639. In some embodiments, the termination sequence that is operably linked to at least one of the site-specific recombinase-encoding polynucleotide,

the selectable marker-encoding polynucleotide, the cell proliferation marker-encoding polynucleotide, the herbicide tolerance polynucleotide, and the polynucleotide of interest is the termination region from the pinII gene. In some of these embodiments, the termination region has the sequence set forth in SEQ ID NO: 1 or an active variant or fragment thereof that is capable of terminating transcription and/or translation in a plant cell.

The expression cassettes may additionally contain 5' leader sequences. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (encephalomyocarditis 5' noncoding region) (Elroy-Stein *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6126-6130); potyvirus leaders, for example, TEV leader (tobacco etch virus) (Gallie *et al.* (1995) *Gene* 165(2):233-238), MDMV leader (maize dwarf mosaic virus) (*Virology* 154:9-20), and human immunoglobulin heavy-chain binding protein (BiP) (Macejak *et al.* (1991) *Nature* 353:90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling *et al.* (1987) *Nature* 325:622-625); tobacco mosaic virus leader (TMV) (Gallie *et al.* (1989) in *Molecular Biology of RNA*, ed. Cech (Liss, New York), pp. 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel *et al.* (1991) *Virology* 81:382-385). See also, Della-Cioppa *et al.* (1987) *Plant Physiol.* 84:965-968.

For example, in some of the embodiments, wherein the herbicide tolerance polynucleotide is a GLYAT polynucleotide, the cauliflower mosaic virus (CaMV) 35S enhancer region or tobacco mosaic virus (TMV) omega 5′ UTR translational enhancer element is included upstream of a promoter that is operably linked (when the excision cassette is excised) to the GLYAT polynucleotide to enhance transcription (see, for example, U.S. Patent Nos. 7,928,296 and 7,622,641, each of which is herein incorporated by reference in its entirety).

In preparing the expression cassette or polynucleotide construct, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA,

removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

Expression cassettes comprise a promoter operably linked to a coding polynucleotide. As used herein, the term "promoter" includes reference to a region of DNA involved in the recognition and binding of RNA polymerase and other proteins to initiate transcription of a coding sequence. Promoters may be naturally occurring promoters, a variant or fragment thereof, or synthetically derived. The term "promoter" refers to the minimal sequences necessary to direct transcription (minimal promoter) as well as sequences comprising the minimal promoter and any number of additional elements, such as operator sequences, enhances, modulators, restriction sites, recombination sites, sequences located in between the minimal promoter and the coding sequence, and sequences of the 5'-untranslated region (5'-UTR), which is the region of a transcript that is transcribed, but is not translated into a polypeptide, which may or may not influence transcription levels in a desired manner. A "plant promoter" refers to a promoter isolated from a plant or a promoter derived therefrom or a heterologous promoter that functions in a plant.

Although according to the invention, the promoter that drives the expression of the site-specific recombinase is an inducible promoter, various types of promoters can be used for the regulation of the expression of the remaining coding polynucleotides in the presently disclosed polynucleotide constructs. The promoter may be selected based on the desired outcome or expression pattern (for a review of plant promoters, see Potenza *et al.* (2004) *In Vitro Cell Dev Biol* 40:1-22).

Constitutive promoters include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Patent No. 6,072,050; the core CaMV 35S promoter (Odell *et al.* (1985) *Nature* 313:810-812); rice actin (McElroy *et al.* (1990) *Plant Cell* 2:163-171); ubiquitin (Christensen *et al.* (1989) *Plant Mol. Biol.* 12:619-632 and Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689); pEMU (Last *et al.* (1991) *Theor. Appl. Genet.* 81:581-588); MAS (Velten *et al.* (1984) *EMBO J.* 3:2723-2730); ALS promoter (U.S. Patent No. 5,659,026), the

Agrobacterium nopaline synthase (NOS) promoter (Bevan *et al.* (1983) *Nucl. Acids Res.* 11:369-385); *Mirabilis* mosaic virus (MMV) promoter (Dey & Maiti (1999) Plant Mol Biol 40:771-782; Dey & Maiti (1999) Transgenics 3:61-70); histone 2B (H2B) (International Application Publication No. WO 99/43797); banana streak virus (BSV) promoter (Remans *et al.* (2005) *Virus Research* 108:177-186); chloris striate mosaic virus (CSMV) promoter (Zhan *et al.* (1993) *Virology* 193:498-502); Cassava vein mosaic virus (CSVMV) promoter (Verdaguer *et al.* (1998) *Plant Mol Biol* 37:1055-1067); figwort mosaic virus (FMV) promoter (U.S. Patent No. 6,018,100), rice alpha-tubulin (OsTUBA1) promoter (Jeon *et al.* (2000) *Plant Physiol* 123:1005-1014); rice cytochrome C (OsCC1) promoter (Jang *et al.* (2002) *Plant Physiol* 129:1473-1481); maize alcohol dehydrogenase1 (ZmADH1) promoter (Kyozuka *et al.* (1990) *Maydica* 35:353-357; an oleosin promoter (*e.g.*, SEQ ID NO: 2 or a variant or fragment thereof) and the like; each of which is herein incorporated by reference in its entirety. Other constitutive promoters are described in, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611; each of which is herein incorporated by reference in its entirety.

In some embodiments, an inducible promoter can be used, such as from a pathogen-inducible promoter. Such promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; *e.g.*, PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. See, for example, Redolfi *et al.* (1983) *Neth. J. Plant Pathol.* 89:245-254; Uknes *et al.* (1992) *Plant Cell* 4:645-656; and Van Loon (1985) *Plant Mol. Virol.* 4:111-116. See also WO 99/43819, herein incorporated by reference. Promoters that are expressed locally at or near the site of pathogen infection include, for example, Marineau *et al.* (1987) *Plant Mol. Biol.* 9:335-342; Matton *et al.* (1989) *Mol Plant-Microbe Interact* 2:325-331; Somsisch *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:2427-2430; Somsisch *et al.* (1988) *Mol. Gen. Genet.* 2:93-98; and Yang (1996) *Proc. Natl. Acad. Sci. USA* 93:14972-14977. See also, Chen *et al.* (1996) *Plant J.* 10:955-966; Zhang *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:2507-2511; Warner *et al.* (1993) *Plant J.* 3:191-201; Siebertz *et al.* (1989) *Plant Cell* 1:961-968; U.S. Patent No. 5,750,386 (nematode-inducible); and the references cited therein.

Additional promoters include the inducible promoter for the maize PRms gene, whose expression is induced by the pathogen *Fusarium moniliforme* (see, for example, Cordero *et al.* (1992) *Physiol. Mol. Plant Path.* 41:189-200). Wound-inducible promoters include potato proteinase inhibitor (pin II) gene (Ryan (1990) *Ann. Rev. Phytopath.* 28:425-449; Duan *et al.* (1996) *Nat Biotechnol* 14:494-498); wun1 and wun2, U.S. Patent No. 5,428,148; win1 and win2 (Stanford *et al.* (1989) *Mol. Gen. Genet.* 215:200-208); systemin (McGurl *et al.* (1992) *Science* 225:1570-1573); WIP1 (Rohmeier *et al.* (1993) *Plant Mol. Biol.* 22:783-792; Eckelkamp *et al.* (1993) *FEBS Lett* 323:73-76); MPI gene (Corderok *et al.* (1994) *Plant J.* 6:141-150); and the like, herein incorporated by reference.

Other inducible promoters useful for regulating the expression of any of the coding sequences of the presently disclosed polynucleotide constructs include stress-inducible promoters, such as those described elsewhere herein.

Chemical-regulated promoters can be used to modulate the expression of a gene in a plant through the application of an exogenous chemical regulator. The promoter may be a chemical-inducible promoter, where application of the chemical induces gene expression, or a chemical-repressible promoter, where application of the chemical represses gene expression. Chemical-inducible promoters are known in the art and include, but are not limited to, the maize In2-2 promoter, which is activated by benzenesulfonamide herbicide safeners (De Veylder *et al.* (1997) *Plant Cell Physiol.* 38:568-77), the maize GST promoter (GST-II-27, WO 93/01294), which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, the PR-1 promoter (Cao *et al.* (2006) *Plant Cell Reports* 6:554-60), which is activated by BTH or benxo(1,2,3)thiaidazole-7-carbothioic acid s-methyl ester, the tobacco PR-1a promoter (Ono *et al.* (2004) *Biosci. Biotechnol. Biochem.* 68:803-7), which is activated by salicylic acid, the copper inducible ACE1 promoter (Mett *et al.* (1993) *PNAS* 90:4567-4571), the ethanol-inducible promoter AlcA (Caddick *et al.* (1988) *Nature Biotechnol* 16:177-80), an estradiol-inducible promoter (Bruce *et al.* (2000) *Plant Cell* 12:65-79), the XVE estradiol-inducible promoter (Zao *et al.* (2000) *Plant J* 24:265-273), the VGE methoxyfenozide inducible promoter (Padidam *et al.* (2003) *Transgenic Res* 12:101-109),

and the TGV dexamethasone-inducible promoter (Bohner *et al.* (1999) *Plant J* 19:87-95). Other chemical-regulated promoters of interest include steroid-responsive promoters (see, for example, the glucocorticoid-inducible promoter in Schena *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:10421-10425 and McNellis *et al.* (1998) *Plant J.* 14(2):247-257) and tetracycline-inducible and tetracycline-repressible promoters (see, for example, Gatz *et al.* (1991) *Mol. Gen. Genet.* 227:229-237; Gatz *et al.* (1992) *Plant J* 2:397-404; and U.S. Patent Nos. 5,814,618 and 5,789,156), herein incorporated by reference.

One particular chemical-inducible promoter that is described in more detail elsewhere herein and that can be used in the presently disclosed compositions and methods, particularly to regulate the expression of the site-specific recombinase, is a promoter responsive to sulfonylurea, wherein the promoter comprises operator sequences capable of binding to a sulfonylurea-responsive transcriptional repressor (SuR) protein, such as those described in U.S. Application Publication Nos. 2010/0105141 and 2011/0287936, each of which is herein incorporated by reference in its entirety.

Tissue-preferred promoters can be utilized to target enhanced expression of a coding polynucleotide within a particular plant tissue. Tissue-preferred promoters include Kawamata *et al.* (1997) *Plant Cell Physiol.* 38(7):792-803; Hansen *et al.* (1997) *Mol. Gen Genet.* 254(3):337-343; Russell *et al.* (1997) *Transgenic Res.* 6(2):157-168; Rinehart *et al.* (1996) *Plant Physiol.* 112(3):1331-1341; Van Camp *et al.* (1996) *Plant Physiol.* 112(2):525-535; Canevascini *et al.* (1996) *Plant Physiol.* 112(2):513-524; Lam (1994) *Results Probl. Cell Differ.* 20:181-196; and Guevara-Garcia *et al.* (1993) *Plant J.* 4(3):495-505.

Leaf-preferred promoters are known in the art. See, for example, Yamamoto *et al.* (1997) *Plant J.* 12:255-265; Kwon *et al.* (1994) *Plant Physiol.* 105:357-67; Yamamoto *et al.* (1994) *Plant Cell Physiol.* 35:773-778; Gotor *et al.* (1993) *Plant J.* 3:509-18; Orozco *et al.* (1993) *Plant Mol. Biol.* 23:1129-1138; and Matsuoka *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:9586-9590. In addition, promoter of cab and rubisco can also be used. See, for example, Simpson *et al.* (1958) *EMBO J 4:*2723-2729 and Timko *et al.* (1988) *Nature 318*:57-58.

Root-preferred promoters are known and can be selected from the many available. See, for example, Hire *et al.* (1992) *Plant Mol. Biol.* 20:207-218 (soybean root-specific glutamine synthase gene); Keller and Baumgartner (1991) *Plant Cell* 3:1051-1061 (root-specific control element in the GRP 1.8 gene of French bean); Sanger *et al.* (1990) *Plant Mol. Biol.* 14:433-443 (root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*); and Miao *et al.* (1991) *Plant Cell* 3:11-22 (full-length cDNA clone encoding cytosolic glutamine synthase (GS), which is expressed in roots and root nodules of soybean). See also Bogusz *et al.* (1990) *Plant Cell* 2:633-641, where two root-specific promoters isolated from hemoglobin genes from the nitrogen-fixing nonlegume *Parasponia andersonii* and the related non-nitrogen-fixing nonlegume *Trema tomentosa* are described. Leach and Aoyagi (1991) describe their analysis of the promoters of the highly expressed rolC and rolD root-inducing genes of *Agrobacterium rhizogenes* (see *Plant Sci* (Limerick) 79:69-76). Teeri *et al.* (1989) used gene fusion to lacZ to show that the *Agrobacterium* T-DNA gene encoding octopine synthase is especially active in the epidermis of the root tip and that the TR2' gene is root specific in the intact plant and stimulated by wounding in leaf tissue (see *EMBO J.* 8:343-350). The TR1' gene, fused to *nptII* (neomycin phosphotransferase II) showed similar characteristics. Additional root-preferred promoters include the VfENOD-GRP3 gene promoter (Kuster *et al.* (1995) *Plant Mol. Biol.* 29:759-772); and rolB promoter (Capana *et al.* (1994) *Plant Mol. Biol.* 25:681-691. See also U.S. Patent Nos. 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732; and 5,023,179. Another root-preferred promoter includes the promoter of the phaseolin gene (Murai *et al.* (1983) *Science 23*:476-482 and Sengopta-Gopalen *et al.* (1988) *Proc. Natl. Acad. Sci. USA 82*:3320-3324.

Seed-preferred promoters include both those promoters active during seed development as well as promoters active during seed germination. See Thompson *et al.* (1989) *BioEssays* 10:108, herein incorporated by reference. Such seed-preferred promoters include, but are not limited to, Cim1 (cytokinin-induced message); cZ19B1 (maize 19 kDa zein); and milps (myo-inositol-1-phosphate synthase); (see WO 00/11177 and U.S. Patent No. 6,225,529; herein incorporated by reference). For dicots, seed-

preferred promoters include, but are not limited to, bean β-phaseolin, napin, β-conglycinin, soybean lectin, cruciferin, and the like. For monocots, seed-preferred promoters include, but are not limited to, maize 15 kDa zein, 22 kDa zein, 27 kDa gamma zein, waxy, shrunken 1, shrunken 2, globulin 1, oleosin, nuc1, etc. See also WO 00/12733, where seed-preferred promoters from *end1* and *end2* genes are disclosed; herein incorporated by reference.

Where low-level expression is desired, weak promoters will be used. Generally, by "weak promoter" is intended a promoter that drives expression of a coding sequence at a low level. By low level is intended at levels of about 1/1000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts. Alternatively, it is recognized that weak promoters also encompasses promoters that are expressed in only a few cells and not in others to give a total low level of expression. Where a promoter is expressed at unacceptably high levels, portions of the promoter sequence can be deleted or modified to decrease expression levels. Such weak constitutive promoters include, for example, the core promoter of the Rsyn7 promoter (WO 99/43838 and U.S. Patent No. 6,072,050), the core 35S CaMV promoter, and the like.

In some embodiments, at least one of the following promoters is a constitutive promoter: the promoter regulating the expression of the herbicide tolerance polypeptide, the promoter operably linked to the cell proliferation marker, and the promoter driving the expression of the selectable marker present within the excision cassette. In particular embodiments, the selectable marker present within the excision cassette of the presently disclosed polynucleotide constructs is operably linked to a constitutive promoter such that the selectable marker is constitutively expressed until excision of the excision cassette, and the same constitutive promoter then regulates the expression of the herbicide tolerance polypeptide upon excision of the cassette. In some of these embodiments, the constitutive promoter is the maize ubiquitin promoter (Christensen *et al.* (1989) *Plant Mol. Biol.* 12:619-632 and Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689), which in some embodiments comprises the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113). In other embodiments, the constitutive

promoter regulating the expression of the selectable marker present within the excision cassette is the enhanced *Mirabilis* mosaic virus (MMV) promoter (Dey & Maiti (1999) Plant Mol Biol 40:771-782; Dey & Maiti (1999) Transgenics 3:61-70). In some embodiments, the polynucleotide encoding a cell proliferation factor (*e.g.*, babyboom polypeptide) is operably linked to a maize ubiquitin promoter (which in some embodiments comprises the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113) or a maize oleosin promoter (*e.g.*, SEQ ID NO: 2 or a variant or fragment thereof).

According to the invention, the promoter that regulates the expression of the site-specific recombinase is an inducible promoter. In some embodiments, the inducible promoter that is operably linked to the site-specific recombinase-encoding polynucleotide comprises a stress-inducible promoter. As used herein, a "stress-inducible promoter" refers to a promoter that initiates transcription when the host cell (e.g., plant cell) or host (e.g., plant or plant part) undergoes stress, including abiotic stress. Non-limiting examples of conditions that can activate stress-inducible promoters include drought, salinity, flood, and suboptimal temperature. Some stress-inducible promoters are only activated by a particular stress (e.g., drought), whereas other stress-inducible promoters can be activated by any type of stress, particularly any type of abiotic stress.

Stress-inducible promoters include those that become activated in response to drought and high salinity (drought-inducible promoters) and cold temperatures (cold-inducible promoters). Some promoters are both drought-inducible and cold-inducible. Many stress-inducible promoters are also activated by abscisic acid (ABA), a phytohormone that is often expressed by plants in response to drought and high-salinity stress. Regulatory pathways by which stress-inducible promoters can become activated include those that are ABA-dependent as well as those that are ABA-independent. Thus, some stress-inducible promoters comprise an ABA-responsive element (ABRE) and respond to ABA. Some of those stress-inducible promoters that are responsive to drought, high salinity, and/or cold temperatures comprise a dehydration-responsive (DRE)/C-repeat (CRT) element. The C-repeat binding factor (CBF)/DREB1

transcription factor, the expression of which is induced by cold stress, and the DREB2 transcription factor, which is induced by dehydration, bind to DRE/CRT elements. In some embodiments, stress-inducible promoters comprise any one of the following *cis*-acting stress-responsive elements: ABRE, CE1, CE3, MYB recognition site (MYBR), MYC recognition site (MYCR), DRE, CRT, low-temperature-responsive element (LTRE), NAC recognition site (NACR), zinc-finger homeodomain recognition site (ZFHDR) and an inducer of CBF expression (ICE) recognition site. Table 1 provides the sequences of these *cis*-acting stress-responsive elements. See Yamaguchi-Shinozaki and Shinozaki (2005) *Trends Plant Sci* 10:1360-1385 and Shinozaki *et al.* (2003) *Curr Opin Plant Biol* 6:410-417, each of which is incorporated by reference in its entirety, for reviews of stress-inducible promoters and the regulatory pathways controlling the same.

Table 1. cis-Acting regulatory elements in stress-inducible gene expression.*

cis element	Sequence (SEQ ID NO:)	Type of transcription factors that bind to *cis* elements	Gene	Stress condition
ABRE	PyACGTGGC (3)	bZIP	Em, RAB16	Water deficit, ABA
CE1	TGCCACCGG (4)	ERF/AP2	HVA1	ABA
CE3	ACGCGTGCCTC (5)	Not known	HVA22	ABA
ABRE	ACGTGTC (6)	bZIP	Osem	ABA
ABRE	ACGTGGC (7), ACGTGTC (8)	bZIP	RD29B	Water deficit, ABA
MYBR	TGGTTAG (9)	MYB	RD22	Water deficit, ABA
MYCR	CACATG (10)	bHLH	RD22	Water deficit, ABA
DRE	TACCGACAT (11)	ERF/AP2	RD29A	Water deficit, cold
CRT	GGCCGACAT (12)	ERF/AP2	Cor15A	Cold
LTRE	GGCCGACGT (13)	ERF/AP2	BN115	Cold
NACR	ACACGCATGT (14)	NAC	ERD1	Water deficit
ZFHDR	Not yet reported	ZFHD	ERD1	Water deficit
ICEr1	GGACACATGTCAGA	Not known	CBF2/	Cold

cis element	Sequence (SEQ ID NO:)	Type of transcription factors that bind to *cis* elements	Gene	Stress condition
	(15)		DREB1C	
ICEr2	ACTCCG (16)	Not known	CBF2/ DREB1C	Cold

*Adopted from Yamaguchi-Shinozaki and Shinozaki (2005) *Trends Plant Sci* 10:1360-1385

In some embodiments, the inducible promoter that is operably linked to the polynucleotide encoding a site-specific recombinase is a cold-inducible promoter. As used herein, a "cold-inducible promoter" is a promoter that is activated at temperatures that are below optimal temperatures for plant growth. In some embodiments, the cold-inducible promoter is one that is induced in response to temperatures less than about 20°C, less than about 19°C, less than about 18°C, less than about 17°C, less than about 16°C, less than about 15°C, less than about 14°C, less than about 13°C, less than about 12°C, less than about 11°C, less than about 10°C, less than about 9°C, less than about 8°C, less than about 7°C, less than about 6°C, less than about 5°C, less than about 4°C, less than about 3°C, less than about 2°C, less than about 1°C, or less than about 0°C.

Cold-inducible promoters may be activated by exposing a plant or plant part to cold temperatures for a period of about 12 hours, about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 3 months, or more. The temperature required or the necessary amount of time the plant or plant part is exposed to the cold temperatures will vary based on, for example, the promoter, the plant species, the type of explant, and the size of the plant tissue, and can be determined by one of skill in the art.

Cold-inducible promoters can comprise a C-repeat (CRT) and/or a low-temperature-responsive element (LTRE), both of which contain an A/GCCGAC motif that forms the core of the DRE sequence, as well. Non-limiting examples of cold-

inducible promoters include the maize *rab17* promoter (Vilardell *et al.* (1990) *Plant Mol Biol* 14:423-432), the *RD29A* promoter (Uno *et al.* (2000) *PNAS* 97:11632-11637), the *Cor15A* promoter (Baker *et al.* (1994) *Plant Mol Biol* 24:701-713), the *BN115* promoter (Jiang *et al.* (1996) *Plant Mol Biol* 30:679-684), and the *CBF2/DREB1C* promoter (Zarka *et al.* (2003) *Plant Physiol* 133:910-918); each of which is herein incorporated by reference in its entirety.

In some embodiments, the inducible promoter that regulates the expression of the site-specific recombinase is a vernalization promoter, which is a promoter that responds to cold exposure to trigger flowering in plants. Vernalization promoters generally require exposure to cold temperatures for an extended period of time (e.g., at least 2 weeks) for activation. In certain embodiments, activation of a vernalization promoter requires exposure to temperatures less than about 20°C, less than about 19°C, less than about 18°C, less than about 17°C, less than about 16°C, less than about 15°C, less than about 14°C, less than about 13°C, less than about 12°C, less than about 11°C, less than about 10°C, less than about 9°C, less than about 8°C, less than about 7°C, less than about 6°C, less than about 5°C, less than about 4°C, less than about 3°C, less than about 2°C, less than about 1°C, or less than about 0°C for at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks, at least 16 weeks, or more. In certain embodiments, activation of a vernalization promoter requires exposure to a temperature of about 4°C for about 2 weeks.

In some embodiments, the vernalization promoter comprises a putative MADS-box protein binding site, referred to herein as CarG-box, the sequence of which is set forth in SEQ ID NO: 114. A non-limiting example of a vernalization promoter is the *Triticum monococcum* VRN1/AP1 promoter set forth in SEQ ID NO: 115 and described in Yan *et al.* (2003) *Proc Natl Acad Sci USA* 100:6263-6268 and U.S. Application Publication No. 2004/0203141, each of which is herein incorporated by reference in its entirety.

In some of those embodiments wherein the inducible promoter that regulates the expression of the site-specific recombinase is a vernalization promoter, the host cell of the polynucleotide construct is a *Brassica* sp., winter wheat, barley, oat, or rye.

In other embodiments, the inducible promoter that regulates the expression of the site-specific recombinase is a drought-inducible promoter. As used herein, a "drought-inducible promoter" or "desiccation-inducible promoter" refers to a promoter that initiates transcription in response to drought conditions, high salinity, and/or dessication of a plant or plant part. Drought-inducible promoters can drive expression in a number of different plant tissues including, but not limited to, root tissue (e.g., root endodermis, root epidermis, or root vascular tissues) and leaf tissue (e.g. epidermis, mesophyll or leaf vascular tissue).

In some embodiments, the drought-inducible promoter comprises a DRE or an early responsive to dehydration 1 (ERD1) cis-acting element (Yamaguchi-Shinozaki and Shinozaki (2004) *Trends Plant Sci* 10:1360-1385; and Shinozaki *et al.* (2003) *Curr Opin Plant Biol* 6:410-417).

The drought-inducible promoter is activated when the plant or plant part comprising the same is desiccated. As used herein, the term "desiccate" refers to a process by which the water content of a plant or plant part is reduced, and can include reference to the natural desiccation process that occurs during the maturation of seeds. Thus, in some embodiments, the drought-inducible promoter is activated in a plant cell comprising the presently disclosed polynucleotide constructs and excision of the excision cassette occurs during the maturation of a seed comprising the plant cell.

A desiccated plant or plant part can comprise about 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.1% or less water than a plant or plant part that has not been dried. The amount of desiccation necessary to activate a drought-inducible promoter or the amount of time needed to desiccate a plant or plant part will vary based on, for example, the promoter, the plant species, the explant type, and the size of the plant tissue.

In some embodiments, a plant or plant part is desiccated and the drought-inducible promoter is activated by exposing the plant or plant part comprising the

drought-inducible promoter to drought conditions. As used herein, "drought" or "drought conditions" can be defined as the set of environmental conditions under which a plant or plant part will begin to suffer the effects of water deprivation, such as decreased stomatal conductance and photosynthesis, decreased growth rate, loss of turgor (wilting), or ovule abortion. For these reasons, plants experiencing drought stress typically exhibit a significant reduction in biomass and yield. Water deprivation may be caused by lack of rainfall or limited irrigation. Alternatively, water deficit may also be caused by high temperatures, low humidity, saline soils, freezing temperatures or water-logged soils that damage roots and limit water uptake to the shoot. Since plant species vary in their capacity to tolerate water deficit, the precise environmental conditions that cause drought stress cannot be generalized.

The drought-inducible promoter may be activated by exposing a plant or plant part to drought conditions for a period of about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 1 week, about 2 weeks, about 3 weeks, or more.

In some embodiments, the plant or plant part is desiccated and the drought-inducible promoter activated by incubating the plant or plant part in the absence of liquid medium and optionally on dry filter paper. In some embodiments, the plant or plant part is desiccated by incubating the plant or plant part in a sealed container with a saturated salt solution (e.g., $(NH_4)_2SO_4$). In some embodiments, the plant or plant part is incubated in the absence of liquid medium, and optionally, on dry filter paper, and in some embodiments, in a sealed container with a saturated salt solution for about 1 day, about 1.5 days, about 2 days, about 2.5 days, about 3 days, about 3.5 days, about 4 days, about 4.5 days, about 5 days, about 5.5 days, about 6 days, about 6.5 days, about 7 days, about 7.5 days, about 8 days, about 8.5 days, about 9 days, about 9.5 days, about 10 days, or more in order to induce the expression of the drought-inducible promoter.

Non-limiting examples of drought-inducible promoters include the promoters of maize *rab17* (Vilardell *et al.* (1990) *Plant Mol Biol* 14:423-432); *Oryza sativa Em* (Guiltinan *et al.* (1990) *Science* 250:267-271); *Rab16* (Mundy *et al.* (1990) *PNAS* 87:406-410); *HVA1* (Hobo *et al.* (1999) *Plant J* 19:679-689); *HVA22* (Su *et al.* (1998) *Plant Physiol* 117:913-922); *RD29B* and *RD29A* (Uno *et al.* (2000) *PNAS* 97:11632-11637);

RD22 (Abe *et al* (1997) *Plant Cell* 9:1859-1868); *Cor15A* (Baker *et al.* (1994) *Plant Mol Biol* 24:701-713); *BN115* (Jiang *et al.* (1996) *Plant Mol Biol* 30:679-684); *ERD1* (Tran *et al.* (2004) *Plant Cell* 16:2481-2498); *Oryza sativa LEA3* (Xiao *et al.* (2007) *Theor Appl Genet* 115:35-46); *Oryza sativa rab16Bj* (Xiao and Xue (2001) *Plant Cell Rep* 20:667-73); *Brassica* LEA3-1 (U.S. Application Publication No. US 2008/0244793); *LEA D7*, *LEA D11*, *LEA D19*, *LEA d34*, and *LEA D113* (Baker *et al.* (1988) *Plant Mol Biol* 11:277-291); *Oryza sativa RAB16* and *Sorghum bicolor DHN2* (Buchanan *et al.* (2004) *Genetics* 168:1639-1654); *Oryza sativa ASR1* (Kuriakose *et al.* (2009) *African J Biotech* 8:4765-73); *Oryza sativa NAC6* (Nakashima *et al.* (2007) *Plant J* 51:617-630); *Oryza sativa SALT* (Garcia *et al.* (1998) *Planta* 207:172-180); *Oryza sativa LIP9* (Aguan *et al.* (1993) *Mol Gen Genet* 240:1-8); *Oryza sativa WS1724* (Takahashi *et al.* (1994) *Plant Mol Biol* 26:339-352); *Oryza sativa WSI18* (Oh *et al.* (2005) *Plant Physiol* 138:341-351); *AREB1*, *AREB2*, and *ABF3* (Yoshida *et al.* (2010) *Plant J* 61:672-685); *Oryza sativa DIP1*, *UGE1*, *R1G1B*, and *RAB21* promoters (Yi *et al.* (2010) *Planta* 232:743-754); cotton *D113* (Luo *et al.* (2008) *Plant Cell Rep* 27:707-717); the *dehydrin* promoter; the *ASI* promoter; the *WGA* promoter; the *P511* promoter; and the *HS70* promoter; the *dehydrin* (DHN) promoter (Robertson *et al.* (1995) *Physiol Plant* 94:470-478); the alpha-amylase/subtilisin inhibitor (ASI) promoter (Furtado *et al.* (2003) *Plant Mol Biol* 52:787-799); the *WGA* promoter; and the *HS70* promoter; each of which is herein incorporated by reference in its entirety.

In some embodiments, the inducible promoter that drives the expression of a site-specific recombinase and subsequent excision of the excision cassette is a Rab17 promoter, such as the maize *rab17* promoter or an active variant or fragment thereof. The maize *rab17* (responsive to abscisic acid) gene (GenBank Accession No. X15994; Vilardell *et al.* (1990) *Plant Mol Biol* 14:423-432; Vilardell *et al.* (1991) *Plant Mol Biol* 17:985-993; each of which is herein incorporated in its entirety) is expressed in late embryos, but its expression can be induced by exposure to abscisic acid, cold temperatures, or water stress. The sequence of the maize *rab17* promoter corresponds to nucleotides 1-558 of GenBank Accession No. X15994, which was disclosed in Vilardell *et al.* (1990) *Plant Mol Biol* 14:423-432 and is set forth in SEQ ID NO: 17. An

alternative maize *rab17* promoter was disclosed in U.S. Patent Nos. 7,253,000 and 7,491,813, each of which is herein incorporated by reference in its entirety, and is set forth in SEQ ID NO: 18. The *rab17* promoter contains four abscisic acid responsive elements (ABRE) (Busk *et al.* (1997) *Plant J* 11:1285-1295, which is herein incorporated by reference in its entirety). The ABRE elements in the maize *rab17* promoter can be found at nucleotides 304-309, 348-353, 363-368, 369-374, 414-419, and 427-432 of SEQ ID NO: 18. The *rab17* promoter also contains drought-responsive elements (DRE), of which the core sequence is identical to the DRE (drought-responsive) and CRT (cold-response elements) elements in *Arabidopsis*. The drought-responsive elements of the maize *rab17* promoter are found at nucleotides 233-238, 299-304, and 322-327 of SEQ ID NO: 18. The CAAT and TATAA box can be found from nucleotides 395 to 398 and 479 to 483 of SEQ ID NO: 18, respectively. In those embodiments wherein the inducible promoter that regulates the expression of the site-specific recombinase is a rab17 promoter, the expression of the recombinase can be induced by desiccating a host cell (e.g., plant cell) or host (e.g., plant or plant part) or exposing the host cell or host to drought conditions, cold temperatures, or abscisic acid.

In some embodiments, the stress-inducible promoter of the presently disclosed polynucleotide constructs has the sequence set forth in SEQ ID NO: 18 or an active variant or fragment thereof. In other embodiments, the stress-inducible promoter of the presently disclosed polynucleotide constructs has the sequence set forth in SEQ ID NO: 17 or 19 or an active variant or fragment thereof.

In some embodiments of the methods and compositions, the polynucleotide constructs comprise active variants or fragments of the maize *rab17* promoter. An active variant or fragment of a maize *rab17* promoter (*e.g.*, SEQ ID NO: 17, 18, 19) is a polynucleotide variant or fragment that retains the ability to initiate transcription in response to drought conditions, desiccation, cold, and/or ABA. In some of these embodiments, the promoter comprises at least one DRE element. In some embodiments, an active fragment of a maize *rab17* promoter may comprise at least about 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 contiguous nucleotides of SEQ ID NO: 17, 18, or 19, or may have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 17, 18, or 19. In particular embodiments, the promoter of the compositions and methods comprises from about -219 to about -102 of the maize *rab17* promoter (corresponding to nucleotides 291 to 408 of SEQ ID NO: 18). In other embodiments, the active maize *rab17* promoter fragment comprises from about -219 to about -80 of the maize *rab17* promoter (nucleotides 291 to 430 of SEQ ID NO: 18), which comprises most of the DRE and ABRE elements.

In some embodiments, the expression of the site-specific recombinase is regulated by a promoter comprising a maize *rab17* promoter or a fragment or variant thereof, and an attachment site, such as an attachment B (attB) site as described in U.S. Application Publication No. 2011/0167516 (which is herein incorporated by reference in its entirety), and in some of these embodiments, the attB site modifies the activity of the maize *rab17* promoter.

As used herein, a "modulator" refers to a polynucleotide that when present between a promoter and a coding sequence, serves to increase or decrease the activity of the promoter. Non-limiting examples of modulators include recombination sites, operators, and insulators.

Attachment sites are site-specific recombination sites found in viral and bacterial genomes that facilitate the integration or excision of the viral genome into and out of its host genome. Non-limiting examples of a viral and bacterial host system that utilize attachment sites is the lambda bacteriophage and *E. coli* system (Weisberg and Landy (1983) In Lambda II, eds. Hendrix *et al.* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) pp.211-250). The modulator of the maize *rab17* promoter can be an *E.coli* attachment site B (attB) site. The attB site can be a naturally occurring *E. coli* attB site or an active variant or fragment thereof or a synthetically derived sequence. Synthetically derived attB sites and active variants and fragments of naturally occurring attB sites are those that are capable of recombining with a bacteriophage lambda attachment P site, a process that is catalyzed by the bacteriophage lambda Integrase (Int) and the *E. coli* Integration Host Factor (IHF) proteins (Landy (1989) *Ann Rev Biochem* 58: 913-949, which is herein incorporated by reference in its entirety). AttB sites typically have a

length of about 25 nucleotides, with a core 15-base pair sequence that is involved in the actual crossover event. Alternatively, active variants and fragments of naturally occurring attB sites are those that are capable of modulating the activity of a promoter. Non-limiting examples of attB sites that can be used include attB1 (SEQ ID NO: 20), attB2 (SEQ ID NO: 21), attB3 (SEQ ID NO: 22), and attB4 (SEQ ID NO: 23), and variants or fragments thereof. In some embodiments, the modulator is an active variant or fragment of an attB site that is capable of modulating (i.e., increasing, decreasing) the activity of a promoter, but is not capable of recombination with an attachment P site. Non-limiting examples of such active variants of an attB site include those having the sequence set forth in SEQ ID NO: 24, 25, or 26.

In some embodiments, the distance of the modulator (e.g., attB site) from the promoter impacts the ability of the modulator to modify the activity of the promoter. The modulator may be contiguous with the promoter and/or the coding polynucleotide. In other embodiments, a linker sequence separates the promoter sequence and the modulator (e.g., attB site). As used herein, a "linker sequence" is a nucleotide sequence that functions to link one functional sequence with another without otherwise contributing to the expression or translation of a coding polynucleotide. Accordingly, the actual sequence of the linker sequence can vary. The linker sequence can comprise plasmid sequences, restriction sites, and/or regions of the 5'-untranslated region (5'-UTR) of the gene from which the promoter is derived. The linker sequence separating the promoter and the modulator (e.g., attB site) can have a length of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 1000 nucleotides or greater. In certain embodiments, a linker sequence of about 133 nucleotides separates the maize *rab17* promoter and the modulator (e.g., attB site). In some embodiments, the linker sequence comprises a fragment of the *rab17* 5'-UTR. The fragment of the 5'-UTR can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 nucleotides, or greater, in length. In certain embodiments, the promoter comprises a linker sequence separating the maize *rab17* promoter and the modulator (e.g., attB site) that comprises 95 nucleotides of the maize *rab17* 5'-UTR. In some of these embodiments, the 95 nucleotide

sequence has the sequence set forth in SEQ ID NO: 27. In certain embodiments, the linker sequence between the maize *rab17* promoter and modulator (e.g., attB site) has the sequence set forth in SEQ ID NO: 28 or a variant or fragment thereof.

In some embodiments, the promoter comprises a linker sequence separating the modulator (e.g., attB site) and the site-specific recombinase-coding polynucleotide. The length and sequence of this linker may also vary and can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 1000 nucleotides or greater in length. In certain embodiments, a linker sequence of about 61 nucleotides separates the modulator (e.g., attB site) and the recombinase-encoding polynucleotide. In certain embodiments, the linker sequence between the modulator (e.g., attB site) and the coding polynucleotide has the sequence set forth in SEQ ID NO: 29 or a variant or fragment thereof. In other embodiments, a linker sequence of about 25 nucleotides separates the modulator (e.g., attB site) and the coding polynucleotide. In certain embodiments, the linker sequence between the modulator (e.g., attB site) and the coding polynucleotide has the sequence set forth in SEQ ID NO: 30.

In certain embodiments, the stress-inducible promoter that regulates the expression of the site-specific recombinase has the sequence set forth in SEQ ID NO: 31 or a variant or fragment thereof.

In other embodiments of the presently disclosed compositions and methods, the inducible promoter that regulates the expression of the site-specific recombinase is a chemical-inducible promoter. In some of these embodiments, the chemical-inducible promoter is a sulfonylurea (SU)-inducible promoter that has at least one operator sequence capable of binding to a sulfonylurea-responsive transcriptional repressor (SuR) protein, such as those disclosed in U.S. Application Publication Nos. 2010/0105141 and 2011/0287936.

As used herein, a "sulfonylurea-responsive transcriptional repressor" or "SuR" refers to a transcriptional repressor protein whose binding to an operator sequence is controlled by a ligand comprising a sulfonylurea compound. The SuR proteins useful in the presently disclosed methods and compositions include those that bind specifically to an operator sequence in the absence of a sulfonylurea ligand.

In some embodiments, the SuR protein is one that specifically binds to a tetracycline operator, wherein the specific binding is regulated by a sulfonylurea compound. Thus, in some embodiments, the sulfonylurea-inducible promoter comprises at least one tetracycline (tet) operator sequence. Tetracycline operator sequences are known in the art and include the tet operator sequence set forth in SEQ ID NO: 32. The tet operator sequence can be located within 0-30 nucleotides 5′ or 3′ of the TATA box of the chemical-regulated promoter, including, for example, within 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 nt of the TATA box. In other instances, the tet operator sequence may partially overlap with the TATA box sequence. In one non-limiting example, the tet operator sequence is SEQ ID NO: 32 or an active variant or fragment thereof.

Useful tet operator containing promoters include, for example, those known in the art (see, *e.g.*, Matzke *et al.* (2003) *Plant Mol Biol Rep* 21:9-19; Padidam (2003) *Curr Op Plant Biol* 6:169-177; Gatz & Quail (1988) *PNAS* 85:1394-1397; Ulmasov *et al.* (1997) *Plant Mol Biol* 35:417-424; Weinmann *et al.* (1994) *Plant J* 5:559-569; each of which is herein incorporated by reference in its entirety). One or more tet operator sequences can be added to a promoter in order to produce a sulfonylurea-inducible promoter. See, for example, Weinmann *et al.* (1994) *Plant J* 5:559-569; Love *et al.* (2000) *Plant J* 21:579-588. In addition, the widely tested tetracycline regulated expression system for plants using the CaMV 35S promoter (Gatz *et al.* (1992) *Plant J* 2:397-404; which is herein incorporated by reference in its entirety) having three tet operators introduced near the TATA box (3XOpT 35S) can be used as the sulfonylurea-inducible promoter.

Thus, a SU-inducible promoter comprising at least one, two, three or more operators capable of binding a SuR (including a tet operator, such as that set forth in SEQ ID NO:32 or an active variant or fragment thereof) can be used to regulate the expression of the site-specific recombinase. Any promoter can be combined with an operator capable of binding a SuR to generate a SU-inducible promoter. In specific embodiments, the promoter is active in plant cells. The promoter can be a constitutive promoter or a non-constitutive promoter. Non-constitutive promoters include tissue-preferred

promoter, such as a promoter that is primarily expressed in roots, leaves, stems, flowers, silks, anthers, pollen, meristem, seed, endosperm, or embryos.

In particular embodiments, the promoter is a plant actin promoter, a banana streak virus promoter (BSV), an MMV promoter, an enhanced MMV promoter (dMMV), a plant P450 promoter, or an elongation factor 1a (EF1A) promoter (U.S. Application Publication No. 20080313776, which is herein incorporated by reference in its entirety).

In those embodiments wherein the inducible promoter that is operably linked to the polynucleotide encoding the site-specific recombinase is a SU-inducible promoter, the host cell further comprises a sulfonylurea-responsive transcriptional repressor (SuR) or the polynucleotide construct comprises a polynucleotide encoding a SuR. Non-limiting examples of SuR polynucleotide and polypeptide sequences include those disclosed in U.S. Application Publication No. 2011/0287936, such as the polypeptide sequences set forth in SEQ ID NOs: 3-419 and the polynucleotide sequences set forth in SEQ ID NOs: 420-836 of U.S. Application Publication No. 2011/0287936, which is herein incorporated by reference in its entirety. Additional non-limiting examples of SuR polynucleotide and polypeptide sequences include those disclosed in U.S. Application Publication No. 2010/0105141, such as the polypeptide sequences set forth in SEQ ID NO: 3-401, 1206-1213, 1228-1233, and 1240-1243 and the polynucleotide sequences set forth in SEQ ID NO: 434-832, 1214-1221, 1222-1227, 1234-1239, and 1244-1247 of U.S. Application Publication No. 2010/0105141, which is herein incorporated by reference in its entirety.

In those embodiments wherein the presently disclosed polynucleotide constructs further comprise a polynucleotide encoding a SuR, the SuR-encoding polynucleotide is operably linked to a promoter that is active in a plant. The promoter may be a constitutive or a non-constitutive promoter, including a tissue-preferred promoter.

In particular embodiments, the promoter that is operably linked to the SuR-encoding polynucleotide comprises operator sequences that are capable of binding to SuR, which allows for autoregulation of the repressor and enhanced induction of the SU-inducible promoter and expression of the site-specific recombinase. See, for example, U.S. Application Publication No. 2011/0287936.

In particular embodiments, the SuR-encoding polynucleotide and optionally, the promoter operably linked thereto, is present within the excision cassette of the presently disclosed polynucleotide constructs, such that the polynucleotide is excised upon induction of the SU-inducible promoter and expression of the site-specific recombinase.

A variety of SU compounds can be used to bind to the SuR and induce the SU-inducible promoter. Sulfonylurea molecules comprise a sulfonylurea moiety (–S(O)2NHC(O)NH(R)–). In sulfonylurea herbicides, the sulfonyl end of the sulfonylurea moiety is connected either directly or by way of an oxygen atom or an optionally substituted amino or methylene group to a typically substituted cyclic or acyclic group. At the opposite end of the sulfonylurea bridge, the amino group, which may have a substituent such as methyl (R being CH_3) instead of hydrogen, is connected to a heterocyclic group, typically a symmetric pyrimidine or triazine ring, having one or two substituents such as methyl, ethyl, trifluoromethyl, methoxy, ethoxy, methylamino, dimethylamino, ethylamino and the halogens. Sulfonylurea herbicides can be in the form of the free acid or a salt. In the free acid form, the sulfonamide nitrogen on the bridge is not deprotonated (*i.e.*, –S(O)2NHC(O)NH(R)), while in the salt form, the sulfonamide nitrogen atom on the bridge is deprotonated, and a cation is present, typically of an alkali metal or alkaline earth metal, most commonly sodium or potassium. Sulfonylurea compounds include, for example, compound classes such as pyrimidinylsulfonylurea compounds, triazinylsulfonylurea compounds, thiadiazolylurea compounds, and pharmaceuticals such as antidiabetic drugs, as well as salts and other derivatives thereof. Examples of pyrimidinylsulfonylurea compounds include amidosulfuron, azimsulfuron, bensulfuron, bensulfuron-methyl, chlorimuron, chlorimuron-ethyl, cyclosulfamuron, ethoxysulfuron, flazasulfuron, flucetosulfuron, flupyrsulfuron, flupyrsulfuron-methyl, foramsulfuron, halosulfuron, halosulfuron-methyl, imazosulfuron, mesosulfuron, mesosulfuron-methyl, nicosulfuron, orthosulfamuron, oxasulfuron, primisulfuron, primisulfuron-methyl, pyrazosulfuron, pyrazosulfuron-ethyl, rimsulfuron, sulfometuron, sulfometuron-methyl, sulfosulfuron, trifloxysulfuron and salts and derivatives thereof. Examples of triazinylsulfonylurea compounds include chlorsulfuron, cinosulfuron, ethametsulfuron, ethametsulfuron-methyl, iodosulfuron, iodosulfuron-methyl,

metsulfuron, metsulfuron-methyl, prosulfuron, thifensulfuron, thifensulfuron-methyl, triasulfuron, tribenuron, tribenuron-methyl, triflusulfuron, triflusulfuron-methyl, tritosulfuron and salts and derivatives thereof. Examples of thiadiazolylurea compounds include buthiuron, ethidimuron, tebuthiuron, thiazafluron, thidiazuron, pyrimidinylsulfonylurea compound (e.g., amidosulfuron, azimsulfuron, bensulfuron, chlorimuron, cyclosulfamuron, ethoxysulfuron, flazasulfuron, flucetosulfuron, flupyrsulfuron, foramsulfuron, halosulfuron, imazosulfuron, mesosulfuron, nicosulfuron, orthosulfamuron, oxasulfuron, primisulftiron, pyrazosulfuron, rimsulfuron, sulfometuron, sulfosulfuron and trifloxysulfuron); a triazinylsulfonylurea compound (e.g., chlorsulfuron, cinosulfuron, ethametsulfuron, iodosulfuron, metsulfuron, prosulfuron, thifensulfuron, triasulfuron, tribenuron, triflusulfuron and tritosulfuron); or a thiadazolylurea compound (e.g., cloransulam, diclosulam, florasulam, flumetsulam, metosulam, and penoxsulam) and salts and derivatives thereof. Examples of antidiabetic drugs include acetohexamide, chlorpropamide, tolbutamide, tolazamide, glipizide, gliclazide, glibenclamide (glyburide), gliquidone, glimepiride and salts and derivatives thereof. In some systems, the SuR polypeptides specifically bind to more than one sulfonylurea compound, so one can chose which SU ligand to apply to the plant.

In some examples, the sulfonylurea compound is selected from the group consisting of chlorsulfuron, ethametsulfuron-methyl, metsulfuron-methyl, thifensulfuron-methyl, sulfometuron-methyl, tribenuron-methyl, chlorimuron-ethyl, nicosulfuron, and rimsulfuron.

In other embodiments, the sulfonylurea compound comprises a pyrimidinylsulfonylurea, a triazinylsulfonylurea, a thiadazolylurea, a chlorosulfuron, an ethametsulfuron, a thifensulfuron, a metsulfuron, a sulfometuron, a tribenuron, a chlorimuron, a nicosulfuron, or a rimsulfuron compound.

In some embodiments, it may be necessary for a plant or plant part that is contacted with a SU in order to induce the SU-inducible promoter to have tolerance to the SU. A host (e.g., a plant or plant part) may be naturally tolerant to the SU ligand, or the host (e.g., the plant or plant part) may be tolerant to the SU ligand as a result of human intervention such as, for example, by the use of a recombinant construct, plant breeding

or genetic engineering. Thus, the host (e.g., the plant or plant part) employed in the various methods disclosed herein can comprise a native or a heterologous sequence that confers tolerance to the sulfonylurea compound.

In some of these embodiments, the presently disclosed polynucleotide constructs can comprise a polynucleotide encoding a sulfonylurea-tolerance polypeptide, which is a polypeptide that when expressed in a host (e.g., plant or plant part) confers tolerance to at least one sulfonylurea. In some of these embodiments, the polynucleotide encoding the SU-tolerance polypeptide is comprised within the excision cassette.

In other embodiments, the herbicide tolerance polypeptide that is expressed upon excision of the excision cassette is a SU-tolerance polypeptide, such that the plant or plant part does not have tolerance to SU prior to the addition of SU to the plant or plant part, but upon the addition of SU, the excision cassette is excised and the SU-tolerance polypeptide is subsequently expressed, which allows for protection of the plant or plant part from damage due to the SU.

Sulfonylurea herbicides inhibit growth of higher plants by blocking acetolactate synthase (ALS), also known as, acetohydroxy acid synthase (AHAS). Thus, in some embodiments, the SU-tolerance polypeptide is an ALS inhibitor-tolerance polypeptide, as described elsewhere herein.

When the inducible promoter of the presently disclosed polynucleotide constructs is activated, a site-specific recombinase is expressed, which catalyzes the excision of the excision cassette comprised within the polynucleotide construct. As used herein, an "excision cassette" refers to a polynucleotide that is flanked by recombination sites that are recombinogenic with one another and directly repeated, such that when acted upon by a site-specific recombinase that recognizes the recombination sites, the nucleotide sequence within the recombination sites is excised from the remaining polynucleotide. The excision cassette of the presently disclosed polynucleotide constructs comprise a first expression cassette comprising a site-specific recombinase-encoding polynucleotide operably linked to an inducible promoter and optionally, at least one of a polynucleotide encoding a selectable marker, a polynucleotide encoding a cell proliferation factor, a

polynucleotide encoding a herbicide tolerance polypeptide, and a polynucleotide of interest.

A site-specific recombinase, also referred to herein as a recombinase, is a polypeptide that catalyzes conservative site-specific recombination between its compatible recombination sites, and includes native polypeptides as well as derivatives, variants and/or fragments that retain activity, and native polynucleotides, derivatives, variants, and/or fragments that encode a recombinase that retains activity. The recombinase used in the methods and compositions can be a native recombinase or a biologically active fragment or variant of the recombinase. For reviews of site-specific recombinases and their recognition sites, see Sauer (1994) *Curr Op Biotechnol* 5:521-527; and Sadowski (1993) *FASEB* 7:760-767, each of which is herein incorporated by reference in its entirety.

Any recombinase system can be used in the presently disclosed methods and compositions. Non-limiting examples of site-specific recombinases include FLP, Cre, S-CRE, V-CRE, Dre, SSV1, lambda Int, phi C31 Int, HK022, R, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, U153, and other site-specific recombinases known in the art, including those described in Thomson and Ow (2006) *Genesis* 44:465-476, which is herein incorporated by reference in its entirety. Examples of site-specific recombination systems used in plants can be found in U.S. Patent Nos. 5,929,301, 6,175,056, 6,331,661; and International Application Publication Nos. WO 99/25821, WO 99/25855, WO 99/25841, and WO 99/25840, the contents of each are herein incorporated by reference.

In some embodiments, the recombinase is a member of the Integrase or Resolvase families, including biologically active variants and fragments thereof. The Integrase family of recombinases has over one hundred members and includes, for example, FLP, Cre, lambda integrase, and R. For other members of the Integrase family, see, for example, Esposito *et al.* (1997) *Nucleic Acids Res* 25:3605-3614; and Abremski *et al.* (1992) *Protein Eng* 5:87-91; each of which are herein incorporated by reference in its entirety. Other recombination systems include, for example, the *Streptomycete* bacteriophage phi C31 (Kuhstoss *et al.* (1991) *J Mol Biol* 20:897-908); the SSV1 site-specific recombination system from *Sulfolobus shibatae* (Maskhelishvili *et al.* (1993) *Mol*

Gen Genet 237:334-342); and a retroviral integrase-based integration system (Tanaka *et al.* (1998) *Gene* 17:67-76). In some embodiments, the recombinase does not require cofactors or a supercoiled substrate. Such recombinases include Cre, FLP, or active variants or fragments thereof.

The FLP recombinase is a protein that catalyzes a site-specific reaction that is involved in amplifying the copy number of the two-micron plasmid of *S. cerevisiae* during DNA replication. FLP recombinase catalyzes site-specific recombination between two FRT sites. The FLP protein has been cloned and expressed (Cox (1993) *Proc Natl Acad Sci USA* 80:4223-4227, which is herein incorporated by reference in its entirety). The FLP recombinase for use in the methods and compositions may be derived from the genus *Saccharomyces*. In some embodiments, a recombinase polynucleotide modified to comprise more plant-preferred codons is used. A recombinant FLP enzyme encoded by a nucleotide sequence comprising maize preferred codons (FLPm) that catalyzes site-specific recombination events is known (the polynucleotide and polypeptide sequence of which is set forth in SEQ ID NO: 33 and 34, respectively; see, *e.g.*, US Patent 5,929,301, which is herein incorporated by reference in its entirety). Additional functional variants and fragments of FLP are known (Buchholz *et al.* (1998) *Nat Biotechnol* 16:657-662; Hartung *et al.* (1998) *J Biol Chem* 273:22884-22891; Saxena *et al.* (1997) *Biochim Biophys Acta* 1340:187-204; Hartley *et al.* (1980) *Nature* 286:860-864; Voziyanov *et al.* (2002) *Nucleic Acids Res* 30:1656-1663; Zhu & Sadowski (1995) *J Biol Chem* 270:23044-23054; and U.S. Patent No. 7,238,854, each of which is herein incorporated by reference in its entirety).

The bacteriophage recombinase Cre catalyzes site-specific recombination between two *lox* sites. The Cre recombinase is known (Guo *et al.* (1997) *Nature* 389:40-46; Abremski *et al.* (1984) *J Biol Chem* 259:1509-1514; Chen *et al.* (1996) *Somat Cell Mol Genet* 22:477-488; Shaikh *et al.* (1977) *J Biol Chem* 272:5695-5702; and, Buchholz *et al.* (1998) *Nat Biotechnol* 16:657-662, each of which is herein incorporated by reference in its entirety). Cre polynucleotide sequences may also be synthesized using plant-preferred codons, for example such sequences (moCre; the polynucleotide and polypeptide sequence of which is set forth in SEQ ID NO: 35 and 36, respectively) are described, for

example, in International Application Publication No. WO 99/25840, which is herein incorporated by reference in its entirety. Variants of the Cre recombinase are known (see, for example U.S. Patent No. 6,890,726; Rufer & Sauer (2002) *Nucleic Acids Res* 30:2764-2772; Wierzbicki *et al.* (1987) *J Mol Biol* 195:785-794; Petyuk *et al.* (2004) *J Biol Chem* 279:37040-37048; Hartung & Kisters-Woike (1998) *J Biol Chem* 273:22884-22891; Santoro & Schultz (2002) *Proc Natl Acad Sci USA* 99:4185-4190; Koresawa *et al.* (2000) *J Biochem* (Tokyo) 127:367-372; and Vergunst *et al.* (2000) *Science* 290:979-982, each of which are herein incorporated by reference in its entirety).

In some embodiments, the recombinase is a S-CRE, V-CRE recombinase (Suzuki & Nakayama (2011) *Nucl Acid Res* 39(8):e49) or Dre recombinase (Sauer & McDermott (2004) *Nucl Acid Res* 32(20):6086-6095), each of which is herein incorporated by reference in its entirety.

In some embodiments, the recombinase is a chimeric recombinase, which is a recombinant fusion protein that is capable of catalyzing site-specific recombination between recombination sites that originate from different recombination systems. For example, if the set of recombination sites comprises a FRT site and a LoxP site, a chimeric FLP/Cre recombinase or active variant or fragment thereof can be used, or both recombinases may be separately provided. Methods for the production and use of such chimeric recombinases or active variants or fragments thereof are described, for example, in International Application Publication No. WO 99/25840; and Shaikh & Sadowski (2000) *J Mol Biol* 302:27-48, each of which are herein incorporated by reference in its entirety.

In other embodiments, a variant recombinase is used. Methods for modifying the kinetics, cofactor interaction and requirements, expression, optimal conditions, and/or recognition site specificity, and screening for activity of recombinases and variants are known, see for example Miller *et al.* (1980) *Cell* 20:721-9; Lange-Gustafson and Nash (1984) *J Biol Chem* 259:12724-32; Christ *et al.* (1998) *J Mol Biol* 288:825-36; Lorbach *et al.* (2000) *J Mol Biol* 296:1175-81; Vergunst *et al.* (2000) *Science* 290:979-82; Dorgai *et al.* (1995) *J Mol Biol* 252:178-88; Dorgai *et al.* (1998) *J Mol Biol* 277:1059-70; Yagu *et al.* (1995) *J Mol Biol* 252:163-7; Sclimente *et al.* (2001) *Nucleic Acids Res* 29:5044-51;

Santoro and Schultze (2002) *Proc Natl Acad Sci USA* 99:4185-90; Buchholz and Stewart (2001) *Nat Biotechnol* 19:1047-52; Voziyanov *et al.* (2002) *Nucleic Acids Res* 30:1656-63; Voziyanov *et al.* (2003) *J Mol Biol* 326:65-76; Klippel *et al.* (1988) *EMBO J* 7:3983-9; Arnold *et al.* (1999) *EMBO J* 18:1407-14; and International Application Publication Nos. WO 03/08045, WO 99/25840, and WO 99/25841; each of which is herein incorporated by reference in its entirety.

By "recombination site" is intended a polynucleotide (native or synthetic/artificial) that is recognized by the recombinase enzyme of interest. As outlined above, many recombination systems are known in the art and one of skill will recognize the appropriate recombination site to be used with the recombinase of interest.

Non-limiting examples of recombination sites include FRT sites including, for example, the native FRT site (FRT1, SEQ ID NO:37), and various functional variants of FRT, including but not limited to, FRT5 (SEQ ID NO:38), FRT6 (SEQ ID NO:39), FRT7 (SEQ ID NO:40), FRT12 (SEQ ID NO: 41), and FRT87 (SEQ ID NO:42). See, for example, International Application Publication Nos. WO 03/054189, WO 02/00900, and WO 01/23545; and Schlake *et al.* (1994) *Biochemistry* 33:12745-12751, each of which is herein incorporated by reference. Recombination sites from the Cre/Lox site-specific recombination system can be used. Such recombination sites include, for example, native LOX sites and various functional variants of LOX.

In some embodiments, the recombination site is a functional variant of a FRT site or functional variant of a LOX site, any combination thereof, or any other combination of recombinogenic or non-recombinogenic recombination sites known. Functional variants include chimeric recombination sites, such as an FRT site fused to a LOX site (see, for example, Luo *et al.* (2007) *Plant Biotech J* 5:263-274, which is herein incorporated by reference in its entirety). Functional variants also include minimal sites (FRT and/or LOX alone or in combination). The minimal native FRT recombination site (SEQ ID NO: 37) has been characterized and comprises a series of domains comprising a pair of 11 base pair symmetry elements, which are the FLP binding sites; the 8 base pair core, or spacer, region; and the polypyrimidine tracts. In some embodiments, at least one

modified FRT recombination site is used. Modified or variant FRT recombination sites are sites having mutations such as alterations, additions, or deletions in the sequence. The modifications include sequence modification at any position, including but not limited to, a modification in at least one of the 8 base pair spacer domain, a symmetry element, and/or a polypyrimidine tract. FRT variants include minimal sites (see, *e.g.*, Broach *et al.* (1982) *Cell* 29:227-234; Senecoff *et al.* (1985) *Proc Natl Acad Sci USA* 82:7270-7274; Gronostajski & Sadowski (1985) *J Biol Chem* 260:12320-12327; Senecoff *et al.* (1988) *J Mol Biol* 201:405-421; and International Application Publication No. WO99/25821), and sequence variants (see, for example, Schlake & Bode (1994) *Biochemistry* 33:12746-12751; Seibler & Bode (1997) *Biochemistry* 36:1740-1747; Umlauf & Cox (1988) *EMBO J* 7:1845-1852; Senecoff *et al.* (1988) *J Mol Biol* 201:405-421; Voziyanov *et al.* (2002) *Nucleic Acids* Res 30:7; International Application Publication Nos. WO 07/011733, WO 99/25854, WO 99/25840, WO 99/25855, WO 99/25853 and WO 99/25821; and U.S. Patent Nos. 7.060,499 and 7,476,539; each of which are herein incorporated by reference in its entirety).

An analysis of the recombination activity of variant LOX sites is presented in Lee *et al.* (1998) *Gene* 216:55-65 and in U.S. Patent No. 6,465,254. Also, see for example, Huang *et al.* (1991) *Nucleic Acids Res* 19:443-448; Sadowski (1995) In *Progress in Nucleic Acid Research and Molecular Biology* Vol. 51, pp. 53-91; U.S. Patent No. 6,465,254; Cox (1989) In *Mobile DNA*, Berg and Howe (eds) American Society of Microbiology, Washington D.C., pp. 116-670; Dixon *et al.* (1995) *Mol Microbiol* 18:449-458; Buchholz *et al.* (1996) *Nucleic Acids Res* 24:3118-3119; Kilby *et al.* (1993) *Trends Genet* 9:413-421; Rossant & Geagy (1995) *Nat Med* 1:592-594; Albert *et al.* (1995) *Plant J* 7:649-659; Bayley *et al.* (1992) *Plant Mol Biol* 18:353-361; Odell *et al.* (1990) *Mol Gen Genet* 223:369-378; Dale & Ow (1991) *Proc Natl Acad Sci USA* 88:10558-10562; Qui *et al.* (1994) *Proc Natl Acad Sci USA* 91:1706-1710; Stuurman *et al.* (1996) *Plant Mol Biol* 32:901-913; Dale *et al.* (1990) *Gene* 91:79-85; and International Application Publication No. WO 01/111058; each of which is herein incorporated by reference in its entirety.

Naturally occurring recombination sites or biologically active variants thereof are of use. Methods to determine if a modified recombination site is recombinogenic are known (see, for example, International Application Publication No. WO 07/011733, which is herein incorporated by reference in its entirety). Variant recognition sites are known, see for example, Hoess *et al.* (1986) *Nucleic Acids Res* 14:2287-300; Albert *et al.* (1995) *Plant J* 7:649-59; Thomson *et al.* (2003) Genesis 36:162-7; Huang et al. (1991) *Nucleic Acids Res* 19:443-8; Siebler and Bode (1997) *Biochemistry* 36:1740-7; Schlake and Bode (1994) *Biochemistry* 33:12746-51; Thygarajan et al. (2001) *Mol Cell Biol* 21:3926-34; Umlauf and Cox (1988) *EMBO J* 7:1845-52; Lee and Saito (1998) *Gene* 216:55-65; International Application Publication Nos. WO 01/23545, WO 99/25851, WO 01/11058, WO 01/07572; and U.S. Patent No. 5,888,732; each of which is herein incorporated by reference in its entirety.

The recombination sites employed in the methods and compositions can be identical or dissimilar sequences, so long as the sites are recombinogenic with respect to one another.

By "recombinogenic" is intended that the set of recombination sites (*i.e.*, dissimilar or corresponding) are capable of recombining with one another. Alternatively, by "non-recombinogenic" is intended the set of recombination sites, in the presence of the appropriate recombinase, will not recombine with one another or recombination between the sites is minimal. Accordingly, it is recognized that any suitable set of recombinogenic recombination sites may be utilized, including a FRT site or functional variant thereof, a LOX site or functional variant thereof, any combination thereof, or any other combination of recombination sites known in the art.

In some embodiments, the recombination sites are asymmetric, and the orientation of any two sites relative to each other will determine the recombination reaction product. Directly repeated recombination sites are those recombination sites in a set of recombinogenic recombination sites that are arranged in the same orientation, such that recombination between these sites results in excision, rather than inversion, of the intervening DNA sequence. Inverted recombination sites are those recombination sites in a set of recombinogenic recombination sites that are arranged in the opposite orientation,

so that recombination between these sites results in inversion, rather than excision, of the intervening DNA sequence. The presently disclosed polynucleotide constructs comprise recombination sites that are recombinogenic with one another and directly repeated so as to result in excision of the excision cassette.

The presently disclosed compositions and methods utilize at least one polynucleotide that confers herbicide tolerance. Tolerance to specific herbicides can be conferred by engineering genes into plants which encode appropriate herbicide metabolizing enzymes and/or insensitive herbicide targets. Such polypeptides are referred to as "herbicide tolerance polypeptides". In some embodiments these enzymes, and the nucleic acids that encode them, originate from a plant. In other embodiments, they are derived from other organisms, such as microbes. *See, e.g.*, Padgette *et al.* (1996) "New weed control opportunities: Development of soybeans with a Roundup Ready® gene" and Vasil (1996) "Phosphinothricin-resistant crops," both in *Herbicide-Resistant Crops*, ed. Duke (CRC Press, Boca Raton, Florida) pp. 54-84 and pp. 85-91.

An "herbicide" is a chemical that causes temporary or permanent injury to a plant. Non-limiting examples of herbicides that can be employed in the various methods and compositions of the invention are discussed in further detail elsewhere herein. A herbicide may be incorporated into the plant or plant part, or it may act on the plant or plant part without being incorporated into the plant or plant part. An "active ingredient" is the chemical in a herbicide formulation primarily responsible for its phytotoxicity and which is identified as the active ingredient on the product label. Product label information is available from the U.S. Environmental Protection Agency and is updated online at the url oaspub.epa.gov/pestlabl/ppls.own; product label information is also available online at the url www.cdms.net.

"Herbicide-tolerant" or "tolerant" in the context of herbicide or other chemical treatment as used herein means that a plant or plant part treated with a particular herbicide or class or subclass of herbicide or other chemical or class or subclass of other chemical will show no significant damage or less damage following that treatment in comparison to an appropriate control plant or plant part. A plant or plant part may be naturally tolerant to a particular herbicide or chemical, or a plant or plant part may be herbicide-

tolerant as a result of human intervention such as, for example, breeding or genetic engineering. An "herbicide-tolerance polypeptide" is a polypeptide that confers herbicide tolerance on a plant or other organism expressing it (*i.e.*, that makes a plant or other organism herbicide-tolerant), and an "herbicide-tolerance polynucleotide" is a polynucleotide that encodes a herbicide-tolerance polypeptide. For example, a sulfonylurea-tolerance polypeptide is one that confers tolerance to sulfonylurea herbicides on a plant or other organism that expresses it, an imidazolinone-tolerance polypeptide is one that confers tolerance to imidazolinone herbicides on a plant or other organism that expresses it; and a glyphosate-tolerance polypeptide is one that confers tolerance to glyphosate on a plant or other organism that expresses it.

Thus, a plant or plant part is tolerant to a herbicide or other chemical if it shows damage in comparison to an appropriate control plant or plant part that is less than the damage exhibited by the control plant or plant part by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, or 1000% or more. In this manner, a plant or plant part that is tolerant to a herbicide or other chemical shows "improved tolerance" in comparison to an appropriate control plant or plant part. Damage resulting from herbicide or other chemical treatment is assessed by evaluating any parameter of plant growth or well-being deemed suitable by one of skill in the art. Damage can be assessed by visual inspection and/or by statistical analysis of suitable parameters of individual plants or plant parts or of a group of plants or plant parts. Thus, damage may be assessed by evaluating, for example, parameters such as plant height, plant weight, leaf color, leaf length, flowering, fertility, silking, yield, seed production, and the like. Damage may also be assessed by evaluating the time elapsed to a particular stage of development (*e.g.*, silking, flowering, or pollen shed) or the time elapsed until a plant has recovered from treatment with a particular chemical and/or herbicide.

In making such assessments, particular values may be assigned to particular degrees of damage so that statistical analysis or quantitative comparisons may be made. The use of ranges of values to describe particular degrees of damage is known in the art, and any suitable range or scale may be used. For example, herbicide injury scores (also

called tolerance scores) can be assigned as set forth in Table 2. In this scale, a rating of 9 indicates that a herbicide treatment had no effect on a crop, *i.e.*, that no crop reduction or injury was observed following the herbicide treatment. Thus, in this scale, a rating of 9 indicates that the crop exhibited no damage from the herbicide and therefore that the crop is tolerant to the herbicide. As indicated above, herbicide tolerance is also indicated by other ratings in this scale where an appropriate control plant exhibits a lower score on the scale, or where a group of appropriate control plants exhibits a statistically lower score in response to a herbicide treatment than a group of subject plants.

Table 2. Herbicide injury scale (1 to 9 scale scoring system).

Rating	**Main categories**	**Detailed description**
9	No Effect	No crop reduction or injury
8	Slight Effect	Slight crop discoloration or stunting
7		Some crop discoloration, stunting, or stunt loss
6		Crop injury more pronounced, but not lasting
5	Moderate Effect	Moderate injury, crop usually recovers
4		Crop injury more lasting, recovery doubtful
3		Lasting crop injury, no recovery

A herbicide does not "significantly damage" a plant or plant part when it either has no effect on a plant or plant part or when it has some effect on a plant or plant part from which the plant later recovers, or when it has an effect which is detrimental but which is offset, for example, by the impact of the particular herbicide on weeds. Thus, for example, a plant or plant part is not "significantly damaged by" a herbicide or other treatment if it exhibits less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% decrease in at least one suitable parameter that is indicative of plant health and/or productivity in comparison to an appropriate control plant or plant part (*e.g.*, an untreated plant or plant part). Suitable parameters that are indicative of plant health and/or productivity include, for example, plant height, plant weight, leaf length, time elapsed to a particular stage of development, flowering, yield, seed production, and the like. The evaluation of a parameter can be by visual inspection and/or by statistical analysis of any suitable parameter. Comparison may be made by

visual inspection and/or by statistical analysis. Accordingly, a plant or plant part is not "significantly damaged by" a herbicide or other treatment if it exhibits a decrease in at least one parameter but that decrease is temporary in nature and the plant or plant part recovers fully within 1 week, 2 weeks, 3 weeks, 4 weeks, or 6 weeks.

Conversely, a plant or plant part is significantly damaged by a herbicide or other treatment if it exhibits more than a 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 150%, 170% decrease in at least one suitable parameter that is indicative of plant health and/or productivity in comparison to an appropriate control plant or plant part. Thus, a plant or plant part is significantly damaged if it exhibits a decrease in at least one parameter and the plant or plant part does not recover fully within 1 week, 2 weeks, 3 weeks, 4 weeks, or 6 weeks.

Damage resulting from a herbicide or other chemical treatment of a plant or plant part can be assessed by visual inspection by one of skill in the art and can be evaluated by statistical analysis of suitable parameters. The plant or plant part being evaluated is referred to as the "test plant" or "test plant part." Typically, an appropriate control plant or plant part is one that expresses the same herbicide-tolerance polypeptide(s) as the plant or plant part being evaluated for herbicide tolerance (*i.e.*, the "test plant") but that has not been treated with herbicide. In some circumstances, the control plant or plant part is one that has been subjected to the same herbicide treatment as the plant or plant part being evaluated (*i.e.*, the test plant or plant part) but that does not express the enzyme intended to provide tolerance to the herbicide of interest in the test plant or plant part. One of skill in the art will be able to design, perform, and evaluate a suitable controlled experiment to assess the herbicide tolerance of a plant or plant part of interest, including the selection of appropriate test plants or plant part, control plants or plant part, and treatments.

Damage caused by a herbicide or other chemical can be assessed at various times after a plant or plant part has been contacted with a herbicide, although in some embodiments, assessment of the plant or plant part for herbicide tolerance occurs during or after rooting/regeneration of the plant or plant part. Often, damage is assessed at about the time that the control plant or plant part exhibits maximum damage. Sometimes, damage is assessed after a period of time in which a control plant or plant part that was

not treated with herbicide has measurably grown and/or developed in comparison to the size or stage at which the treatment was administered. Damage can be assessed at various times, for example, at 12 hours or at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 days, or three weeks, four weeks, or longer after the test plant or plant part was treated with herbicide. Any time of assessment is suitable as long as it permits detection of a difference in response to a treatment of test and control plants or plant parts.

Thus, as used herein, a "test plant" or "test plant part" is one which has been transformed with the presently disclosed polynucleotide constructs or is a plant or plant part which is descended from a plant or plant part so altered and which comprises the herbicide tolerance polynucleotide.

A "control" or "control plant" or "control plant part" provides a reference point for measuring changes in phenotype of the subject plant or plant part, and may be any suitable plant or plant part. A control plant or plant part may comprise, for example: (a) a wild-type plant or plant part, *i.e.*, an untransformed plant of the same genotype as the test plant or plant part prior to transformation; (b) a plant or plant part of the same genotype as the starting material but which has been transformed with a null construct (*i.e.*, with a construct which has no known effect on the trait of interest, such as a construct comprising a marker gene); (c) a plant or plant part which is a non-transformed segregant among progeny of a subject plant or plant part; (d) a plant or plant part which is genetically identical to the subject plant or plant part but which is not exposed to the same treatment (*e.g.*, herbicide treatment) as the subject plant or plant part; (e) the subject plant or plant part itself, under conditions in which the herbicide tolerance polynucleotide is not expressed; or (f) the subject plant or plant part itself, under conditions in which it has not been exposed to a particular treatment such as, for example, a herbicide or combination of herbicides and/or other chemicals. In some instances, an appropriate control maize plant or plant part comprises a NK603 event (Nielson *et al.* (2004) *European Food Research and Technology* 219:421-427 and Ridley *et al.* (2002) *Journal of Agriculture and Food Chemistry* 50: 7235-7243), an elite stiff stalk inbred plant, a P3162 plant (Pioneer Hi-Bred International), a 39T66 plant (Pioneer Hi-Bred International), or a 34M91 plant

(Pioneer Hi-Bred International). In some instances, an appropriate control soybean plant or plant part is a "Jack" soybean plant (Illinois Foundation Seed, Champaign, Illinois).

The herbicide tolerance polypeptides used in the presently disclosed compositions and methods can confer tolerance to any respective herbicide. In some embodiments, the herbicide tolerance polypeptide confers tolerance to a herbicide selected from the group consisting of glyphosate, an ALS inhibitor (e.g., a sulfonylurea), an acetyl Co-A carboxylase inhibitor, a synthetic auxin, a protoporphyrinogen oxidase (PPO) inhibitor herbicide, a pigment synthesis inhibitor herbicide, a phosphinothricin acetyltransferase or a phytoene desaturase inhibitor, a glutamine synthase inhibitor, a hydroxyphenylpyruvatedioxygenase inhibitor, and a protoporphyrinogen oxidase inhibitor.

One herbicide which has been studied extensively is N-phosphonomethylglycine, commonly referred to as glyphosate. Glyphosate is a broad spectrum herbicide that kills both broadleaf and grass-type plants due to inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (also referred to as "EPSP synthase" or "EPSPS"), an enzyme which is part of the biosynthetic pathway for the production of aromatic amino acids, hormones, and vitamins. Glyphosate-resistant transgenic plants have been produced which exhibit a commercially viable level of glyphosate resistance due to the introduction of a modified *Agrobacterium* CP4 EPSPS. This modified enzyme is targeted to the chloroplast where, even in the presence of glyphosate, it continues to synthesize EPSP from phosphoenolpyruvic acid ("PEP") and shikimate-3-phosphate. CP4 glyphosate-resistant soybean transgenic plants are presently in commercial use (*e.g.*, as sold by Monsanto under the name "Roundup Ready®").

In some embodiments, the presently disclosed methods and compositions utilize a polynucleotide that encodes a herbicide tolerance polypeptide that confers tolerance to glyphosate. Various sequences which confer tolerance to glyphosate can be employed in the presently disclosed methods and compositions. In some embodiments, the herbicide tolerance polypeptide that confers resistance to glyphosate has glyphosate transferase activity. As used herein, a "glyphosate transferase" polypeptide has the ability to transfer the acetyl group from acetyl CoA to the N of glyphosate, transfer the propionyl group of

propionyl CoA to the N of glyphosate, or to catalyze the acetylation of glyphosate analogs and/or glyphosate metabolites, *e.g.*, aminomethylphosphonic acid. Methods to assay for this activity are disclosed, for example, in U.S. Publication No. 2003/0083480, U.S. Publication No. 2004/0082770, and U.S. Patent No. 7,405,074, WO2005/012515, WO2002/36782 and WO2003/092360. In one embodiment, the transferase polypeptide comprises a glyphosate-N-acetyltransferase "GLYAT" polypeptide.

As used herein, a GLYAT polypeptide or enzyme comprises a polypeptide which has glyphosate-N-acetyltransferase activity ("GLYAT" activity), *i.e.*, the ability to catalyze the acetylation of glyphosate. In specific embodiments, a polypeptide having glyphosate-N-acetyltransferase activity can transfer the acetyl group from acetyl CoA to the N of glyphosate. In addition, some GLYAT polypeptides transfer the propionyl group of propionyl CoA to the N of glyphosate. Some GLYAT polypeptides are also capable of catalyzing the acetylation of glyphosate analogs and/or glyphosate metabolites, *e.g.*, aminomethylphosphonic acid. GLYAT polypeptides are characterized by their structural similarity to one another, *e.g.*, in terms of sequence similarity when the GLYAT polypeptides are aligned with one another. Exemplary GLYAT polypeptides and the polynucleotides encoding them are known in the art and particularly disclosed, for example, in U.S. App. Publ. No. 2003/0083480, and U.S. Patent Nos. 7,462,481, 7,531,339, 7,622,641, and 7,405,074, each of which is herein incorporated by reference in its entirety. In some embodiments, GLYAT polypeptides used in the presently disclosed methods and compositions comprise the amino acid sequence set forth in: SEQ ID NO: 43, 44, 45, 46, 48, or 50. In some embodiments, the GLYAT polynucleotide that encodes the GLYAT polypeptide that is used in the presently disclosed methods and compositions are set forth in SEQ ID NO: 47 or 49. As discussed in further detail elsewhere herein, the use of fragments and variants of GLYAT polynucleotides and other known herbicide-tolerance polynucleotides and polypeptides encoded thereby is also encompassed by the present invention.

Active variants of SEQ ID NOS: 43, 44, 45, 46, 48, or 50 which retain glyphosate N-acetyltranserase activity include sequences which generate a similarity score of at least 430 using the BLOSUM62 matrix, a gap existence penalty of 11, and a gap extension

penalty of 1 when optimally aligned with any one of SEQ ID NO. Some aspects of the invention pertain to GAT polypeptides comprising an amino acid sequence that can be optimally aligned with an amino acid sequence selected from the group consisting of SEQ ID NOS: 43, 44, 45, 46, 48, and 50 to generate a similarity score of at least 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 505, 510, 515, 520, 525, 530, 535, 540, 545, 550, 555, 560, 565, 570, 575, 580, 585, 590, 595, 600, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, or 760 using the BLOSUM62 matrix, a gap existence penalty of 11, and a gap extension penalty of 1. Two sequences are "optimally aligned" when they are aligned for similarity scoring using a defined amino acid substitution matrix (e.g., BLOSUM62), gap existence penalty and gap extension penalty so as to arrive at the highest score possible for that pair of sequences.

Plants expressing GLYAT that have been treated with glyphosate contain the glyphosate metabolite N-acetylglyphosate ("NAG"). The presence of N-acetylglyphosate can serve as a diagnostic marker for the presence of an active GLYAT gene in a plant and can be evaluated by methods known in the art, for example, by mass spectrometry or by immunoassay. Generally, the level of NAG in a plant containing a GLYAT gene that has been treated with glyphosate is correlated with the activity of the GLYAT gene and the amount of glyphosate with which the plant has been treated.

Polynucleotides that encode glyphosate tolerance polypeptides that can be used in the presently disclosed methods and compositions include those that encode a glyphosate oxido-reductase enzyme as described more fully in U.S. Patent Nos. 5,776,760 and 5,463,175, which are incorporated herein by reference in their entireties for all purposes.

Other herbicides commonly used for commercial crop production include glufosinate (phosphinothricin) and acetolactate synthase (ALS) chemistry such as the sulfonylurea herbicides. Glufosinate is a broad spectrum herbicide which acts on the chloroplast glutamate synthase enzyme. Glufosinate-tolerant transgenic plants have been produced which carry the *bar* gene from *Streptomyces hygroscopicus*. The enzyme encoded by the *bar* gene has N-acetylation activity and modifies and detoxifies glufosinate. Glufosinate-tolerant plants are presently in commercial use (*e.g.*, as sold by

Bayer under the name "Liberty Link®"). As described elsewhere herein, sulfonylurea herbicides inhibit growth of higher plants by blocking acetolactate synthase (ALS). Plants containing particular mutations in ALS are tolerant to the ALS herbicides including sulfonylureas.

In some embodiments, the herbicide tolerance polypeptide that is utilized in the presently disclosed methods and compositions is an ALS inhibitor-tolerance polypeptide. As used herein, an "ALS inhibitor-tolerance polypeptide" comprises any polypeptide which when expressed in a plant confers tolerance to at least one ALS inhibitor. A variety of ALS inhibitors are known and include, for example, sulfonylurea, imidazolinone, triazolopyrimidines, pryimidinyoxy(thio)benzoates, and/or sulfonylaminocarbonyltriazolinone herbicides. Additional ALS inhibitors are known and are disclosed elsewhere herein. It is known in the art that ALS mutations fall into different classes with regard to tolerance to sulfonylureas, imidazolinones, triazolopyrimidines, and pyrimidinyl(thio)benzoates, including mutations having the following characteristics: (1) broad tolerance to all four of these groups; (2) tolerance to imidazolinones and pyrimidinyl(thio)benzoates; (3) tolerance to sulfonylureas and triazolopyrimidines; and (4) tolerance to sulfonylureas and imidazolinones.

Various ALS inhibitor-tolerance polypeptides can be employed. In some embodiments, the ALS inhibitor-tolerance polynucleotides contain at least one nucleotide mutation resulting in one amino acid change in the ALS polypeptide. In specific embodiments, the change occurs in one of seven substantially conserved regions of acetolactate synthase. See, for example, Hattori *et al.* (1995) *Molecular Genetics and Genomes* 246:419-425; Lee *et al.* (1998) *EMBO Journal* 7:1241-1248; Mazur *et al.* (1989) *Ann. Rev. Plant Phys.* 40:441-470; and U.S. Patent No. 5,605,011, each of which is incorporated by reference in their entirety. The ALS inhibitor-tolerance polypeptide can be encoded by, for example, the SuRA or SuRB locus of ALS. In specific embodiments, the ALS inhibitor-tolerance polypeptide comprises the C3 ALS mutant, the HRA ALS mutant, the S4 mutant or the S4/HRA mutant or any combination thereof. Different mutations in ALS are known to confer tolerance to different herbicides and groups (and/or subgroups) of herbicides; see, e.g., Tranel and Wright (2002) *Weed*

Science 50:700-712. See also, U.S. Patent No. 5,605,011, 5,378,824, 5,141,870, 5,013,659, and 7,622,641, each of which is herein incorporated by reference in their entirety. See also, SEQ ID NO:51 comprising a soybean HRA sequence; SEQ ID NO:52 comprising a maize HRA sequence; and SEQ ID NO:53 comprising an *Arabidopsis* HRA sequence. The HRA mutation in ALS finds particular use in one embodiment of the invention. The mutation results in the production of an acetolactate synthase polypeptide which is resistant to at least one ALS inhibitor chemistry in comparison to the wild-type protein. For example, a plant expressing an ALS inhibitor-tolerant polypeptide may be tolerant of a dose of sulfonylurea, imidazolinone, triazolopyrimidines, pryimidinyloxy(thio)benzoates, and/or sulfonylaminocarbonyltriazolinone herbicide that is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 50, 70, 80, 100, 125, 150, 200, 500, or 1000 times higher than a dose of the herbicide that would cause damage to an appropriate control plant. In some embodiments, an ALS inhibitor-tolerant polypeptide comprises a number of mutations.

In some embodiments, the ALS inhibitor-tolerance polypeptide confers tolerance to sulfonylurea and imidazolinone herbicides. Sulfonylurea and imidazolinone herbicides inhibit growth of higher plants by blocking acetolactate synthase (ALS), also known as, acetohydroxy acid synthase (AHAS). For example, plants containing particular mutations in ALS (*e.g.*, the S4 and/or HRA mutations) are tolerant to sulfonylurea herbicides. The production of sulfonylurea-tolerant plants and imidazolinone-tolerant plants is described more fully in U.S. Patent Nos. 5,605,011; 5,013,659; 5,141,870; 5,767,361; 5,731,180; 5,304,732; 4,761,373; 5,331,107; 5,928,937; and 5,378,824; and international publication WO 96/33270, which are incorporated herein by reference in their entireties for all purposes. In specific embodiments, the ALS inhibitor-tolerance polypeptide comprises a sulfonamide-tolerant acetolactate synthase (otherwise known as a sulfonamide-tolerant acetohydroxy acid synthase) or an imidazolinone-tolerant acetolactate synthase (otherwise known as an imidazolinone-tolerant acetohydroxy acid synthase).

Often, a herbicide-tolerance polynucleotide that confers tolerance to a particular herbicide or other chemical or a plant expressing it will also confer tolerance to other

herbicides or chemicals in the same class or subclass, for example, a class or subclass set forth in Table 3.

Table 3: Abbreviated version of HRAC Herbicide Classification

I. ALS Inhibitors (WSSA Group 2)
A. Sulfonylureas
1. Azimsulfuron
2. Chlorimuron-ethyl
3. Metsulfuron-methyl
4. Nicosulfuron
5. Rimsulfuron
6. Sulfometuron-methyl
7. Thifensulfuron-methyl
8. Tribenuron-methyl
9. Amidosulfuron
10. Bensulfuron-methyl
11. Chlorsulfuron
12. Cinosulfuron
13. Cyclosulfamuron
14. Ethametsulfuron-methyl
15. Ethoxysulfuron
16. Flazasulfuron
17. Flupyrsulfuron- methyl
18. Foramsulfuron
19. Imazosulfuron
20. Iodosulfuron-methyl
21. Mesosulfuron-methyl
22. Oxasulfuron
23. Primisulfuron-methyl
24. Prosulfuron
25. Pyrazosulfuron-ethyl
26. Sulfosulfuron
27. Triasulfuron
28. Trifloxysulfuron
29. Triflusulfuron-methyl
30. Tritosulfuron
31. Halosulfuron-methyl
32. Flucetosulfuron
B. Sulfonylaminocarbonyltriazolinones
1. Flucarbazone
2. Procarbazone
C. Triazolopyrimidines
1. Cloransulam-methyl
2. Flumetsulam
3. Diclosulam
4. Florasulam
5. Metosulam
6. Penoxsulam
7. Pyroxsulam
D. Pyrimidinyloxy(thio)benzoates
1. Bispyribac
2. Pyriftalid
3. Pyribenzoxim
4. Pyrithiobac
5. Pyriminobac-methyl
E. Imidazolinones
1. Imazapyr
2. Imazethapyr
3. Imazaquin
4. Imazapic
5. Imazamethabenz-methyl
6. Imazamox
II. Other Herbicides--Active Ingredients/ Additional Modes of Action
A. Inhibitors of Acetyl CoA carboxylase (ACCase) (WSSA Group 1)
1. Aryloxyphenoxypropionates ('FOPs')
a. Quizalofop-P-ethyl
b. Diclofop-methyl
c. Clodinafop-propargyl
d. Fenoxaprop-P-ethyl
e. Fluazifop-P-butyl
f. Propaquizafop
g. Haloxyfop-P-methyl
h. Cyhalofop-butyl
i. Quizalofop-P-ethyl
2. Cyclohexanediones ('DIMs')
a. Alloxydim
b. Butroxydim
c. Clethodim
d. Cycloxydim
e. Sethoxydim
f. Tepraloxydim
g. Tralkoxydim
B. Inhibitors of Photosystem II—HRAC Group C1/ WSSA Group 5
1. Triazines
a. Ametryne
b. Atrazine
c. Cyanazine
d. Desmetryne
e. Dimethametryne
f. Prometon
g. Prometryne
h. Propazine
i. Simazine
j. Simetryne
k. Terbumeton

l. Terbuthylazine
m. Terbutryne
n. Trietazine
2. Triazinones
a. Hexazinone
b. Metribuzin
c. Metamitron
3. Triazolinone
a. Amicarbazone
4. Uracils
a. Bromacil
b. Lenacil
c. Terbacil
5. Pyridazinones
a. Pyrazon
6. Phenyl carbamates
a. Desmedipham
b. Phenmedipham
C. Inhibitors of Photosystem II--HRAC Group C2/WSSA Group 7
1. Ureas
a. Fluometuron
b. Linuron
c. Chlorobromuron
d. Chlorotoluron
e. Chloroxuron
f. Dimefuron
g. Diuron
h. Ethidimuron
i. Fenuron
j. Isoproturon
k. Isouron
l. Methabenzthiazuron
m. Metobromuron
n. Metoxuron
o. Monolinuron
p. Neburon
q. Siduron
r. Tebuthiuron
2. Amides
a. Propanil
b. Pentanochlor
D. Inhibitors of Photosystem II--HRAC Group C3/ WSSA Group 6
1. Nitriles
a. Bromofenoxim
b. Bromoxynil
c. Ioxynil
2. Benzothiadiazinone (Bentazon)
a. Bentazon
3. Phenylpyridazines
a. Pyridate

b. Pyridafol
E. Photosystem-I-electron diversion (Bipyridyliums) (WSSA Group 22)
1. Diquat
2. Paraquat
F. Inhibitors of PPO (protoporphyrinogen oxidase) (WSSA Group 14)
1. Diphenylethers
a. Acifluorfen-Na
b. Bifenox
c. Chlomethoxyfen
d. Fluoroglycofen-ethyl
e. Fomesafen
f. Halosafen
g. Lactofen
h. Oxyfluorfen
2. Phenylpyrazoles
a. Fluazolate
b. Pyraflufen-ethyl
3. N-phenylphthalimides
a. Cinidon-ethyl
b. Flumioxazin
c. Flumiclorac-pentyl
4. Thiadiazoles
a. Fluthiacet-methyl
b. Thidiazimin
5. Oxadiazoles
a. Oxadiazon
b. Oxadiargyl
6. Triazolinones
a. Carfentrazone-ethyl
b. Sulfentrazone
7. Oxazolidinediones
a. Pentoxazone
8. Pyrimidindiones
a. Benzfendizone
b. Butafenicil
9. Others
a. Pyrazogyl
b. Profluazol
G. Bleaching: Inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS) (WSSA Group 12)
1. Pyridazinones
a. Norflurazon
2. Pyridinecarboxamides
a. Diflufenican
b. Picolinafen
3. Others
a. Beflubutamid
b. Fluridone
c. Flurochloridone
d. Flurtamone

H. Bleaching: Inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD) (WSSA Group 28)
1. Triketones
a. Mesotrione
b. Sulcotrione
2. Isoxazoles
a. Isoxachlortole
b. Isoxaflutole
3. Pyrazoles
a. Benzofenap
b. Pyrazoxyfen
c. Pyrazolynate
4. Others
a. Benzobicyclon
I. Bleaching: Inhibition of carotenoid biosynthesis (unknown target) (WSSA Group 11 and 13)
1. Triazoles (WSSA Group 11)
a. Amitrole
2. Isoxazolidinones (WSSA Group 13)
a. Clomazone
3. Ureas
a. Fluometuron
3. Diphenylether
a. Aclonifen
J. Inhibition of EPSP Synthase
1. Glycines (WSSA Group 9)
a. Glyphosate
b. Sulfosate
K. Inhibition of glutamine synthetase
1. Phosphinic Acids
a. Glufosinate-ammonium
b. Bialaphos
L. Inhibition of DHP (dihydropteroate) synthase (WSSA Group 18)
1 Carbamates
a. Asulam
M. Microtubule Assembly Inhibition (WSSA Group 3)
1. Dinitroanilines
a. Benfluralin
b. Butralin
c. Dinitramine
d. Ethalfluralin
e. Oryzalin
f. Pendimethalin
g. Trifluralin
2. Phosphoroamidates
a. Amiprophos-methyl
b. Butamiphos
3. Pyridines
a. Dithiopyr
b. Thiazopyr
4. Benzamides
a. Pronamide
b. Tebutam
5. Benzenedicarboxylic acids
a. Chlorthal-dimethyl
N. Inhibition of mitosis/microtubule organization WSSA Group 23)
1. Carbamates
a. Chlorpropham
b. Propham
c. Carbetamide
O. Inhibition of cell division (Inhibition of very long chain fatty acids as proposed mechanism; WSSA Group 15)
1. Chloroacetamides
a. Acetochlor
b. Alachlor
c. Butachlor
d. Dimethachlor
e. Dimethanamid
f. Metazachlor
g. Metolachlor
h. Pethoxamid
i. Pretilachlor
j. Propachlor
k. Propisochlor
l. Thenylchlor
2. Acetamides
a. Diphenamid
b. Napropamide
c. Naproanilide
3. Oxyacetamides
a. Flufenacet
b. Mefenacet
4. Tetrazolinones
a. Fentrazamide
5. Others
a. Anilofos
b. Cafenstrole
c. Indanofan
d. Piperophos
P. Inhibition of cell wall (cellulose) synthesis
1. Nitriles (WSSA Group 20)
a. Dichlobenil
b. Chlorthiamid
2. Benzamides (isoxaben (WSSA Group 21))
a. Isoxaben
3. Triazolocarboxamides (flupoxam)
a. Flupoxam
Q. Uncoupling (membrane disruption):

(WSSA Group 24)
1. Dinitrophenols
a. DNOC
b. Dinoseb
c. Dinoterb
R. Inhibition of Lipid Synthesis by other than ACC inhibition
1. Thiocarbamates (WSSA Group 8)
a. Butylate
b. Cycloate
c. Dimepiperate
d. EPTC
e. Esprocarb
f. Molinate
g. Orbencarb
h. Pebulate
i. Prosulfocarb
j. Benthiocarb
k. Tiocarbazil
l. Triallate
m. Vernolate
2. Phosphorodithioates
a. Bensulide
3. Benzofurans
a. Benfuresate
b. Ethofumesate
4. Halogenated alkanoic acids (WSSA Group 26)
a. TCA
b. Dalapon
c. Flupropanate
S. Synthetic auxins (IAA-like) (WSSA Group 4)
1. Phenoxycarboxylic acids
a. Clomeprop
b. 2,4-D
c. Mecoprop
2. Benzoic acids
a. Dicamba
b. Chloramben

c. TBA
3. Pyridine carboxylic acids
a. Clopyralid
b. Fluroxypyr
c. Picloram
d. Tricyclopyr
4. Quinoline carboxylic acids
a. Quinclorac
b. Quinmerac
5. Others (benazolin-ethyl)
a. Benazolin-ethyl
T. Inhibition of Auxin Transport
1. Phthalamates; semicarbazones (WSSA Group 19)
a. Naptalam
b. Diflufenzopyr-Na
U. Other Mechanism of Action
1. Arylaminopropionic acids
a. Flamprop-M-methyl /-isopropyl
2. Pyrazolium
a. Difenzoquat
3. Organoarsenicals
a. DSMA
b. MSMA
4. Others
a. Bromobutide
b. Cinmethylin
c. Cumyluron
d. Dazomet
e. Daimuron-methyl
f. Dimuron
g. Etobenzanid
h. Fosamine
i. Metam
j. Oxaziclomefone
k. Oleic acid
l. Pelargonic acid
m. Pyributicarb

The presently disclosed methods and compositions can utilize multiple herbicide tolerance polynucleotides. That is, the presently disclosed polynucleotide constructs can comprise more than one coding polynucleotide for a herbicide tolerance polypeptide. In some embodiments, the polynucleotide construct comprises more than one polynucleotide that encodes the same type of herbicide tolerance polypeptide (i.e., more than one GLYAT). In other embodiments, the polynucleotide constructs comprise more than one

herbicide-tolerance coding polynucleotide, wherein each of the coding polynucleotides encodes for a distinct type of herbicide tolerance polypeptide (of a different class or subclass). In some embodiments, the polynucleotide construct comprises at least a first and a second polynucleotide encoding a herbicide tolerance polypeptide, wherein the first and the second polynucleotide encodes a first and a second herbicide tolerance polypeptide that confer tolerance to a first and a second herbicide, wherein the first and second herbicide have different mechanisms of action.

In some of those embodiments wherein the presently disclosed polynucleotide constructs comprise at least two herbicide tolerance polynucleotides, at least two herbicide tolerance polynucleotides are located outside of the excision cassette. In other embodiments, the polynucleotide construct comprises a herbicide tolerance polynucleotide outside of the excision cassette that becomes operably linked to its promoter upon excision of the excision cassette and a second herbicide tolerance polypeptide within the excision cassette.

In some embodiments, the presently disclosed methods and compositions utilize polynucleotides that confer tolerance to glyphosate and at least one ALS inhibitor herbicide. In other embodiments, the presently disclosed methods and compositions utilize polynucleotides that confer tolerance to glyphosate and at least one ALS inhibitor herbicide, as well as, tolerance to at least one additional herbicide.

In addition to glyphosate and ALS inhibitors, the presently disclosed polynucleotide constructs can comprise polynucleotides that encode herbicide tolerance polypeptides that confer tolerance to other types of herbicides. Such additional herbicides, include but are not limited to, an acetyl Co-A carboxylase inhibitor such as quizalofop-P-ethyl, a synthetic auxin such as quinclorac, a protoporphyrinogen oxidase (PPO) inhibitor herbicide (such as sulfentrazone), a pigment synthesis inhibitor herbicide such as a hydroxyphenylpyruvate dioxygenase inhibitor (*e.g.*, mesotrione or sulcotrione), a phosphinothricin acetyltransferase or a phytoene desaturase inhibitor like diflufenican or pigment synthesis inhibitor.

In some embodiments, the presently disclosed polynucleotide constructs comprise polynucleotides encoding polypeptides conferring tolerance to herbicides which inhibit

the enzyme glutamine synthase, such as phosphinothricin or glufosinate (*e.g.*, the *bar* gene or *pat* gene). Glutamine synthetase (GS) appears to be an essential enzyme necessary for the development and life of most plant cells, and inhibitors of GS are toxic to plant cells. Glufosinate herbicides have been developed based on the toxic effect due to the inhibition of GS in plants. These herbicides are non-selective; that is, they inhibit growth of all the different species of plants present. The development of plants containing an exogenous phosphinothricin acetyltransferase is described in U.S. Patent Nos. 5,969,213; 5,489,520; 5,550,318; 5,874,265; 5,919,675; 5,561,236; 5,648,477; 5,646,024; 6,177,616; and 5,879,903, which are incorporated herein by reference in their entireties for all purposes. Mutated phosphinothricin acetyltransferase having this activity are also disclosed. In certain embodiments a maize-optimized PAT gene is used. In some of these embodiments, the maize-optimized PAT gene has the sequence set forth in SEQ ID NO: 54. In some embodiments, the PAT gene is used as a selectable marker as described elsewhere herein and is present within the excision cassette.

In still other embodiments, the presently disclosed polynucleotide constructs comprise polynucleotides encoding polypeptides conferring tolerance to herbicides which inhibit protox (protoporphyrinogen oxidase). Protox is necessary for the production of chlorophyll, which is necessary for all plant survival. The protox enzyme serves as the target for a variety of herbicidal compounds. These herbicides also inhibit growth of all the different species of plants present. The development of plants containing altered protox activity which are resistant to these herbicides are described in U.S. Patent Nos. 6,288,306; 6,282,837; and 5,767,373; and international publication WO 01/12825, which are incorporated herein by reference in their entireties for all purposes.

In still other embodiments, the presently disclosed polynucleotide constructs may comprise polynucleotides encoding polypeptides involving other modes of herbicide resistance. For example, hydroxyphenylpyruvatedioxygenases are enzymes that catalyze the reaction in which para-hydroxyphenylpyruvate (HPP) is transformed into homogentisate. Molecules which inhibit this enzyme and which bind to the enzyme in order to inhibit transformation of the HPP into homogentisate are useful as herbicides. Plants more resistant to certain herbicides are described in U.S. Patent Nos. 6,245,968;

6,268,549; and 6,069,115; and international publication WO 99/23886, which are incorporated herein by reference in their entireties for all purposes. Mutated hydroxyphenylpyruvatedioxygenase having this activity are also disclosed.

In some embodiments, the methods and compositions can further comprise at least one cell proliferation factor. Expression of a cell proliferation factor, such as babyboom can enhance the transformation frequency of otherwise recalcitrant plants or plant parts. A polynucleotide encoding a cell proliferation factor can be co-transformed into a plant or plant part with the presently disclosed polynucleotide constructs. In other embodiments, the presently disclosed polynucleotide constructs comprise at least one polynucleotide encoding a cell proliferation factor. In some of these embodiments, the at least one polynucleotide encoding a cell proliferation factor is located within the excision cassette of the polynucleotide construct, such that the polynucleotide is excised when the site-specific recombinase is expressed.

As used herein, a "cell proliferation factor" is a polypeptide or a polynucleotide capable of stimulating growth of a cell or tissue, including but not limited to promoting progression through the cell cycle, inhibiting cell death, such as apoptosis, stimulating cell division, and/or stimulating embryogenesis. The polynucleotides can fall into several categories, including but not limited to, cell cycle stimulatory polynucleotides, developmental polynucleotides, anti-apoptosis polynucleotides, hormone polynucleotides, or silencing constructs targeted against cell cycle repressors or pro-apoptotic factors. The following are provided as non-limiting examples of each category and are not considered a complete list of useful polynucleotides for each category: 1) cell cycle stimulatory polynucleotides including plant viral replicase genes such as RepA, cyclins, E2F, prolifera, cdc2 and cdc25; 2) developmental polynucleotides such as Lec1, Kn1 family, WUSCHEL, Zwille, BBM, Aintegumenta (ANT), FUS3, and members of the Knotted family, such as Kn1, STM, OSH1, and SbH1; 3) anti-apoptosis polynucleotides such as CED9, Bcl2, Bcl-X(L), Bcl-W, A1, McL-1, Mac1, Boo, and Bax-inhibitors; 4) hormone polynucleotides such as IPT, TZS, and CKI-1; and 5) silencing constructs targeted against cell cycle repressors, such as Rb, CKl, prohibitin, and wee1, or stimulators of apoptosis such as APAF-1, bad, bax, CED-4, and caspase-3, and repressors of plant developmental

transitions, such as Pickle and WD polycomb genes including FIE and Medea. The polynucleotides can be silenced by any known method such as antisense, RNA interference, cosuppression, chimerplasty, or transposon insertion.

The polynucleotide encoding the cell proliferation factor may be native to the cell or heterologous. Any of a number of cell proliferation factors can be used. In certain embodiments, those cell proliferation factors that are capable of stimulating embryogenesis are used to enhance transformation efficiency. Such cell proliferation factors are referred to herein as embryogenesis-stimulating polypeptides and they include, but are not limited to, babyboom polypeptides.

In some embodiments, the cell proliferation factor is a member of the AP2/ERF family of proteins. The AP2/ERF family of proteins is a plant-specific class of putative transcription factors that regulate a wide variety of developmental processes and are characterized by the presence of an AP2 DNA binding domain that is predicted to form an amphipathic alpha helix that binds DNA (PFAM Accession PF00847). The AP2/ERF proteins have been subdivided into distinct subfamilies based on the presence of conserved domains. Initially, the family was divided into two subfamilies based on the number of DNA binding domains, with the ERF subfamily having one DNA binding domain, and the AP2 subfamily having 2 DNA binding domains. As more sequences were identified, the family was subsequently subdivided into five subfamilies: AP2, DREB, ERF, RAV, and others. (Sakuma *et al.* (2002) *Biochem Biophys Res Comm* 290:998-1009).

Members of the APETALA2 (AP2) family of proteins function in a variety of biological events, including but not limited to, development, plant regeneration, cell division, embryogenesis, and cell proliferation (see, *e.g.*, Riechmann and Meyerowitz (1998) *Biol Chem* 379:633-646; Saleh and Pagés (2003) *Genetika* 35:37-50 and Database of Arabidopsis Transciption Factors at daft.cbi.pku.edu.cn). The AP2 family includes, but is not limited to, AP2, ANT, Glossy15, AtBBM, BnBBM, and maize ODP2/BBM.

U.S. Application Publication No. 2011/0167516, which is herein incorporated by reference in its entirety, describes an analysis of fifty sequences with homology to a maize BBM sequence (also referred to as maize ODP2 or ZmODP2, the polynucleotide and

amino acid sequence of the maize BBM is set forth in SEQ ID NO: 55 and 56, respectively; the polynucleotide and amino acid sequence of another ZmBBM is set forth in SEQ ID NO: 58 and 59, respectively). The analysis identified three motifs (motifs 4-6; set forth in SEQ ID NOs: 61-63), along with the AP2 domains (motifs 2 and 3; SEQ ID NOs: 64 and 65) and linker sequence that bridges the AP2 domains (motif 1; SEQ ID NO: 66), that are found in all of the BBM homologues. Thus, motifs 1-6 distinguish these BBM homologues from other AP2-domain containing proteins (*e.g.*, WRI, AP2, and RAP2.7) and these BBM homologues comprise a subgroup of AP2 family of proteins referred to herein as the BBM/PLT subgroup. In some embodiments, the cell proliferation factor that is used in the methods and compositions is a member of the BBM/PLT group of AP2 domain-containing polypeptides. In these embodiments, the cell proliferation factor comprises two AP2 domains and motifs 4-6 (SEQ ID NOs: 61-63) or a fragment or variant thereof. In some of these embodiments, the AP2 domains have the sequence set forth in SEQ ID NOs: 64 and 65 or a fragment or variant thereof, and in particular embodiments, further comprises the linker sequence of SEQ ID NO: 66 or a fragment or variant thereof. In other embodiments, the cell proliferation factor comprises at least one of motifs 4-6 or a fragment or variant thereof, along with two AP2 domains, which in some embodiments have the sequence set forth in SEQ ID NO: 64 and/or 65 or a fragment or variant thereof, and in particular embodiments have the linker sequence of SEQ ID NO: 66 or a fragment or variant thereof. Based on the phylogenetic analysis, the subgroup of BBM/PLT polypeptides can be subdivided into the BBM, AIL6/7, PLT1/2, AIL1, PLT3, and ANT groups of polypeptides.

In some embodiments, the cell proliferation factor is a babyboom (BBM) polypeptide, which is a member of the AP2 family of transcription factors. The BBM protein from *Arabidopsis* (AtBBM) is preferentially expressed in the developing embryo and seeds and has been shown to play a central role in regulating embryo-specific pathways. Overexpression of AtBBM has been shown to induce spontaneous formation of somatic embryos and cotyledon-like structures on seedlings. See, Boutiler *et al.* (2002) *The Plant Cell* 14:1737-1749. The maize BBM protein also induces embryogenesis and promotes transformation (See, U.S. Patent No. 7,579,529, which is herein incorporated by

reference in its entirety). Thus, BBM polypeptides stimulate proliferation, induce embryogenesis, enhance the regenerative capacity of a plant, enhance transformation, and as demonstrated herein, enhance rates of targeted polynucleotide modification.

In some embodiments, the babyboom polypeptide comprises two AP2 domains and at least one of motifs 7 and 10 (set forth in SEQ ID NO: 67 and 68, respectively) or a variant or fragment thereof. In certain embodiments, the AP2 domains are motifs 2 and 3 (SEQ ID NOs: 64 and 65, respectively) or a fragment or variant thereof, and in particular embodiments, the babyboom polypeptide further comprises a linker sequence between AP2 domain 1 and 2 having motif 1 (SEQ ID NO: 66) or a fragment or variant thereof. In particular embodiments, the BBM polypeptide further comprises motifs 4-6 (SEQ ID NOs 61-63) or a fragment or variant thereof. The BBM polypeptide can further comprise motifs 8 and 9 (SEQ ID NOs: 69 and 70, respectively) or a fragment or variant thereof, and in some embodiments, motif 10 (SEQ ID NO: 68) or a variant or fragment thereof. In some of these embodiments, the BBM polypeptide also comprises at least one of motif 14 (set forth in SEQ ID NO: 71), motif 15 (set forth in SEQ ID NO: 72), and motif 19 (set forth in SEQ ID NO: 73), or variants or fragments thereof. The variant of a particular amino acid motif can be an amino acid sequence having at least about 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater sequence identity with the motif disclosed herein. Alternatively, variants of a particular amino acid motif can be an amino acid sequence that differs from the amino acid motif by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids.

Non-limiting examples of babyboom polynucleotides and polypeptides that can be used in the methods and compositions include the *Arabidopsis thaliana* AtBBM (SEQ ID NOs: 74 and 75), *Brassica napus* BnBBM1 (SEQ ID NOs: 76 and 77), *Brassica napus* BnBBM2 (SEQ ID NOs: 78 and 79), *Medicago truncatula* MtBBM (SEQ ID NOs: 80 and 81), *Glycine max* GmBBM (SEQ ID NOs: 82 and 83), *Vitis vinifera* VvBBM (SEQ ID NOs: 84 and 85), *Zea mays* ZmBBM (SEQ ID NOs: 55 and 56 and genomic sequence set forth in SEQ ID NO: 57; or SEQ ID NOs: 58 and 59 and genomic sequence set forth in SEQ ID NO: 60) and ZmBBM2 (SEQ ID NOs: 101 and 102), *Oryza sativa* OsBBM (polynucleotide sequences set forth in SEQ ID NOs: 86 and 87; amino acid sequence set

forth in SEQ ID NO: 89; and genomic sequence set forth in SEQ ID NO: 88), OsBBM1 (SEQ ID NOs: 90 and 91), OsBBM2 (SEQ ID NOs: 92 and 93), and OsBBM3 (SEQ ID NOs: 94 and 95), *Sorghum bicolor* SbBBM (SEQ ID NOs: 96 and 97 and genomic sequence set forth in SEQ ID NO: 98) and SbBBM2 (SEQ ID NOs: 99 and 100) or active fragments or variants thereof. In particular embodiments, the cell proliferation factor is a maize BBM polypeptide (SEQ ID NO: 56, 59, or 102) or a variant or fragment thereof, or is encoded by a maize BBM polynucleotide (SEQ ID NO: 55, 57, 121, 116, or 101) or a variant or fragment thereof.

Thus, in some embodiments, a polynucleotide encoding a cell proliferation factor has a nucleotide sequence having at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the nucleotide sequence set forth in SEQ ID NO: 82, 96, 84, 80, 55, 101, 86, 90, 92, 94, 74, 76, 78, 99, 57, 60, 88, 87, 58, or 98 or the cell proliferation factor has an amino acid sequence having at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the amino acid sequence set forth in SEQ ID NO: 83, 97, 85, 81, 56, 102, 89, 91, 93, 95, 75, 77, 79, 59, or 100. In some of these embodiments, the cell proliferation factor has at least one of motifs 7 and 10 (SEQ ID NO: 67 and 68, respectively) or a variant or fragment thereof at the corresponding amino acid residue positions in the babyboom polypeptide. In other embodiments, the cell proliferation factor further comprises at least one of motif 14 (set forth in SEQ ID NO: 71), motif 15 (set forth in SEQ ID NO: 72), and motif 19 (set forth in SEQ ID NO: 73) or a variant or fragment thereof at the corresponding amino acid residue positions in the babyboom polypeptide.

In other embodiments, other cell proliferation factors, such as, Lec1, Kn1 family, WUSCHEL (*e.g.*, WUS1, the polynucleotide and amino acid sequence of which is set forth in SEQ ID NO: 103 and 104; WUS2, the polynucleotide and amino acid sequence of which is set forth in SEQ ID NO: 105 and 106; WUS2 alt, the polynucleotide and amino acid sequence of which is set forth in SEQ ID NO: 107 and 108; WUS3, the polynucleotide and amino acid sequence of which is set forth in SEQ ID NO: 109 and 110), Zwille, and Aintegumeta (ANT), may be used alone, or in combination with a

babyboom polypeptide or other cell proliferation factor. See, for example, U.S. Application Publication No. 2003/0135889, International Application Publication No. WO 03/001902, and U.S. Patent No. 6,512,165, each of which is herein incorporated by reference.

In some embodiments, the polynucleotide construct comprises a polynucleotide encoding a Wuschel polypeptide (see International Application Publication No. WO 01/23575 and U.S. Patent No. 7,256,322, each of which are herein incorporated by reference in its entirety). In certain embodiments, the polynucleotide encoding the Wuschel polypeptide has the sequence set forth in SEQ ID NO: 103, 105, 107, or 109 (WUS1, WUS2, WUS2 alt, or WUS3, respectively) or an active variant or fragment thereof. In particular embodiments, the Wuschel polypeptide has the sequence set forth in SEQ ID NO: 104, 106, 108, or 110 (WUS1, WUS2, WUS2 alt, or WUS3, respectively) or an active variant or fragment thereof. In some of these embodiments, the polynucleotide encoding a Wuschel polypeptide is operably linked to a promoter active in the plant, including but not limited to the maize In2-2 promoter or a nopaline synthase promoter.

When multiple cell proliferation factors are used, or when a babyboom polypeptide is used along with any of the abovementioned polypeptides, the polynucleotides encoding each of the factors can be present on the same expression cassette or on separate expression cassettes. When two or more factors are coded for by separate expression cassettes, the expression cassettes can be provided to the plant simultaneously or sequentially. In some embodiments, the polynucleotide construct comprises a polynucleotide encoding a babyboom polypeptide and a polynucleotide encoding a Wuschel polypeptide within the excision cassette such that the cell proliferation factors enhance the transformation frequency of the polynucleotide construct, but are subsequently excised upon desiccation of the transformed plant cell/tissue.

In some embodiments, herbicide tolerance polynucleotides can serve as a selectable marker for the identification of plants or plant parts that further comprise a polynucleotide of interest. Thus, in certain embodiments, the presently disclosed

polynucleotide constructs can further comprise a polynucleotide of interest. In some embodiments, the polynucleotide of interest is operably linked to a promoter that is active in a plant cell. The promoter that is operably linked to the polynucleotide of interest can be a constitutive promoter, an inducible promoter, or a tissue-preferred promoter.

In certain embodiments, the polynucleotide of interest, and optionally the operably linked promoter, are located outside of the excision cassette on the polynucleotide construct. In other embodiments, the polynucleotide of interest and optionally its operably linked promoter are located within the excision cassette and the herbicide tolerance polynucleotide serves as a selectable marker to identify those plants or plant parts from which the polynucleotide of interest has been excised.

The polynucleotide of interest may impart various changes in the organism, particularly plants, including, but not limited to, modification of the fatty acid composition in the plant, altering the amino acid content of the plant, altering pathogen resistance, and the like. These results can be achieved by providing expression of heterologous products, increased expression of endogenous products in plants, or suppressed expression of endogenous products in plants.

General categories of polynucleotides of interest include, for example, those genes involved in information, such as zinc fingers, those involved in communication, such as kinases, those involved in biosynthetic pathways, and those involved in housekeeping, such as heat shock proteins. More specific categories of transgenes, for example, include sequences encoding important traits for agronomics, insect resistance, disease resistance, sterility, grain characteristics, oil, starch, carbohydrate, phytate, protein, nutrient, metabolism, digestability, kernel size, sucrose loading, and commercial products.

Traits such as oil, starch, and protein content can be genetically altered in addition to using traditional breeding methods. Modifications include increasing content of oleic acid, saturated and unsaturated oils, increasing levels of lysine and sulfur, providing essential amino acids, and also modification of starch. Protein modifications to alter amino acid levels are described in U.S. Patent Nos. 5,703,049, 5,885,801, 5,885,802, and 5,990,389 and WO 98/20122, herein incorporated by reference.

Insect resistance genes may encode resistance to pests such as rootworm, cutworm, European Corn Borer, and the like. Such genes include, for example, *Bacillus thuringiensis* toxic protein genes (U.S. Patent Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; and Geiser *et al.* (1986) *Gene* 48:109); lectins (Van Damme *et al.* (1994) *Plant Mol. Biol.* 24:825); and the like.

Genes encoding disease resistance traits include detoxification genes, such as against fumonosin (U.S. Patent No. 5,792,931); avirulence (avr) and disease resistance (R) genes (Jones *et al.* (1994) *Science 266*:789; Martin *et al.* (1993) *Science* 262:1432; and Mindrinos *et al.* (1994) *Cell* 78:1089); and the like.

Sterility genes can also be encoded in an expression cassette and provide an alternative to physical detasseling. Examples of genes used in such ways include male tissue-preferred genes and genes with male sterility phenotypes such as QM, described in U.S. Patent No. 5,583,210. Other genes include kinases and those encoding compounds toxic to either male or female gametophytic development.

Commercial traits can also be encoded on a gene or genes that could, for example increase starch for ethanol production, or provide expression of proteins.

Although the herbicide tolerance polynucleotide can serve as a selectable marker to aid in the identification of transgenic plants that comprise a polynucleotide of interest or lack a polynucleotide of interest, an additional selectable marker may be present in the excision cassette of the presently disclosed polynucleotide constructs that aids in the selection of transgenic plants or plant parts at an earlier point in development when most herbicide selection systems are less efficient. In general, the selectable marker that is present within the excision cassette is one that allows for efficient selection in early stages of plant development and production (e.g., during the tissue proliferation stage of transgenic plant production). For example, the expression of a fluorescent protein can be used to select plants or plant parts that comprise a presently disclosed polynucleotide construct during or prior to tissue proliferation. Proliferating the tissue to a certain mass is generally necessary before regeneration of the tissue into a plant. The expression of the site-specific recombinase is then induced before herbicide selection, which in general, occurs during or after the regeneration of the provided cells or tissues into plants.

"Regenerating" or "regeneration" of a plant cell is the process of growing a plant from the plant cell (e.g., plant protoplast, callus or explant).

Marker genes that can be present within the excision cassette include polynucleotides encoding products that provide resistance against otherwise toxic compounds (e.g. antibiotic resistance) such as those encoding neomycin phosphotransferase II (NEO or nptII) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D), including but not limited to, the selectable marker gene phosphinothricin acetyl transferase (PAT) (Wohlleben *et al.* (1988) *Gene* 70:25-37), which confers resistance to the herbicide Bialaphos. In certain embodiments, the selectable marker that is present within the excision cassette is not a herbicide tolerance polynucleotide.

As used herein, "antibiotic resistance polypeptide" refers to a polypeptide that confers resistance or tolerance to an antibiotic compound to a host cell comprising or secreting the polypeptide.

Additional selectable marker-encoding polynucleotides include those that encode products that can be readily identified, including but not limited to phenotypic markers such as β-galactosidase, and visual markers, such as fluorescent proteins. As used herein, a "fluorescent protein" or "fluorescent polypeptide" refers to a polypeptide that is capable of absorbing radiation (e.g., light at a wavelength in the visible spectrum) at one wavelength and emitting radiation as light at a different wavelength. Non-limiting examples of fluorescent protein include green fluorescent protein (GFP) (Su et *al.* (2004) *Biotechnol Bioeng 85:*610-9 and Fetter *et al.* (2004) *Plant Cell 16:*215-28), cyan florescent protein (CYP) (Bolte *et al.* (2004) *J. Cell Science 117*:943-54 and Kato *et al.* (2002) *Plant Physiol 129*:913-42), red fluorescent protein, and yellow florescent protein (PhiYFP™ from Evrogen, see, Bolte *et al.* (2004) *J. Cell Science 117*:943-54). For additional selectable markers, see generally, Yarranton (1992) *Curr. Opin. Biotech.* 3:506-511; Christopherson *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6314-6318; Yao *et al.* (1992) *Cell* 71:63-72; Reznikoff (1992) *Mol. Microbiol.* 6:2419-2422; Barkley *et al.* (1980) in *The Operon*, pp. 177-220; Hu *et al.* (1987) *Cell* 48:555-566; Brown *et al.* (1987) *Cell*

49:603-612; Figge *et al.* (1988) *Cell* 52:713-722; Deuschle *et al.* (1989) *Proc. Natl. Acad. Aci. USA* 86:5400-5404; Fuerst *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:2549-2553; Deuschle *et al.* (1990) *Science* 248:480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:1917-1921; Labow *et al.* (1990) *Mol. Cell. Biol.* 10:3343-3356; Zambretti *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3952-3956; Baim *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:5072-5076; Wyborski *et al.* (1991) *Nucleic Acids Res.* 19:4647-4653; Hillenand-Wissman (1989) *Topics Mol. Struc. Biol.* 10:143-162; Degenkolb *et al.* (1991) *Antimicrob. Agents Chemother.* 35:1591-1595; Kleinschnidt *et al.* (1988) *Biochemistry* 27:1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Oliva *et al.* (1992) *Antimicrob. Agents Chemother.* 36:913-919; Hlavka *et al.* (1985) *Handbook of Experimental Pharmacology*, Vol. 78 (Springer-Verlag, Berlin); Gill *et al.* (1988) *Nature* 334:721-724. Such disclosures are herein incorporated by reference.

The presently provided methods and compositions can also utilize metabolic enzymes as selectable markers. The term "metabolic enzymes" as it relates to selectable markers refer to enzymes that confer a selectable metabolic advantage to cells. Cells expressing the metabolic enzyme are then positively selected for the ability to metabolize and utilize a particular chemical compound that cannot otherwise be metabolized or utilized by other cells not comprising the enzyme. Non-limiting examples of metabolic enzymes for use as selectable markers include D-amino oxidase (encoded by the *doa1* gene), which catalyzes the oxidative deamination of various D-amino acids (see, for example, Erikson *et al.* (2004) *Nature Biotechnology* 22:455-458, which is herein incorporated by reference in its entirety); cyanamide hydratase (encoded by the *cah* gene), which converts cyanamide into urea as a fertilizer source (see, for example, U.S. Patent No. 6,268,547, which is herein incorporated by reference in its entirety); and phosphomannose isomerase (encoded by the *pmi* gene), which catalyzes the reversible inter-conversion of mannose-6-phosphate and fructose-6-phosphate, allowing plant cells to utilize mannose as a carbon source (see, for example, Joersbo *et al.* (1998) *Molecular Breeding* 4:11-117, which is herein incorporated by reference in its entirety).

In some embodiments, the excision cassette comprises more than one selectable marker-coding polynucleotide. In some of these embodiments, the excision cassette comprises both a visual marker and an antibiotic resistance or herbicidal resistance selectable marker. In some of these embodiments, the excision cassette comprises a maize optimized PAT-coding polynucleotide (such as the sequence set forth in SEQ ID NO: 54) or a polynucleotide encoding neomycin phosphotransferase II (NEO or nptII), and a polynucleotide encoding a fluorescent protein, such as yellow fluorescent protein.

The selectable marker-encoding polynucleotide within the excision cassette is operably linked to a promoter that is active in a plant cell. This promoter can be present within or outside of the excision cassette. In some of the embodiments wherein the promoter that is operably linked to the selectable marker-encoding polynucleotide is outside of the excision cassette, this same promoter will become operably linked to the herbicide tolerance polynucleotide after excision of the excision cassette.

In certain embodiments, the promoter that is operably linked to the selectable marker-encoding polynucleotide present within the excision cassette is a constitutive promoter such that the selectable marker will be constitutively expressed in the plant or plant part until excision of the excision cassette. In some of these embodiments, the constitutive promoter is a maize ubiquitin promoter, which in some embodiments comprises the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113).

During the selection of the plant or plant part that expresses the selectable marker that is found within the excision cassette, the plant or plant part can be cultured in the presence of a selection agent. As used herein, a "selection agent" refers to a compound that when contacted with a plant or plant part allows for the identification of a plant or plant part expressing a selectable marker, either positively or negatively. For example, a selection agent for an antibiotic resistance polynucleotide is the antibiotic to which the polynucleotide confers resistance. As a further non-limiting example, a selection agent for a metabolizing enzyme selectable marker is the compound that can only be metabolized and utilized by the cell that expresses the selectable marker.

In particular embodiments wherein the polynucleotide construct is designed for transformation of maize, the polynucleotide construct comprises, outside of the excision cassette, the expression cassettes for a GLYAT polypeptide and an ALS-inhibitor tolerance polypeptide as present in the T-DNA region of plasmid PHP24279 described in U.S. Patent No. 7,928,296, which is herein incorporated by reference in its entirety. In these embodiments, the polynucleotide construct comprises the *glyat4621* gene that was derived from the soil bacterium *Bacillus licheniformis* and was synthesized by a gene shuffling process to optimize the acetyltransferase activity of the GLYAT4621 enzyme (Castle *et al.* (2004) *Science* 304:1151-1154). The polynucleotide construct further comprises a ZM-HRA expression cassette comprising a modified maize acetolactate synthase gene, *zm-hra* (*Zea mays*-highly resistant allele), encoding the ZM-HRA protein, which confers tolerance to a range of ALS-inhibiting herbicides, such as sulfonylureas. In these embodiments, the *glyat4621* gene cassette and the *zm-hra* gene cassette are in reverse orientation. The expression of the *glyat4621* gene is controlled by the ubiquitin regulatory region from maize (*ubi*ZM1 promoter (SEQ ID NO: 111), 5'UTR (SEQ ID NO: 112), and intron (SEQ ID NO: 112) (Christensen *et al.* (1992)) and the *pin*II terminator (An et al. (1989) *Plant Cell* 1:115-122). The expression of the *zm-hra* gene is controlled by the native maize acetolactate synthase promoter (*zm-als* promoter) (Fang *et al.* (2000)). The terminator for the *zm-hra* gene is the 3' terminator sequence from the proteinase inhibitor II gene of *Solanum tuberosum* (*pin*II terminator). Upstream of both cassettes are three copies of the enhancer region from the cauliflower mosaic virus (CaMV 35S enhancer, US Application No. 11/508,045, herein incorporated by reference) providing expression enhancement to both cassettes.

In certain embodiments wherein the polynucleotide construct is designed for transformation of soybean (*Glycine max*), the polynucleotide construct comprises, outside of the excision cassette, the expression cassettes for a GLYAT polypeptide and an ALS-inhibitor tolerance polypeptide as present in the *Not* I-*Asc* I fragment of plasmid PHP20163 described in U.S. Patent No. 7,622,641, which is herein incorporated by reference in its entirety. In these embodiments, the polynucleotide construct comprises the glyphosate acetyltransferase (*glyat*) gene derived from *Bacillus licheniformis* and a

modified version of the soybean acetolactate synthase gene (*zm-hra*). The *glyat* gene was functionally improved by a gene shuffling process to optimize the kinetics of glyphosate acetyltransferase (GLYAT) activity for acetylating the herbicide glyphosate. The *glyat* gene is under the control of the SCP1 promoter and Tobacco Mosaic Virus (TMV) omega 5′ UTR translational enhancer element and the proteinase inhibitor II (*pin*II) terminator from *Solanum tuberosum*. The *zm-hra* gene is under the control of the S-adenosyl-L-methionine synthetase (SAMS) promoter and the acetolactate synthase (*gm-als*) terminator, both from soybean.

In other embodiments wherein the polynucleotide construct is designed for transformation of *Brassica*, the polynucleotide construct comprises the expression cassette for a GLYAT polypeptide as present in the plasmid PHP28181 described in U.S. Appl. Publ. No. 2012/0131692, which is herein incorporated by reference in its entirety. In these embodiments, the polynucleotide construct comprises the *glyat4621* gene, which was derived from the soil bacterium *Bacillus licheniformis* and was synthesized by a gene shuffling process to optimize the acetyltransferase activity of the GLYAT4621 enzyme (Castle, *et al.*, (2004) *Science* 304:1151-1154). The expression of the *glyat4621* gene is controlled by the UBQ10 regulatory region from Arabidopsis and the *pin*II terminator. In some of these embodiments, the polynucleotide construct further comprises an expression cassette for an ALS inhibitor tolerance polypeptide.

The presently disclosed compositions and methods can utilize fragments or variants of known polynucleotide or polypeptide sequences. By "fragment" is intended a portion of the polynucleotide or a portion of an amino acid sequence and hence protein encoded thereby. Fragments of a polynucleotide may retain the biological activity of the native polynucleotide and, for example, have promoter activity (promoter fragments), or are capable of stimulating proliferation, inducing embryogenesis, modifying the regenerative capacity of a plant (cell proliferation factor fragments), are capable of conferring herbicide tolerance (herbicide tolerance polypeptide fragments) or catalyzing site-specific recombination (site-specific recombinase fragments). In those embodiments wherein the polynucleotide encodes a polypeptide, fragments of the polynucleotide may encode protein fragments that retain the biological activity of the native protein.

Alternatively, fragments of a polynucleotide that are useful as hybridization probes generally do not retain biological activity or encode fragment proteins that retain biological activity. Thus, fragments of a nucleotide sequence may range from at least about 20, 50, 100, 150, 200, 250, 300, 400, 500 nucleotides, or greater.

A fragment of a polynucleotide that encodes a biologically active portion of a cell proliferation factor, for example, will encode at least 15, 25, 30, 50, 100, 150, 200, 250, 300, 400, 500 contiguous amino acids, or up to the total number of amino acids present in the full-length cell proliferation factor. Fragments of a coding polynucleotide that are useful as hybridization probes or PCR primers generally need not encode a biologically active portion of a polypeptide.

"Variants" is intended to mean substantially similar sequences. For polynucleotides, a variant comprises a polynucleotide having deletions at the 5' and/or 3' end; deletion and/or addition of one or more nucleotides at one or more internal sites in the native polynucleotide; and/or substitution of one or more nucleotides at one or more sites in the native polynucleotide. As used herein, a "native" polynucleotide or polypeptide comprises a naturally occurring nucleotide sequence or amino acid sequence, respectively. For polynucleotides encoding polypeptides conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence the polypeptide (*e.g.*, cell proliferation factor). Naturally occurring variants such as these can be identified with the use of well-known molecular biology techniques, such as, for example, with polymerase chain reaction (PCR) and hybridization techniques. Variant polynucleotides also include synthetically derived polynucleotides, such as those generated, for example, by using site-directed mutagenesis. Generally, variants of a particular will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to that particular polynucleotide as determined by sequence alignment programs and parameters.

Variants of a particular polynucleotide that encodes a polypeptide can also be evaluated by comparison of the percent sequence identity between the polypeptide encoded by a variant polynucleotide and the polypeptide encoded by the particular

polynucleotide. Percent sequence identity between any two polypeptides can be calculated using sequence alignment programs and parameters. Where any given pair of polynucleotides is evaluated by comparison of the percent sequence identity shared by the two polypeptides they encode, the percent sequence identity between the two encoded polypeptides is at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity.

"Variant" protein is intended to mean a protein derived from the native protein by deletion of one or more amino acids at the N-terminal and/or C-terminal end of the native protein; deletion and/or addition of one or more amino acids at one or more internal sites in the native protein; and/or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins retain the desired biological activity of the native protein. For example, variant cell proliferation factors stimulate proliferation and variant babyboom polypeptides are capable of stimulating proliferation, inducing embryogenesis, modifying the regenerative capacity of a plant, increasing the transformation efficiency in a plant, increasing or maintaining the yield in a plant under abiotic stress, producing asexually derived embryos in a plant, and/or enhancing rates of targeted polynucleotide modification. Such variants may result from, for example, genetic polymorphism or from human manipulation. Biologically active variants of a native protein will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the amino acid sequence for the native protein as determined by sequence alignment programs and parameters. A biologically active variant of a native protein may differ from that protein by as few as 1-15 amino acid residues, as few as 1-10, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

Where appropriate, the coding polynucleotides may be optimized for increased expression in the transformed plant. That is, the coding polynucleotides can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell and Gowri (1990) *Plant Physiol.* 92:1-11 for a discussion of host-preferred codon usage. Methods are available in the art for synthesizing plant-preferred genes.

See, for example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray *et al.* (1989) *Nucleic Acids Res.* 17:477-498, herein incorporated by reference.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

The following terms are used to describe the sequence relationships between two or more polynucleotides or polypeptides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", and, (d) "percentage of sequence identity."

(a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two polynucleotides. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent sequence identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local alignment algorithm of Smith *et al.* (1981) *Adv. Appl. Math.* 2:482; the global

alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453; the search-for-local alignment method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 872264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the GCG Wisconsin Genetics Software Package, Version 10 (available from Accelrys Inc., 9685 Scranton Road, San Diego, California, USA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins *et al.* (1988) *Gene* 73:237-244 (1988); Higgins *et al.* (1989) *CABIOS* 5:151-153; Corpet *et al.* (1988) *Nucleic Acids Res.* 16:10881-90; Huang *et al.* (1992) *CABIOS* 8:155-65; and Pearson *et al.* (1994) *Meth. Mol. Biol.* 24:307-331. The ALIGN program is based on the algorithm of Myers and Miller (1988) *supra*. A PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used with the ALIGN program when comparing amino acid sequences. The BLAST programs of Altschul *et al* (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) *supra*. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleotide sequence encoding a protein of the invention. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a protein or polypeptide of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra*. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (*e.g.*, BLASTN for

nucleotide sequences, BLASTX for proteins) can be used. See www.ncbi.nlm.nih.gov. Alignment may also be performed manually by inspection.

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

GAP uses the algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453, to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of matched bases and the fewest gaps. It allows for the provision of a gap creation penalty and a gap extension penalty in units of matched bases. GAP must make a profit of gap creation penalty number of matches for each gap it inserts. If a gap extension penalty greater than zero is chosen, GAP must, in addition, make a profit for each gap inserted of the length of the gap times the gap extension penalty. Default gap creation penalty values and gap extension penalty values in Version 10 of the GCG Wisconsin Genetics Software Package for protein sequences are 8 and 2, respectively. For nucleotide sequences the default gap creation penalty is 50 while the default gap extension penalty is 3. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 200. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or greater.

GAP presents one member of the family of best alignments. There may be many members of this family, but no other member has a better quality. GAP displays four

figures of merit for alignments: Quality, Ratio, Identity, and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the GCG Wisconsin Genetics Software Package is BLOSUM62 (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

(c) As used herein, "sequence identity" or "identity" in the context of two polynucleotides or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (*e.g.*, charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, *e.g.*, as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which

does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

In hybridization techniques, all or part of a known polynucleotide is used as a probe that selectively hybridizes to other corresponding polynucleotides present in a population of cloned genomic DNA fragments or cDNA fragments (*i.e.,* genomic or cDNA libraries) from a chosen organism. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P, or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the babyboom polynucleotide. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

For example, the entire coding polynucleotide, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to a corresponding coding polynucleotide and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among the particular family of coding polynucleotide sequences and are optimally at least about 10 nucleotides in length, and most optimally at least about 20 nucleotides in length. Such probes may be used to amplify corresponding coding polynucleotides from a chosen plant by PCR. This technique may be used to isolate additional coding sequences from a desired plant or as a diagnostic assay to determine the presence of coding sequences in a plant. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (*e.g.*, at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, optimally less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours. The duration of the wash time will be at least a length of time sufficient to reach equilibrium.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: $T_m = 81.5°C + 16.6 (\log M) + 0.41 (\%GC) - 0.61$

(% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with ≥90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is optimal to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes,* Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

The presently disclosed polynucleotide constructs can be introduced into a host cell. By "host cell" is meant a cell, which comprises a heterologous nucleic acid sequence. Host cells may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells. In some examples, host cells are

monocotyledonous or dicotyledonous plant cells. In particular embodiments, the monocotyledonous host cell is a sugarcane host cell.

An intermediate host cell may be used, for example, to increase the copy number of the cloning vector and/or to mediate transformation of a different host cell. With an increased copy number, the vector containing the nucleic acid of interest can be isolated in significant quantities for introduction into the desired plant cells. In one embodiment, plant promoters that do not cause expression of the polypeptide in bacteria are employed.

Prokaryotes most frequently are represented by various strains of *E. coli;* however, other microbial strains may also be used. Commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding sequences, include such commonly used promoters as the beta lactamase (penicillinase) and lactose (lac) promoter systems (Chang *et al.* (1977) *Nature 198*:1056), the tryptophan (trp) promoter system (Goeddel *et al.* (1980) *Nucleic Acids Res. 8*:4057) and the lambda derived P L promoter and N-gene ribosome binding site (Shimatake *et al.* (1981) *Nature 292*:128). The inclusion of selection markers in DNA vectors transfected in *E. coli* is also useful. Examples of such markers include genes specifying resistance to ampicillin, tetracycline, or chloramphenicol.

The vector is selected to allow introduction into the appropriate host cell. Bacterial vectors are typically of plasmid or phage origin. Appropriate bacterial cells are infected with phage vector particles or transfected with naked phage vector DNA. If a plasmid vector is used, the bacterial cells are transfected with the plasmid vector DNA. Expression systems for expressing a protein are available using *Bacillus sp.* and *Salmonella* (Palva *et al.* (1983) *Gene 22*:229-235); Mosbach *et al.* (1983) *Nature 302*:543-545).

Methods are provided for regulating the expression of a herbicide tolerance polynucleotide, wherein a host cell is provided that comprises a presently disclosed polynucleotide construct and the expression of the site-specific recombinase is induced, thereby excising the excision cassette and allowing for the operable linkage of the

herbicide tolerance polynucleotide and its promoter and the expression of the herbicide tolerance polynucleotide.

Such methods allow for the delay of the expression of a herbicide tolerance polynucleotide until a point in development at which herbicide selection is more effective.

Thus, methods are further provided for selecting a herbicide tolerant plant cell, wherein a population of plant cells are provided, wherein at least one plant cell within the population comprises a presently disclosed polynucleotide construct, inducing the expression of the recombinase, and contacting the population of cells with a herbicide to which the herbicide tolerant polypeptide confers tolerance in order to select for the herbicide tolerant plant cell.

As used herein, the term "population of plant cells" may refer to any one of the following: a grouping of individual plant cells; a grouping of plant cells present within a single tissue, plant or plant part; a population of plants; a population of plant tissues either from the same plant or different plants; a population of seeds either from the same plant or different plants; or a population of plant parts either from the same plant or different plants. The provided population of plant cells, plant tissues, plants, or plant parts may be contacted with the herbicide. Alternatively, the provided population of plant cells may be cultured into a population of plant tissues or a population of plants, which is then exposed to the herbicide. Likewise, a provided population of plant seeds may be planted to produce a population of plants, which is then exposed to the herbicide.

In some embodiments, the provided population of plant cells is cultured into a population of plant tissues or plants prior to, during, or after the induction step, and the population of plant tissues or plants is then contacted with the herbicide. In some of these embodiments, the population of plant tissues is contacted with the herbicide during the regeneration of the tissues into plants or the population of plants that were regenerated from the population of plant tissues is contacted with the herbicide.

In certain embodiments, the provided population of plant cells is a population of immature or mature seeds. In some of these embodiments, the provided population of seeds is planted prior to, during, or after the induction step to produce a population of

plants, and the population of plants are contacted with the herbicide. In those embodiments wherein the provided population of plant cells is a population of immature seeds and the inducible promoter that regulates the expression of the site-specific recombinase is a drought-inducible promoter, the drought-inducible promoter is activated in response to the natural desiccation that occurs during the maturation of the immature seed into a mature seed.

In other embodiments, the provided population of plant cells is a population of plant tissues and these plant tissues are cultured into a population of plants prior to, during, or after the induction step and the population of plants are then contacted with the herbicide.

In yet other embodiments, the provided population of plant cells is a population of plants.

In some embodiments, the provision of a plant or plant part comprising a presently disclosed polynucleotide construct comprises introducing the polynucleotide construct into the plant or plant part.

"Introducing" is intended to mean presenting to the organism, such as a plant, or the cell the polynucleotide or polypeptide in such a manner that the sequence gains access to the interior of a cell of the organism or to the cell itself. The methods and compositions do not depend on a particular method for introducing a sequence into an organism or cell, only that the polynucleotide or polypeptide gains access to the interior of at least one cell of the organism. Methods for introducing polynucleotides or polypeptides into plants or plant parts are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

"Stable transformation" is intended to mean that the nucleotide construct introduced into a plant integrates into a genome of the plant and is capable of being inherited by the progeny thereof. "Transient transformation" is intended to mean that a polynucleotide is introduced into the plant and does not integrate into a genome of the plant or a polypeptide is introduced into a plant.

Protocols for introducing polypeptides or polynucleotide sequences into plants may vary depending on the type of plant or plant cell, *i.e.*, monocot or dicot, targeted for transformation. Suitable methods of introducing polypeptides and polynucleotides into plant cells include microinjection (Crossway *et al.* (1986) *Biotechniques* 4:320-334), electroporation (Riggs *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606, *Agrobacterium*-mediated transformation (U.S. Patent No. 5,563,055 and U.S. Patent No. 5,981,840), direct gene transfer (Paszkowski *et al.* (1984) *EMBO J.* 3:2717-2722), and ballistic particle acceleration (see, for example, U.S. Patent Nos. 4,945,050; U.S. Patent No. 5,879,918; U.S. Patent No. 5,886,244; and, 5,932,782; Tomes *et al.* (1995) in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin); McCabe *et al.* (1988) *Biotechnology* 6:923-926); and Lec1 transformation (WO 00/28058). Also see Weissinger *et al.* (1988) *Ann. Rev. Genet.* 22:421-477; Sanford *et al.* (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou *et al.* (1988) *Plant Physiol.* 87:671-674 (soybean); McCabe *et al.* (1988) *Bio/Technology* 6:923-926 (soybean); Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P:175-182 (soybean); Singh *et al.* (1998) *Theor. Appl. Genet.* 96:319-324 (soybean); Datta *et al.* (1990) *Biotechnology* 8:736-740 (rice); Klein *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein *et al.* (1988) *Biotechnology* 6:559-563 (maize); U.S. Patent Nos. 5,240,855; 5,322,783; and, 5,324,646; Klein *et al.* (1988) *Plant Physiol.* 91:440-444 (maize); Fromm *et al.* (1990) *Biotechnology* 8:833-839 (maize); Hooykaas-Van Slogteren *et al.* (1984) *Nature* 311:763-764; U.S. Patent No. 5,736,369 (cereals); Bytebier *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet *et al.* (1985) in *The Experimental Manipulation of Ovule Tissues,* ed. Chapman *et al.* (Longman, New York), pp. 197-209 (pollen); Kaeppler *et al.* (1990) *Plant Cell Rep* 9:415-418 and Kaeppler *et al.* (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); D'Halluin *et al.* (1992) *Plant Cell* 4:1495-1505 (electroporation); Li *et al.* (1993) *Plant Cell Rep* 12:250-255 and Christou and Ford (1995) *Annals of Botany* 75:407-413 (rice); Osjoda *et al.* (1996) *Nat Biotechnol* 14:745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

In specific embodiments, the polynucleotide constructs can be provided to a plant using a variety of transient transformation methods. Such transient transformation methods include, but are not limited to, the introduction of the polynucleotide construct directly into the plant. Such methods include, for example, microinjection or particle bombardment. See, for example, Crossway *et al.* (1986) *Mol Gen. Genet. 202*:179-185; Nomura *et al.* (1986) *Plant Sci. 44:53-58*; Hepler *et al.* (1994) *Proc. Natl. Acad. Sci. 91*:2176-2180 and Hush *et al.* (1994) *J Cell Sci 107*:775-784, all of which are herein incorporated by reference. Alternatively, the polynucleotide construct can be transiently transformed into the plant using techniques known in the art. Such techniques include viral vector system and the precipitation of the polynucleotide in a manner that precludes subsequent release of the DNA. Thus, the transcription from the particle-bound DNA can occur, but the frequency with which it is released to become integrated into the genome is greatly reduced. Such methods include the use of particles coated with polyethylimine (PEI; Sigma #P3143).

In other embodiments, the polynucleotide construct may be introduced into plants or plant parts by contacting plants or plant parts with a virus or viral nucleic acids. Generally, such methods involve incorporating a nucleotide construct within a viral DNA or RNA molecule. It is recognized that the proteins encoded by the various coding polynucleotides of the polynucleotide construct may be initially synthesized as part of a viral polyprotein, which later may be processed by proteolysis *in vivo* or *in vitro* to produce the desired recombinant protein. Further, it is recognized that promoters also encompass promoters utilized for transcription by viral RNA polymerases. Methods for introducing polynucleotides into plants and expressing a protein encoded therein, involving viral DNA or RNA molecules, are known in the art. See, for example, U.S. Patent Nos. 5,889,191, 5,889,190, 5,866,785, 5,589,367, 5,316,931, and Porta *et al.* (1996) *Molecular Biotechnology 5*:209-221; herein incorporated by reference.

Other methods of introducing polynucleotides into a plant or plant part can be used, including plastid transformation methods, and the methods for introducing polynucleotides into tissues from seedlings or mature seeds.

Methods are known in the art for the targeted insertion of a polynucleotide at a specific location in the plant genome. In one embodiment, the insertion of the polynucleotide at a desired genomic location is achieved using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840, WO99/25855, and WO99/25853, all of which are herein incorporated by reference. Briefly, the polynucleotide can be contained in a transfer cassette flanked by two non-recombinogenic recombination sites. The transfer cassette is introduced into a plant or plant part having stably incorporated into its genome a target site which is flanked by two non-recombinogenic recombination sites that correspond to the sites of the transfer cassette. An appropriate recombinase is provided and the transfer cassette is integrated at the target site. The polynucleotide construct is thereby integrated at a specific chromosomal position in the plant genome.

The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick *et al.* (1986) *Plant Cell Rep* 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved. In this manner, transformed seed (also referred to as "transgenic seed") having a nucleotide construct, for example, an expression cassette, stably incorporated into their genome is provided. Thus, compositions of the invention include plant cells, plant tissues, plant parts, and plants comprising the presently disclosed polynucleotide constructs. Likewise, the methods of the invention can be performed in plant cells, plant tissues, plant parts, and plants.

In certain embodiments the presently disclosed polynucleotide constructs can be stacked with any combination of polynucleotide sequences of interest in order to create plants with a desired trait. A trait, as used herein, refers to the phenotype derived from a particular sequence or groups of sequences. Plants that have various stacked combinations of traits can be created by any method including, but not limited to, cross-

breeding plants by any conventional or TopCross methodology, or genetic transformation. If the sequences are stacked by genetically transforming the plants, the polynucleotide sequences of interest can be combined at any time and in any order. For example, a transgenic plant comprising one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The traits can be introduced simultaneously in a co-transformation protocol with the polynucleotides of interest provided by any combination of transformation cassettes. For example, if two sequences will be introduced, the two sequences can be contained in separate transformation cassettes (trans) or contained on the same transformation cassette (cis). Expression of the sequences can be driven by the same promoter or by different promoters. In certain cases, it may be desirable to introduce a transformation cassette that will suppress the expression of a polynucleotide of interest. This may be combined with any combination of other suppression cassettes or overexpression cassettes to generate the desired combination of traits in the plant. It is further recognized that polynucleotide sequences can be stacked at a desired genomic location using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840, WO99/25855, and WO99/25853, all of which are herein incorporated by reference.

Any plant species can be transformed, including, but not limited to, monocots and dicots. Examples of plant species of interest include, but are not limited to, corn (*Zea mays*), *Brassica* sp. (*e.g.*, *B. napus, B. rapa, B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor, Sorghum vulgare*), millet (*e.g.*, pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum* spp.), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense*, *Gossypium hirsutum*), sweet potato (*Ipomoea batatus*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera*

indica), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats (*Avena*), barley (*Hordeum*), *Arabidopsis*, switchgrass, vegetables, ornamentals, grasses, and conifers.

Vegetables include tomatoes (*Lycopersicon esculentum*), lettuce (*e.g.*, *Lactuca sativa*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus* spp.), and members of the genus *Cucumis* such as cucumber (*C. sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*). Ornamentals include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia (*Euphorbia pulcherrima*), and chrysanthemum.

Conifers that may be employed in practicing the present invention include, for example, pines such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (C*hamaecyparis nootkatensis*). In specific embodiments, plants of the present invention are crop plants (for example, corn, alfalfa, sunflower, *Brassica*, soybean, cotton, safflower, peanut, sorghum, wheat, millet, tobacco, etc.). sugarcane (*Saccharum* spp.). In other embodiments, the plants are maize, rice, sorghum, barley, wheat, millet, oats, sugarcane, turfgrass, or switch grass. In specific embodiments, the plant is sugarcane.

Other plants of interest include grain plants that provide seeds of interest, oil-seed plants, and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, etc. Oil-seed plants include cotton, soybean, safflower, sunflower, *Brassica*, maize, alfalfa, palm, coconut, etc. Leguminous plants include beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, etc.

In certain embodiments, the plant or plant part is a winter wheat plant or plant part. As used herein, "winter wheat" refers to wheat plants or plant parts that require an extended period of low temperatures to be able to flower. Non-limiting examples of winter wheat include *Triticum aestivum* and *Triticum monococcum*.

As used herein, the term "plant part" refers to plant cells, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants such as embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit, kernels, ears, cobs, husks, stalks, roots, root tips, anthers, and the like, as well as the parts themselves. Grain is intended to mean the mature seed produced by commercial growers for purposes other than growing or reproducing the species. Progeny, variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise the introduced polynucleotides.

Methods are also provided for increasing transformation frequency, wherein a host cell is provided that comprises a presently disclosed polynucleotide construct comprising an excision cassette separating a polynucleotide encoding a herbicide tolerance polypeptide from its promoter, wherein the excision cassette comprises a polynucleotide encoding a site-specific recombinase that when expressed can excise the excision cassette. The population of plant cells comprising the polynucleotide construct is cultured in the absence of a herbicide to which the herbicide tolerance polypeptide confers herbicide resistance for a period of time sufficient for the population of plant cells to proliferate, followed by the induction of the expression of the site-specific recombinase, thereby excising the excision cassette and allowing for the operable linkage of the herbicide tolerance polynucleotide and its promoter and the expression of the herbicide tolerance polynucleotide allowing for the direct herbicide selection, thereby the transformation frequency is increased compared to a comparable plant cell not comprising the excision cassette and selected directly by herbicide selection. In some embodiments, the herbicide is glyphosate. In some embodiments, the induction comprises desiccating the population of plant cells. In some embodiments the induction comprises cold treatment.

By "period of time sufficient for the population cells to proliferate" is intended to mean that the population of cells has proliferated to a size and quality to produce transgenic events at an optimal level. The time period sufficient for the cells to proliferate may vary depending on the plant species, cultivar, explant and proliferation medium. In some embodiments, the population of plant cells is cultured in the absence of the herbicide to which the herbicide tolerance polypeptide confers herbicide resistance for about 1 hour to about 12 weeks, about 1 day to about 12 weeks, about 1 week to about 12 weeks, or about 1 week to 6 weeks, including but not limited to about 1 hour, 2, hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, and 12 weeks. In other embodiments, the population of plant cells is cultured in the absence of the herbicide to which the herbicide tolerance polypeptide confers herbicide resistance for about 1 day to about 6 weeks, about 1 day to about 2 weeks, about 1 day to about 4 weeks, about 2 days to about 6 weeks, about 4 days to about 6 weeks, about 1 week to about 6 weeks, about 2 weeks to about 6 weeks, about 2 weeks to about 4 weeks, or about 2 weeks to about 3 weeks prior to excision.

"Transformation frequency" refers to the percentage of plant cells that are successfully transformed with a heterologous nucleic acid after performance of a transformation protocol on the cells to introduce the nucleic acid. In some embodiments, transformation further includes a selection protocol to select for those cells that are expressing one or more proteins encoded by a heterologous nucleic acid of interest. In some embodiments, transformation makes use of a "vector," which is a nucleic acid molecule designed for transformation into a host cell.

An increased "transformation efficiency," as used herein, refers to any improvement, such as an increase in transformation frequency, increased quality events frequency, labor saving, and/or decrease in ergonomic impact that impact overall efficiency of the transformation process by reducing the amount of resources required.

In general, upon use of the methods taught herein, transformation frequency is increased by at least about 3%, 5%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% or greater, or even 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-fold or more, than the transformation frequency relative to a control. The "control" provides a reference point for measuring changes in phenotype of the subject plant or plant cell, e.g., transformation frequency/efficiency, callus quality or transformation process time. The control may include, for example, plant cells transformed with a corresponding nucleic acid without the excision cassette.

In certain embodiments, the plant or plant part useful in the presently disclosed methods and compositions is recalcitrant. As used herein, a "recalcitrant plant" or "recalcitrant plant part" is a plant or plant part in which the average transformation frequency using typical transformation methods is relatively low, and typically less than about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, or 30%. The transformation of species, varieties or cultivars recalcitrant to transformation is time consuming, laborious, and inefficient compared to the transformation of non-recalcitrant varieties, with respect to one or more methods of transformation (e.g., *Agrobacterium*-mediated transformation). Non-limiting examples of species recalcitrant to *Agrobacterium*-mediated transformation include, but are not limited to, species of *Lolium* (rye grass), elite varieties of maize, cultivars of sugarcane, species of rice (especially Indica), and various turf grass species. In some embodiments, the recalcitrant plant or plant part is unable to be transformed in the absence of a cell proliferation factor. In certain embodiments, the recalcitrant plant or plant part is an elite maize inbred or a cell or tissue thereof. In other embodiments, the recalcitrant plant or plant part is the sugarcane cultivar CP96-1252, CP01-1372, CPCL97-2730, HoCP85-845, or CP89-2143 or a cell or tissue thereof.

In some embodiments of the present methods the recalcitrant plant part is an explant from a model or recalcitrant inbred or cultivar. In some embodiments of the present methods and compositions, the explant is from a recalcitrant inbred having a type I callus genotype. In some embodiments of the present methods and compositions, the explant is from a recalcitrant maize inbred having a type I callus genotype. Callus in

grasses can be classified as type I or type II, based upon color, texture, regeneration system, and the amount of time required for callus initiation. The morphology of callus has been reported and described in the agronomically important monocot crops such as maize (Armstrong et al. (1985) *Planta* 164:207-214; Assam (2001) *Arab J Biotechnol* 4:247 256; Frame et al. (2000) *In Vitro Cell Dev Biol-Plant* 36:21-29; Lu et al. (1982) *L. Theor Appl Genet* 62:109-112; McCain et al. (1988) *Bot Gazette* 149:16-20; Songstad et al. (1992) *Am J Bat* 79:761-764; Welter et al. (1995) *Plant Cell Rep* 14:725-729; each of which is herein incorporated by reference in its entirety), rice (Chen et al. (1985) *Plant Cell Tissue Organ Cult* 4:51-51; Nakamura et al. (1989) *Japan J Crop Sci* 58:395-403; Rueb et al. (1994)*Plant Cell Tissue Organ Cult* 36:259-264; each of which is herein incorporated by reference in its entirety), sorghum (Jeoung et al. (2002) *Hereditas* 137:20-28; which is herein incorporated by reference in its entirety), sugarcane (Guiderdoni et al. (1988) *Plant Cell Tissue Organ Cult* 14:71-88; which is herein incorporated by reference in its entirety), wheat (Redway et al. (1990) *Theor Appl Genet* 79:609-617; which is herein incorporated by reference in its entirety), and various nonfood grasses. Type I callus is the typical and most prevalent callus formed in monocot species. It is characterized by compact form, slow-growth, white to light yellow in color, and highly organized. This callus is composed almost entirely of cytoplasmic meristematic cells that lack large vacuoles. In maize, type I callus can only be maintained for a few months and cannot be used in suspension cultures; whereas, type II callus can be maintained in culture for extended periods of time and is able to form cell suspensions. Type II callus derived from maize has been described as soft, friable, rapidly growing and exceedingly regenerative but is typically formed at lower frequencies than type I callus. Embryogenic suspension cells can be initiated from type II callus, which few maize lines can form. Although the ability to form type II callus can be backcrossed into agronomically important maize lines, in practice this is time consuming and difficult. Moreover, even for those lines that can form type II callus, the method requires a great deal of time and labor and is, therefore, impractical. Normally, recalcitrant inbred or cultivar genotypes that produce type I callus have low transformation frequencies. Typically with maize type I inbreds large numbers of embryos or other explants must be

screened to identify sufficient quantities of events, which is expensive and labor intensive.

It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a polynucleotide" is understood to represent one or more polynucleotides. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

Throughout this specification and the claims, the words "comprise," "comprises," and "comprising" are used in a non-exclusive sense, except where the context requires otherwise.

As used herein, the term "about," when referring to a value is meant to encompass variations of, in some embodiments ± 50%, in some embodiments ± 20%, in some embodiments ± 10%, in some embodiments ± 5%, in some embodiments ± 1%, in some embodiments ± 0.5%, and in some embodiments ± 0.1% from the specified amount, as such variations are appropriate to perform the disclosed methods or employ the disclosed compositions.

Further, when an amount, concentration, or other value or parameter is given as either a range, preferred range, or a list of upper preferable values and lower preferable values, this is to be understood as specifically disclosing all ranges formed from any pair of any upper range limit or preferred value and any lower range limit or preferred value, regardless of whether ranges are separately disclosed. Where a range of numerical values is recited herein, unless otherwise stated, the range is intended to include the endpoints thereof, and all integers and fractions within the range. It is not intended that the scope of the presently disclosed subject matter be limited to the specific values recited when defining a range.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1. Glyphosate Selection of Transformed Maize Inbred PHR03

Immature embryos from maize inbred PHR03 were harvested 9-13 days post-pollination with embryo sizes ranging from 0.8 – 2.5 mm length and were co-cultivated with *Agrobacterium* strain LBA4404 containing the vector PHP29204 or *Agrobacterium* strain LBA4404 containing the vector PHP32269 on PHI-T medium for 2-4 days in dark conditions. PHP29204: Ubi:DsRed + Ubi:GAT4602. PHP32269: Ubi:PMI + Ubi:MOPAT::YFP. Ubi refers to the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113). The tissues were then transferred to DBC3 medium without selection for one week, and then to DBC3 medium with 0.25 mM or 0.5 mM glyphosate for 3 weeks, and then DBC3 medium with 0.5 mM glyphosate for another 3-4 weeks. The embryos were then transferred to PHI-RF maturation medium with 0.1 mM glyphosate for 2-3 weeks until shoots formed, at which point, the shoots were transferred to MSB medium in Phytatrays containing 100 mg/L cefotaxime for rooting. Plants with good roots were transferred to soil for further growth and a glyphosate spray test. For PMI selection using PHP32269, DBC3 medium containing 12.5 g/L mannose and 5g/L maltose was used for selection. PHI-RF maturation medium without any selective agent or sugar modifications was used for regeneration.

PHI-T medium contains 0.1 μM copper in MS salts 4.3 mg/L, Nicotinic acid 0.5 mg/L, Pyridoxine HCl 0.5 mg/L, Thiamine HCl 1 mg/L, Myo-inositol 100 mg/L, 2,4-D 2 mg/L, Sucrose 20 g/L, Glucose 10 g/L, L-proline 700 mg/L, MES 0.5 g/L, Acetosyringone 100 μM, Ascorbic acid 10 mg/L and Agar 8.0 g/L.

PHI-RF is 4.3 g/L MS salts (GIBCO BRL 11117-074), 0.5 mg/L nicotinic acid, 0.1 mg/L thiamine HCl, 0.5 mg/L pyridoxine HCl, 2.0 mg/L glycine, 0.1 g/L myo-inositol, 0.49 μM cupric sulfate, 0.5 mg/L zeatin (Sigma Z-0164), 1 mg/L IAA, 26.4 μg/L ABA, thidiazuron 0.1 mg/L, 60 g/L sucrose, 100 mg/L cefotaxime, 8 g/L agar, pH 5.6.

Table 4. Transformation frequency of maize inbred PHR03 with PHP29204 or PHP32269.

Vector	No. of embryos	No. of T_0 events	% Transformation	No. single copy events	% Single Copy Events
PHP29204	300	21	7	13	61.9
PHP32269	90	36	40	16	44.4

The transformation frequency with PHP29204 with glyphosate selection was only 7% in the maize inbred PHR03. Overall, glyphosate selection did not provide for a clean selection, a lot of non-transformed tissues were growing, and the morphology of both transformed and non-transformed tissues was irregular.

Example 2. *Agrobacterium*-mediated Sugarcane Transformation Using a Standard Test Vector Without Developmental Genes

Media for Plant Transformation:

Liquid DBC3(M5G) contains MS salts (4.3 g/L) plus maltose (30 g/L); glucose (5 g/L); thiamine-HC1 (1 mg/mL); myo-inositol(0.25 g/L); N-Z-amine-A (casein hydrolysate) (1 g/L); proline (0.69 g/L); $CuSO_4$ (4.9 μM); 2,4-D (1.0 mg/L); BAP (0.5 mg/L); Adjust volume to 1 L with ddH2O; pH 5.8 — Adjust pH with 1 M KOH; autoclave.

DBC3 contains MS salts (4.3 g/L) plus maltose (30 g/L); thiamine-HC1 (1 mg/mL); myo-inositol (0.25 g/L); N-Z-amine-A (casein hydrolysate) (1 g/L); proline (0.69 g/L); $CuSO_4$ (4.9 μM); 2,4-D (1.0 mg/L); BAP (0.5 mg/L); Adjust volume to 1 L with ddH_2O; pH 5.8 — Adjust pH with 1 M KOH; Phytagel (3.5 g/L); autoclave.

DBC6 contains MS salts (4.3 g/L) plus maltose (30 g/L); thiamine-HC1 (1 mg/mL); myo-inositol(0.25 g/L); N-Z-amine-A (casein hydrolysate) (1 g/L); proline (0.69 g/L); $CuSO_4$ (4.9 μM); 2,4-D (0.5 mg/L); BAP (2.0 mg/L); Adjust volume to 1 L with ddH_2O; pH 5.8 — Adjust pH with 1 M KOH; Phytagel (3.5 g/L); autoclave.

MSB contains MS salts and vitamins (4.43 g/L) plus sucrose (20 g/L); myo-inositol (1.0 g/L); indole-3-butyric acid (IBA, 0.5 mg/L); Adjust volume to 1 L with ddH_2O; pH 5.8 — Adjust pH with 1 M KOH; Phytagel (3.5 g/L); autoclave.

Preparation of Agrobacterium Suspension:

Agrobacterium tumefaciens harboring a binary vector from a -80° frozen aliquot was streaked out onto solid PHI-L or LB medium containing an appropriate antibiotic and cultured at 28°C in the dark for 2-3 days. A single colony or multiple colonies were picked from the master plate and streaked onto a plate containing PHI-M medium and incubated at 28°C in the dark for 1-2 days. *Agrobacterium* cells were collected from the solid medium using 5 mL 10 mM $MgSO_4$ medium (*Agrobacterium* infection medium) plus 100 μM acetosyringone. One mL of the suspension was transferred to a spectrophotometer tube and the OD_{500nm} of the suspension was adjusted to 0.35-0.40 at 550 nm using the same medium.

Agrobacterium Infection and Co-cultivation:

Good quality callus tissues induced from *in vitro*-cultured plantlets were collected in an empty Petri dish and exposed to air in the hood for about 30 minutes. Tissue that is younger than 2 months old is considered ideal for transformation. One mL *Agrobacterium* suspension was added to the Petri dish, the tissues were broken or chopped into small pieces, and an additional 1-3 mL *Agrobacterium* (AGL1) suspension was then added to cover all the tissues. The Petri dish was placed into a transparent polycarbonate desiccator container, and the container was covered and connected to an in-house vacuum system for 20 minutes. After infection, the *Agrobacterium* suspension was drawn off from the Petri dish and the tissues were transferred onto 2 layers of VWR 415 filter paper (7.5 cm diameter) of a new Petri dish and 0.7-2.0 mL liquid DBC3 (M5G) medium plus 100 μM acetosyringone was added for cocultivation depending on the amount of tissue collected. The top layer of filter paper containing the infected tissues was transferred to a fresh layer of filter paper of another new Petri dish. The infected tissues were incubated at 21°C in the dark for 3 days.

Selection and Plant Regeneration:

Callus tissues were transferred to first round selection DBC3 containing antibiotics (timentin and cefotaxime) and 3 mg/L bialaphos (Meiji Seika, Tokyo, Japan).

Tissues were transferred to 2nd round selection DBC6 containing antibiotics and 3-5 mg/L bialaphos and subcultured for 3 weeks at 26-28°C in dark or dim light conditions. At the 3rd round selection on DBC6 medium containing antibiotics and bialaphos, tissues were broken into smaller pieces and exposed to bright light conditions (30-150 μmol m^{-2} sec^{-1}) for 2-3 weeks. Shoot-elongated tissues were broken into small pieces and transferred to MSB regeneration/rooting medium containing antibiotics and 3 mg/L bialaphos. Single plantlets were separated and transferred to soil.

Table 5 shows the results of transformation experiments using 7 U.S. sugarcane cultivars. CP89-2376 and CP88-1762 had >100% transformation frequency at the T_0 plant level using a standard vector containing DsRED and PAT (or moPAT) while the remaining 5 cultivars, CP96-1252, CP01-1372, CPCL97-2730, HoCP85-845 and CP89-2143, were recalcitrant in transformation.

Table 5. Transformation Frequencies at T_0 Plant Level in 7 U.S. Sugarcane Cultivars Using a Standard Test Vector.

CP96-1252	**CP01-1372**	**CP89-2376**	**CPCL97-2730**	**HoCP85-845**	**CP89-2143**	**CP88-1762**
n.t.*	n.t.	75.0% (6/8)	n.t.	n.t.	n.t.	n.t.
0% (0/8)	0% (0/8)	100.0% (8/8)	0% (0/8)	n.t.	n.t.	n.t.
n.t.	n.t.	87.5% (7/8)	n.t.	n.t.	n.t.	n.t.
n.t.	n.t.	150.0% (12/8)	n.t.	0% (0/8)	n.t.	n.t.
n.t.	n.t.	n.t.	n.t.	n.t.	0% (0/8)	62.5% (5/8)
n.t.	n.t.	100.0% (8/8)	n.t.	n.t.	0% (0/8)	137.5% (11/8)
n.t.	n.t.	187.5% (15/8)	n.t.	n.t.	n.t.	137.5% (11/8)

Transformation Frequency = (# transgenic events / # explants infected with *Agrobacterium*) x 100%
*n.t.: not tested

Confirmation of Transgenic Events:

The putative stable callus/green tissues/regenerating plants were identified based on the visible RFP marker gene expression. All of these putative transgenic callus tissues were transferred to medium for plant regeneration under standard regeneration conditions. The final confirmation of stable transformation frequency was determined based on molecular analysis such as PCR and Southern blot hybridization.

Example 3. Sugarcane Transformation Using a Developmental Gene (DevGene) Vector PHP35648 and Excision Test

A DevGene binary vector (PHP35648, Figure 1) with the BBM/WUS gene cassette was initially compared with a standard vector containing PAT or moPAT plus DsRED without the BBM/WUS gene cassette for transformation frequency using two *Agrobacterium* strains, AGL1 and LBA4404, in cultivar CP89-2376 and the recalcitrant cultivar CP01-1372 (Table 6). The DevGene binary vector contains Ubi::LoxP::CFP+Rab17Pro-attb1::Cre+Nos::ZmWUS2+Ubi::ZmBBM-LoxP::YFP+Ubi::MOPAT (Figure 1); each gene cassette has a 3' terminator. The Lox cassette containing CFP::Cre::WUS::BBM can be excised by Cre recombinase controlled by the Rab17 promoter. The PHP35648 vector was designed to demonstrate the excision efficiency of the excision cassette using visual markers. The PHP35648 excision cassette comprises the cyan fluorescent protein (CFP) controlled by the ubiquitin promoter (comprising the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113)), which is located outside of the loxP site flanking the excision cassette (see Figure 1). Transformants comprising the excision cassette can be visually identified by the presence of the cyan fluorescent protein (CFP). When the excision cassette is excised, the yellow fluorescent protein (YFP) is expressed under the regulation of the ubiquitin promoter. Transformants lacking the excision cassette can be visually identified by the presence of the yellow fluorescent protein (YFP). The ratio of

cyan fluorescent protein (CFP) to yellow fluorescent protein (YFP) can be used to demonstrate the excision efficiency. In PHP35648, the ubiquitin promoter controlling the expression of the moPAT gene product was included outside of the excision cassette as a positive selection to reduce the number of escapes.

Callus tissues of all 5 sugarcane cultivars were induced and maintained on DBC3 medium. Tissues were infected with *Agrobacterium* containing the DevGene binary vector PHP35648 in liquid 10 mM $MgSO_4$ plus 100 μM acetosyringone and then co-cultivated with liquid DBC3 (M5G) medium plus 100 μM acetosyringone on filter paper in Petri dishes at 21°C in the dark. Three days after co-cultivation, the tissues were transferred to DBC3 containing 100 mg/L cefotaxime and 150 mg/L timentin for AGL1 and DBC3 containing 100 mg/L carbenicillin for LBA4404, and incubated at 26°C (±1°C) in the dark or dim light for 3-7 days. Afterwards, the tissues were transferred to the same media as the previous step plus 3 or 5 mg/L bialaphos. After 2 to 3 weeks, the tissues were transferred to 2nd round selection DBC6 containing antibiotics and 3-5 mg/L bialaphos. After two months from the initiation of the experiment, transformation frequency was calculated by the number of tissues showing CFP-expressing sectors divided by the number of explants infected by *Agrobacterium*. AGL1 was more efficient in transformation than LBA4404 in both CP89-2376 and CP01-1372 (Table 6, rows 1 and 2). There was also a genotype difference in transformation frequency; the CP89-2376 cultivar had a much higher transformation frequency than the recalcitrant cultivar CP01-1372 using either of the *Agrobacterium* strains.

AGL1 containing the DevGene binary vector PHP35648 was also used to test sugarcane germplasm screening in another set of four experiments (Table 6, rows 3-6) using 5 different cultivars (CP96-1252, CP01-1372, CP89-2376, CPCL97-2730 and HoCP85-845). Callus tissues of all 5 cultivars tested were induced and maintained on DBC3 medium and tissues were infected with AGL1 containing the developmental gene binary vector PHP35648. The use of developmental genes dramatically increased transformation frequency in all 5 cultivars tested. Transformation frequencies in the most amenable cultivar, CP89-2376, using a standard binary vector averaged 116.7% (56/48) (Table 6). In contrast, an average transformation frequency in CP89-2376 from the 5

experiments using the DevGene binary vector PHP35648 was >2,512.5% (>1,005 events/40 tissues infected) (see Table 6, rows 2-6). An increase in transformation frequency was also observed in the recalcitrant cultivars CP96-1252, CP01-1372, CPCL97-2730 and HoCP85-845; with transformation frequencies ranging from 62.5% to 1250.0% using AGL1 while no transgenic events were obtained using the standard vector without the BBM/WUS gene cassette from these cultivars (Table 6, row 7).

Table 6. Transformation Frequency in Sugarcane Using a BBM/WUS Developmental Gene Vector PHP35648.

Agrobacterium		Sugarcane Cultivar				
Strain	**Binary Vector**	**CP96-1252**	**CP01-1372**	**CP89-2376**	**CPCL97-2730**	**HoCP85-845**
AGL1	DG[a]	n.t.[c]	37.5% (3/8)	n.t.	n.t.	n.t.
LBA4404	DG	n.t.	0% (0/8)	n.t.	n.t.	n.t.
AGL1	DG	n.t.	>1,250.0% (>100/8)	>6,250.0% (>500/8)	n.t.	n.t.
LBA4404	DG	n.t.	12.5% (1/8)	>1,500% (>120/8)	n.t.	n.t.
AGL1	DG	n.t.	n.t.	687.5% (>55/8)	n.t.	n.t.
AGL1	DG	n.t.	n.t.	>2,500% (>200/8)	175.0% (14/8)	n.t.
AGL1	DG	150.0% (12/8)	62.5% (5/8)	>625.0% (>50/8)	62.5% (6/8)	n.t.
AGL1	DG	n.t.	n.t.	>2,500% (>200/8)	n.t.	187.5% (15/8)
AGL1	**Std[b]**	**0% (0/8)**	**0% (0/8)**	**116.7% (56/48)**	**0% (0/8)**	**0% (0/8)**

Each transformation treatment had 8 pieces of callus tissues 0.4-0.5 cm in size.
DG[a]: developmental gene vector with BBM/WUS gene cassette
Std[b]: standard vector without BBM/WUS gene cassette
n.t.[c].: not tested

Excision of the LoxP cassette by dessication monitored by visual markers

Transgenic callus tissues were desiccated on dry filter papers for one day to induce excision of the Lox cassette containing CFP::Cre::WUS::BBM by Cre recombinase driven by the Rab17 promoter (Figure 1). Excision was monitored by observing YFP expression on desiccated transgenic callus events by the presence of the UBI:loxP:YFP junction formed as a result of excision (Figure 1). Cre excision occurred on 83 of 87 transgenic events (95.4%) (Table 7). Plants from some transgenic events after excision were regenerated on MSB plus 1-3 mg/L bialaphos and antibiotics.

Table 7. Excision Efficiency of the BBM/WUS Gene Cassette in Transgenic Sugarcane Events by Desiccation.

Sugarcane Cultivar	***Agrobacterium* Strain**	**Binary Vector**	**Excision Efficiency (%)**
CP89-2376	AGL1	DG[a]	93% (40/43)
CP89-2376	LBA4404	DG	100% (25/25)
CP01-1372	AGL1	DG	100% (13/13)
CP01-1372	LBA4404	DG	0% (0/1)
CP89-2376	AGL1	DG	100% (5/5)
Average			95.4% (83/87)

DG[a]: developmental gene (DevGene) vector PHP35648 with BBM/WUS gene cassette

Example 4. Sugarcane Excision Induction and Plant Regeneration from Transformed Callus/Green Tissue Events Generated Using a Developmental Gene (DevGene) Vector PHP54561

Generation of Transgenic Events:

A new DevGene binary vector PHP54561 with the BBM/WUS gene cassette was designed as described in Figure 2. The DevGene binary vector PHP54561 contains Ubi::LoxP-moPAT+Ubi:YFP+Rab17Pro-attb1:Cre+Nos:ZmWUS2+Ubi:ZmBBM-LoxP::GLYAT (Figure 2); each gene cassette has a 3' terminator. The Lox cassette

containing moPAT+Ubi:YFP+Rab17Pro-attb1:Cre+Nos:ZmWUS2+Ubi:ZmBBM can be excised by Cre recombinase controlled by the Rab17 promoter. The PHP54561 excision cassette was designed to test the excision efficiency directly by glyphosate tolerance (see Figure 2). The yellow florescent protein (YFP) was included in the PHP54561 excision cassette as a visual marker and moPAT as a selection marker prior to excision (see Figure 2). Ubi refers to the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113).

Callus tissues of two U.S. sugarcane cultivars, CP88-1762, CP01-1372 and 1 Australian cultivar, KQ228, were induced and maintained on DBC3 or DBC6 medium. Tissues were infected with *Agrobacterium* containing the DevGene binary vector PHP54561 in liquid 10 mM $MgSO_4$ plus 100 μM acetosyringone and then co-cultivated with liquid DBC3 (M5G) medium plus 100 μM acetosyringone on the filter paper in Petri dishes at 21°C in the dark. Three days after co-cultivation, the tissues of CP88-1762/CP01-1372 and KQ228 were transferred to DBC3 and DBC6 containing 100 mg/L cefotaxime and 150 mg/L timentin, respectively, and incubated at 26°C (±1°C) in the dark or dim light for 3-7 days. Afterwards, the tissues were transferred to the same media as the previous step plus 3 or 5 mg/L bialaphos. After 2 to 3 weeks, the tissues were transferred to 2nd round selection DBC6 containing antibiotics and 3-5 mg/L bialaphos. YFP-expressing sectors were transferred to the same medium for proliferation. After two months from the initiation of the experiment, transformation frequency was calculated by the number of tissues showing YFP-expressing sectors divided by the number of explants infected by *Agrobacterium*. Table 8 demonstrated transformation frequency at the T_0 tissue level in 3 sugarcane cultivars. CP88-1762, an amenable cultivar had 405% transformation. Two recalcitrant cultivars, CP01-1372 and KQ228 also had high transformation frequencies, 885% and 130%, respectively.

Table 8. Transformation Frequencies at the T_0 Tissue Level in Sugarcane with Bialaphos Selection before Excision.

Cultivar	Txn Frequency (%)
CP01-1372*	270% (27/10)
CP01-1372*	1500% (150/10)
Total	885% (177/20)
CP88-1762	400% (40/10)
CP88-1762	410% (41/10)
Total	405% (81/20)
KQ228*	10% (1/10)
KQ228*	250% (25/10)
Total	130% (26/20)

*CP01-1372 and KQ228 are recalcitrant commercial cultivars.

Excision of LoxP Cassette by Desiccation and Plant Regeneration with Glyphosate Selection:

Transgenic tissues (0.3-0.5 mm in diameter) were transferred to an empty 60 mm x 25 mm Petri dish containing a piece of sterilized glass filter paper (VWR Glass Microfibre filter, 691). The Petri dish was covered with a lid and placed in a container with a tight-seal cover. A Petri dish (or beaker) with ~20 mL of sterilized water with the lid open was placed in the container. The container was kept in a dark culture room for 1-2.5 days at 28°C; the desiccation period was dependent on the degree or size of tissues. After 1-2.5 days of desiccation treatment, the desiccated tissues were transferred to DBC6 proliferation medium with antibiotics and 100 μM glyphosate. The plates were kept in

dim (10-50 μmol m^{-2} sec^{-1}) to moderately bright light at 26-28°C for 2-3 weeks (Figure 3). If necessary, tissues were subcultured for another round on the same medium for another 2-3 weeks to get small green shoots; the plates was kept in a higher intensity of light at 26-28°C. Tissues with shoots were picked up and placed onto MSB regeneration/rooting medium containing antibiotics and 20-30 μM glyphosate in A175 Agar (PhytoTechnology Lab) as a gelling agent. Tissues were cultured under bright light conditions (50-200 μmol m^{-2} sec^{-1}) for 3-4 weeks at 26-28°C. When shoots were strong enough, single plantlets were separated and transferred to soil. In general, plants with complete excision exhibited a normal phenotype with greener and faster growth, while plantlets from tissues without excision of the developmental genes or having incomplete excision usually showed a stunted phenotype or bleached shoots, indicating susceptibility to glyphosate (Figures 4 and 5). Plants with a normal phenotype were transferred to soil for further growth, glyphosate spray test and molecular assay.

Table 9 shows LoxP cassette excision efficiency in transgenic events of 3 sugarcane cultivars, CP88-1762, CP01-1372 and KQ228, based on glyphosate resistance of the events. Excision efficiencies ranged from 32% to 68% in these 3 cultivars.

Table 9. Excision Efficiency with Glyphosate Selection of Transgenic Sugarcane Events by Desiccation.

Cultivar	Transformation Frequency*	# of events desiccated	# of events with green elongated shoots on glyphosate	Excision Efficiency (# of events excised/# of events desiccated)
CP01-1372	270% (27/10)	12	8	66.7% (8/12)
CP01-1372	1500% (150/10)	41	28	68.3% (28/41)
Total	885% (177/20)	53	36	67.9% (36/53)
CP88-1762	400% (40/10)	15	6	40.0% (6/15)
CP88-1762	410% (41/10)	38	20	52.6% (20/38)
Total	405% (81/20)	53	26	49.1% (26/53)
KQ228	10% (1/10)	1	0	0% (0/1)
KQ228	250% (25/10)	21	7	33.3% (7/21)
Total	130% (26/20)	22	7	31.8% (7/22)

*bialaphos selection before excision

Glyphosate Resistance Confirmation by Glyphosate Spray Test:

T_0 plantlets were then moved to soil and spray tested with 4X glyphosate to confirm excision/ glyphosate resistance. All 72 independent T_0 events from 3 sugarcane cultivars (Table 9) showed strong glyphosate resistance while plants of 3 nontransgenic cultivars were completely killed by glyphosate spray. The final confirmation of stable

transformation frequency is determined based on molecular analysis such as PCR and Southern blot hybridization.

Example 5. Corn Excision Induction and Plant Regeneration from Desiccated T_1 Immature Embryos

Corn Transformation:

A corn elite inbred, PHR03 was transformed with *Agrobacterium* strain AGL1 containing the excision vector PHP54353. The PHP54353 vector contains Ubi::LoxP-Ds RED+Rab17-attB::CRE-LoxP::GLYAT (Figure 6). The Lox cassette containing Ds RED+Rab17-attB::CRE can be excised by Cre recombinase controlled by the Rab17 promoter. The PHP54353 excision cassette was designed to demonstrate direct glyphosate selection. Ubi refers to the maize ubiquitin promoter (UBI1ZM PRO; SEQ ID NO: 111), the ubiquitin 5' UTR (UBI1ZM 5UTR; SEQ ID NO: 112), and ubiquitin intron 1 (UBIZM INTRON1; SEQ ID NO: 113).

Immature embryos from maize inbred PHR03 were harvested 9-13 days post-pollination with embryo sizes ranging from 0.8 – 2.5 mm length and were co-cultivated with *Agrobacterium* strain AGL1 containing the excision vector PHP54353 on PHI-T medium for 3 days in dark conditions. These embryos were then transferred to DBC3 medium containing 100 mg/L cefotaxime in dim light conditions. RFP-expressing sectors were picked up and proliferated on the same medium. When the tissue proliferation period for each transgenic event was sufficient, tissues were moved to PHI-RF maturation medium. Regenerating shoots were transferred to MSB medium in Phytatrays containing 100 mg/L cefotaxime for rooting. Plants with good roots were transferred to soil for further growth, glyphosate spray test and molecular assay.

PHI-T medium contains 0.1 μM copper in MS salts 4.3 mg/L, Nicotinic acid 0.5 mg/L, Pyridoxine HCl 0.5 mg/L, Thiamine HCl 1 mg/L, Myo-inositol 100 mg/L, 2,4-D 2 mg/L, Sucrose 20 g/L, Glucose 10 g/L, L-proline 700 mg/L, MES 0.5 g/L, Acetosyringone 100 μM, Ascorbic acid 10 mg/L and Agar 8.0 g/L.

PHI-RF is 4.3 g/L MS salts (GIBCO BRL 11117-074), 0.5 mg/L nicotinic acid, 0.1 mg/L thiamine HCl, 0.5 mg/L pyridoxine HCl, 2.0 mg/L glycine, 0.1 g/L myo-inositol, 0.49 μM cupric sulfate, 0.5 mg/L zeatin (Sigma Z-0164), 1 mg/L IAA, 26.4 μg/L ABA, thidiazuron 0.1 mg/L, 60 g/L sucrose, 100 mg/L cefotaxime, 8 g/L agar, pH 5.6.

Immature Embryo Isolation, Desiccation, Selection and Regeneration:

Sterilized immature embryos with 2.0-3.5 mm were placed scutellum side down on sterile fiber glass filter paper in a Petri dish. 300 μL of DBC6 liquid medium with 100 mg/L cefotaxime was added to the filter paper to prevent over drying. Plates were wrapped with Parafilm and checked for expression of DsRed before desiccation in order to compare expression after desiccation. Plates were moved into a sterile laminar hood unwrapped and let stand for 2-4 days until the embryos appeared darker and shrunken, and were desiccated. Embryos were then placed scutellum side down onto MSA regeneration medium containing 100 mg/L cefotaxime with 10-50 uM glyphosate for selection. Five to 10 days later, DsRed expression is checked in the emerging shoots.

Example 6. Natural Desiccation and Excision in Transgenic Mature Corn Seed

Immature embryos of maize inbred PHR03 were transformed with the excision vector AGL1/PHP54353, the expression of DsRed was visually confirmed, and T_0 plantlets were regenerated as described in Example 5. Before moving the T_0 plantlets to soil, the expression of DsRed was again visually confirmed.

Glyphosate resistance confirmation

To confirm that the natural desiccation process that occurs during seed maturation would in fact allow for the excision of DsRed and resistance to glyphosate, seeds collected from T_0 plants crossed with wild-type PHR03 pollen were germinated in soil. By planting seeds straight to soil without any treatments, excision would be a result of natural processes.

Three random events were chosen to be tested by this method. Five mature T_1 seeds each from the following events, PHP54353 T_0 event numbers 6, 7, and 10 were placed in small pots with Metro Mix soil (Sun Gro Horticulture, McFarland, CA) with

fertilizer and placed in the greenhouse. After plants had germinated and grown to about 12-18 cm (10-12 days after planting), the plants were then sprayed with glyphosate + surfactant at 2X or 4X concentration (1X is equivalent to what is used in the field). Before spraying, all pots were evenly spaced and positioned to ensure that they would receive an even distribution of glyphosate. The distance between the sprayer nozzle and the apical meristem of the plants was approximately 18 inches. Within 10-12 days, it was visibly evident which plants were not affected by the herbicide and which plants had been severely damaged.

The results of the spray test are presented in Table 10. From visible spray test results, all wild-type PHR03 plants had been severely damaged, as predicted. It was also clear that 2 out of 4 plants from event number 6 had no signs of damage and continued to grow at a normal rate having not lost any leaf tissue. However, all 5 plants from event number 7 did show damage equivalent to that of the wild-type PHR03 plants, which was not expected. All 4 plants from event number 10 also showed damage equivalent to that of the wild-type PHR03 plants. When the T_0 plants were analyzed for the presence of the DsRED and GLYAT genes, it was discovered that event number 10 did not have the DsRED gene and although the T_0 plant had the GLYAT gene, presumably GLYAT was not expressed because it was not operably linked to a promoter (see Table 10). In event number 13, 3 out of 5 plants showed damage and 2 out of 5 plants were tolerant.

Table 10. Glyphosate Spray Test on Plants Germinated from T_1 Mature Corn Seed

Lab event #	**DS-RED2INT QPCR of T_0**	**GLYAT QPCR of T_0**	**Glyphosate Spray Test**
6	+	+	2/4 plants damaged; 2/4 plants tolerant
7	+	+	5/5 plants damaged
10	-	+	4/4 plants damaged
13	+	+	3/5 plants damaged; 2/5 plants tolerant
Wild-type	-	-	4/4 plants damaged

Example 7. Tobacco Excision Induction and Plant Regeneration from Transformed Tissue Events

Tobacco Transformation

Young leaves are harvested from *in vitro*-cultured tobacco plants and cut into 0.5-1 cm size as an *Agrobacterium* infection target. AGL1/PHP55062 (a standard excision vector, Figure 8) is used for transformation. Transgenic tobacco (cv. Petite havana) plants are generated following the leaf disc method described by Horsch et al. (1985) *Science* 227:1229-1231, which is herein incorporated by reference in its entirety, and 50 mg/L hygromycin B was used for selection.

Excision of LoxP Cassette by Desiccation and Plant Regeneration with Glyphosate Selection

Tobacco desiccation experiments are conducted to induce excision from transformed tissue events and transformed plants are regenerated. Once tissue from each event having visual marker expression has reached a sufficient size when grown on selection medium with hygromycin, desiccation experiments can be conducted. Tissues (0.3-0.5mm in diameter) are sliced and transferred to an empty 60 mm x 25 mm Petri dish containing a piece of sterilized glass filter paper (VWR Glass Microfibre filter, 691). The Petri dish is covered and placed in a container with a tight-seal cover. An open Petri dish with 15 mL of sterilized water is placed in the container. The container is placed in a dark culture room at 28°C. After 2-3 days of desiccation treatment, the tissues are either directly transferred to regeneration medium or selection medium with antibiotics and 20-50 uM glyphosate using Phytagel as a gelling agent for 2-3 weeks with sealed plates for proliferation and regeneration. The tissues are transferred to regeneration medium with antibiotics and 20-50 uM glyphosate for another 2-4 weeks to generate shoots. Plates are placed in higher intensity light at 26-28°C. When shoots are strong enough, single plantlets are separated and transferred to soil. Leaf samples are collected for qPCR analysis.

Example 8. Tobacco Excision Induction and Plant Regeneration from Desiccated T_1 Immature Seeds

T_1 immature seeds from transgenic tobacco plants are isolated, sterilized with 15% Clorox + 2 drops of Tween 20 and rinsed with autoclaved water 3 times. Sterilized immature seeds are placed on sterile fiber glass filter paper in a Petri dish. The Petri dish is covered and moved into a sterile laminar hood unwrapped and incubated for 1-2 days until the seeds are desiccated. Desiccated immature seeds are then placed onto regeneration medium containing 100 mg/L cefotaxime and with 20-50 μM glyphosate for selection. One to 2 weeks later, DsRed expression is checked in the emerging shoots. Immature seeds that have been properly desiccated have very weak or no DsRed expression as the gene is excised via the LoxP sites. Both transgenic and nontransgenic seeds without desiccation treatment will germinate well on glyphosate-free medium while germination will be completely inhibited for both of them on 20-50 μM glyphosate. Immature seeds that successfully underwent gene excision by desiccation will have glyphosate resistance and regenerate on medium containing 20-50 μM glyphosate.

Healthy plantlets are transferred to regeneration medium in Phytatrays containing 100 mg/L cefotaxime and 20-50 μM glyphosate for further selection and growth.

Example 9. Natural Desiccation and Excision in Transgenic Mature Tobacco Seeds

Mature seed sterilization, Selection/ Regeneration:

T_1 mature tobacco seed transformed with AGL1/ PHP55062 are sterilized with 20% Clorox + 2 drops Tween 20 and rinsed with autoclaved water 3 times. Sterilized seeds are then transferred to regeneration medium containing 100 mg/L cefotaxime with 20-50 μM glyphosate for selection. After 5-10 days, DsRed expression is checked in the emerging shoots. Seeds that have been excised will no longer have DsRed expression as the gene is cleaved via the Lox P sites. Those seeds that are successfully excised of DsRed will have glyphosate resistance and regenerate on medium containing glyphosate. Once seeds have healthy shoot and root formation, the plantlets are moved to soil or another regeneration medium containing 100 mg/L cefotaxime in Phytatrays with 20 or 50 μM glyphosate for further selection and growth.

Sowing dry tobacco T_1 seeds straight to soil and glyphosate resistance confirmation:

To confirm that the natural desiccation process that occurs during seed maturation would in fact allow for the excision of DsRed and resistance to glyphosate, seeds collected from T_0 tobacco plants are germinated in soil. By planting seeds straight to soil without any treatments, excision would truly be a result of natural processes. After plants have germinated and grown to about 10-15 cm, the plants are sprayed with glyphosate + surfactant at 2X or 4X concentration (1X is equivalent to what is used in the field). Within 10-12 days, it is visibly evident which plants are not affected by the herbicide and which plants are severely damaged.

Example 10. Soybean Excision Induction and Plant Regeneration from Transformed Tissue Events

Soybean Transformation:

Soybean (cv. Jack) mature seeds are sterilized and sliced into half longitudinally and half-seeds are used as an *Agrobacterium* infection target. *Agrobacterium* strain AGL1 containing the PHP55062 vector (a standard excision vector, Figure 8) is used for transformation. Alternatively, soybean embryogenic suspension cultures are transformed with the PHP55062 vector via *Agrobacterium*-mediated transformation as described herein or by the method of particle gun bombardment (Klein *et al.* (1987) *Nature*, 327:70, which is herein incorporated by reference in it entirety).

Transgenic soybean plants are generated following the method described in U.S. Patent No. 7,473,822, which is herein incorporated by reference in its entirety, and 5 to 30 mg/L hygromycin B is used for selection.

Excision of LoxP Cassette by Desiccation and Plant Regeneration with Glyphosate Selection:

Soybean desiccation experiments are conducted to induce excision from transformed tissue events and transformed plants are regenerated. Once tissue from each event having visual marker expression has reached a sufficient size when grown on

selection medium with hygromycin, desiccation experiments can be conducted. Tissues (0.3-0.5mm in diameter) are sliced and transferred to an empty 60 mm x 25 mm Petri dish containing a piece of sterilized glass filter paper (VWR Glass Microfibre filter, 691). The Petri dish is covered and placed in a container with a tight-seal cover. An open Petri dish with 15 mL of sterilized water is placed in the container. The container is placed in a dark culture room at 28°C. After 2-3 days of desiccation treatment, the tissues are either directly transferred to regeneration medium with antibiotics and 20-50 μM glyphosate using Phytagel as a gelling agent for 2-3 weeks with sealed plates for proliferation and regeneration. The tissues are transferred to regeneration medium with antibiotics and 20-50 μM glyphosate for another 2-4 weeks to generate shoots. Plates are placed in higher intensity light at 26-28°C. When shoots are strong enough, single plantlets are separated and transferred to soil. Leaf samples were collected for qPCR analysis.

Example 11. Soybean Excision Induction and Plant Regeneration from Desiccated T_1 Immature Seeds

T_1 immature pods from transgenic soybean plants are harvested, sterilized with 15% Clorox + 2 drops of Tween 20 and rinsed with autoclaved water 3 times. Immature seeds are isolated from sterilized pods and placed on sterile fiber glass filter paper in a Petri dish. The Petri dish is covered and moved into a sterile laminar hood unwrapped and incubated for 1-2 days until the seeds are desiccated. Desiccated immature seeds are then placed onto regeneration medium containing 100 mg/L cefotaxime and with 20-50 μM glyphosate for selection. One to 2 weeks later, DsRed expression is checked in the emerging shoots. Immature seeds that have been properly desiccated will have very weak or no DsRed expression as the gene is excised via the LoxP sites. Both transgenic and nontransgenic seeds without desiccation treatment will germinate well on glyphosate-free medium while germination will be completely inhibited for both of them on 20-50 μM glyphosate. Immature seeds that successfully underwent gene excision by desiccation will have glyphosate resistance and regenerate on medium containing 20-50 μM glyphosate.

Healthy plantlets are transferred to regeneration medium in Phytatrays containing 100 mg/L cefotaxime and 20-50 uM glyphosate for further selection and growth.

Example 12. Natural Desiccation and Excision of Transgenic Mature Soybean Seeds

Mature seed sterilization, Selection/ Regeneration:

T_1 mature soybean seed transformed with AGL1/PHP55062 are sterilized with 20% Clorox + 2 drops Tween 20 and rinsed with autoclaved water 3 times. Sterilized seeds are then transferred to regeneration medium containing 100 mg/L cefotaxime with 20-50 μM glyphosate for selection. After 5-10 days, DsRed expression is checked in the emerging shoots. Seeds that have been excised will no longer have DsRed expression as the gene is cleaved via the Lox P sites. Those seeds that are successfully excised of DsRed will have glyphosate resistance and regenerate on medium containing glyphosate. Once seeds have healthy shoot and root formation, the plantlets are moved to soil or another regeneration medium containing 100 mg/L cefotaxime in Phytatrays with 20 or 50 μM glyphosate for further selection and growth.

Sowing dry soybean T_1 seeds straight to soil and glyphosate resistance confirmation:

To confirm that the natural desiccation process that occurs during seed maturation would in fact allow for the excision of DsRed and resistance to glyphosate, seeds collected from T_0 soybean plants are germinated in soil. By planting seeds straight to soil without any treatments, excision would be a result of truly natural processes. After plants have germinated and grown to about 10-15 cm, the plants are sprayed with glyphosate + surfactant at 2X or 4X concentration (1X is equivalent to what is used in the field). Within 10 days, it is visibly evident which plants are not affected by the herbicide and which plants are severely damaged.

Example 13. *Agrobacterium*-mediated Transformation of Wheat using Immature Embryos (IEs) with Standard and Sand treatments

Preparation of Agrobacterium Suspension:

Agrobacterium tumefaciens harboring vector of interest was streaked from a -80° frozen aliquot onto solid LB medium containing selection (kanamycin or spectinomycin). The *Agrobacterium* was cultured on the LB plate at 21°C in the dark for 2-3 days. A single colony was selected from the master plate and was streaked onto an 810D medium plate containing selection and it was incubated at 28°C in the dark overnight. A sterile spatula was used to collect *Agrobacterium* cells from the solid medium and cells were suspended in ~ 5mL wheat infection medium (WI4) with 400 uM acetosyringone (As) (Table 1). The OD of the suspension was adjusted to 0.1 at 600 nm using the same medium.

Wheat Immature Embryo Transformation:

Material preparation, sterilization and sand treatment

4-5 spikes were collected, containing immature seeds with 1.5-2.5mm embryos. Immature seeds/wheat grains were then isolated from the spike by pulling downwards on the awn and removing both sets of bracts (the lemma and palea). Wheat grains were surface-sterilized for 15 min in 20% (v/v) bleach (5.25% sodium hypochlorite) plus 1 drop of Tween 20, and then they were washed in sterile water 2-3 times. Immature embryos (IEs) were isolated from the wheat grains and were placed in 1.5 ml of the WI4 medium into 2 mL micro-centrifuge tubes. Immature embryos were isolated and placed in 1 mL of WI4 medium with 0.25 mL of autoclaved sand. The 2 mL microcentrifuge tubes containing the immature embryos were centrifuged at 6k for 30 seconds, vortexed at 4.5, 5 or 6 for 10 seconds, and then centrifuged at 6k for 30 seconds. Embryos were let stood for 20 minutes.

Embryo treatments with sand and Infection

WI4 medium was drawn off, and 1.0 ml of *Agrobacterium* suspension was added to the 2 mL microcentrifuge tubes containing the immature embryos. Embryos were let to stand for 20 minutes. The suspension of *Agrobacterium* and immature embryos was poured onto wheat co-cultivation medium, WC21 (Table 2). Any embryos left in the tube were transferred to the

plate using a sterile spatula. The immature embryos were placed embryo axis side down on the media, and it was ensured that the embryos were immersed in the solution. The plate was sealed with Parafilm tape and incubated in the dark at 25°C for 3 days of co-cultivation.

Media Scheme and selection

After 3 days of co-cultivation immature embryos were transferred embryo axis side down to DBC4 green tissue (GT) induction medium containing 100 mg/L cefotaxime (PhytoTechnology Lab., Shawnee Mission, KS) (Table 3). All embryos were then incubated at 26-28°C in dim light for two weeks, then were transfered to DBC6 tissue (GT) induction medium containing 100 mg/L cefotaxime for another two weeks (Table 4). Regenerable sectors appear 3-4 weeks after transformation and will be ready for regeneration after being isolated. Regenerable sectors were cut from the non-transformed tissues and placed on regeneration media MSA with 100 mg/L cefotaxime (Table 5). Sectors on MSA medium should be placed in bright light for 1.5- 2 weeks or until roots and elongated shoots have formed. After sectors have developed into small plantlets they were transferred to Phyta trays until plantlets are ready to be transferred to soil. During each transfer plantlets were checked for marker gene expression and any non-expressing or chimeric tissues were removed.

Table 11. Liquid Wheat Infection Medium

WI4	
DI water	1000mL
MS salt + Vitamins	4.43 g
Maltose	30 g
Glucose	10 g
MES	1.95 g
2,4-D (0.5 mg/L)	1 ml
Picloram (10 mg/ml)	200 µl
BAP (1 mg/L)	0.5 ml
Adjust PH to 5.8 with KOH	

Table 12. Wheat Co-cultivation Medium

WC21	
DI water	1000mL
MS salt + Vitamins	4.43 g
Maltose	30 g
MES	1.95 g
2,4-D (0.5 mg/L)	1 ml
Picloram (10 mg/ml)	200 µl
BAP (1 mg/L)	0.5 ml
50X CuSO4 (0.1 M)	49 µl
Adjust PH to 5.8 with KOH Add 3.5 g/L of Phytagel	

Post sterilization	
Acetosyringone (1 M)	400 µl

Post sterilization	
Acetosyringone (1 M)	400 µl

Table 13. DBC 4 medium

DBC4	
dd H20	1000mL
MS salt	4.3 g
Maltose	30 g
Myo-inositol	0.25 g
N-Z-Amine-A	1 g
Proline	0.69 g
Thiamine-HCl (0.1 mg/mL)	10 mL
50X CuSO4 (0.1 M)	49 µL
2,4-D (0.5mg/mL)	2 mL
BAP	1 mL
Adjust PH to 5.8 with KOH Add 3.5 g/L of Phytagel Post sterilization	
Cefotaxime (100 mg/ml)	1 ml

Table 14. DBC 6 medium

DBC6	
dd H20	1000mL
MS salt	4.3 g
Maltose	30 g
Myo-inositol	0.25 g
N-Z-Amine-A	1 g
Proline	0.69 g
Thiamine-HCl (0.1 mg/mL)	10 mL
50X CuSO4 (0.1 M)	49 µL
2,4-D (0.5 mg/mL)	1 mL
BAP	2 mL
Adjust PH to 5.8 with KOH Add 3.5 g/L of Phytagel Post sterilization	
Cefotaxime (100 mg/ml)	1 ml

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Table 15. Regeneration MSA medium

MSA	
dd H20	1000mL
MS salt + Vitamins(M519)	4.43 g
Sucorse	20g
Myo- Inositol	1 g
Adjust PH to 5.8 with KOH Add 3.5 g/L of Phytagel Post steriliaztion	
Cefotaxime (100 mg/ml)	1 ml

Wheat *Agrobacterium*-mediated transformation using immature embryos were conducted with standard treatments and sand treatments to compare the transformation frequencies at T0 plant level.

Table 16 shows the transformation frequencies at T0 plant level (T0) for transformation experiments with standard and sand treatments using Standard vector for Pioneer elite spring wheat variety SBC0456D; the binary vectors are difficult constructs for transformation because the visual marker is driven by weal promoter for selection. All experiments were performed with 4.5-6 vortex speed for both standard and sand treatments. Data showed that T0 frequencies ranged from 0% to 1.2% for standard treatments. For sand treatments, T0 frequencies ranged from 5.9% to 6.8%. Results indicated that experiments conducted with sand treatments had higher transformation frequencies comparing to standard treatments.

Table 16. *Agrobacterium*-mediated transformation of immature embryos using standard vector with standard and sand treatments

Treatments	**Standard Vortex at 4.5**	**0.25mL sand Vortex at 4.5**	**Standard Vortex at 5**	**0.25mL sand Vortex at 5**	**Standard Vortex at 6**	**0.25mL sand Vortex at 6**
Transformation Frequency (T0)	0% (0/52)	5.9% (3/51)	0% (0/46)	18.6% (8/43)	0% (0/48)	13.3% (6/45)
			0% (0/54)	3.7% (2/54)	0% (0/66)	1.4% (1/72)
			2.8% (2/71)	1.5% (1/65)		
Average	**0% (0/52)**	**5.9% (3/51)**	**1.2% (2/171)**	**6.8% (11/162)**	**0% (0/114)**	**6.0% (7/117)**

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

THAT WHICH IS CLAIMED:

1. A polynucleotide construct comprising:

a) an excision cassette, comprising an expression cassette A (EC_A) comprising:

i) a coding polynucleotide A (CP_A) encoding a site specific recombinase; and

ii) an inducible promoter A (P_A) operably linked to the CP_A;

b) a first and a second recombination site flanking the excision cassette;

c) a coding polynucleotide B (CP_B) encoding a herbicide tolerance polypeptide; and

d) a promoter B (P_B), wherein the P_B is operably linked to the CP_B after excision of the excision cassette.

2. The polynucleotide construct of claim 1, wherein the inducible promoter P_A is selected from the group consisting of a stress-inducible promoter and a chemical-inducible promoter.

3. The polynucleotide construct of claim 2, wherein said chemical-inducible promoter comprises a promoter comprising a tet operator.

4. The polynucleotide construct of claim 3, wherein said polynucleotide construct further comprises a coding polynucleotide F (CP_F) encoding a sulfonylurea-responsive transcriptional repressor protein, wherein said CP_F is operably linked to a promoter active in a plant cell.

5. The polynucleotide construct of claim 2, wherein the stress-inducible promoter can be induced in response to cold, drought, high salinity, desiccation, or a combination thereof.

6. The polynucleotide construct of claim 2, wherein the stress-inducible promoter comprises a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence having the sequence set forth in SEQ ID NO: 18;

b) a nucleotide sequence having at least 70% sequence identity to the sequence set forth in SEQ ID NO: 18;

c) a nucleotide sequence comprising at least 50 contiguous nucleotides of the sequence set forth in SEQ ID NO: 18;

d) the nucleotide sequence set forth in nucleotides 291-430 of SEQ ID NO: 18; and

e) a nucleotide sequence having at least 70% sequence identity to the sequence set forth in nucleotides 291-430 of SEQ ID NO: 18.

7. The polynucleotide construct of claim 1, wherein the P_B is a constitutive promoter.

8. The polynucleotide construct of claim 7, wherein the P_B is selected from the group consisting of a ubiquitin promoter, an oleosin promoter, an actin promoter, and a *Mirabilis* mosaic virus (MMV) promoter.

9. The polynucleotide construct of claim 1, wherein the excision cassette further comprises a coding polynucleotide C (CP_C) encoding a selectable marker, wherein the CP_C is operably linked to a promoter active in a plant cell.

10. The polynucleotide construct of claim 9, wherein the CP_C is operably linked to P_B prior to excision of the excision cassette.

11. The polynucleotide construct of claim 9, wherein the excision cassette further comprises a promoter C (P_C) operably linked to the CP_C.

12. The polynucleotide construct of claim 11, wherein the P_C is a constitutive promoter.

13. The polynucleotide construct of claim 9, wherein the selectable marker is selected from the group consisting of a fluorescent protein, an antibiotic resistance polypeptide, a herbicide tolerance polypeptide, and a metabolic enzyme.

14. The polynucleotide construct of claim 1, wherein the herbicide tolerance polypeptide encoded by CP_B comprises a glyphosate-N-acetyltransferase (GLYAT) polypeptide or an ALS inhibitor-tolerance polypeptide.

15. The polynucleotide construct of claim 14, wherein said ALS inhibitor-tolerance polypeptide comprises the highly resistant ALS (HRA) mutation of acetolactate synthase.

16. The polynucleotide construct of claim 1, wherein the excision cassette further comprises a coding polynucleotide D (CP_D) encoding a cell proliferation factor operably linked to a promoter active in a plant cell.

17. The polynucleotide construct of claim 16, wherein the cell proliferation factor is selected from a WUSCHEL polypeptide and a babyboom polypeptide.

18. The polynucleotide construct of claim 17, wherein the babyboom polypeptide comprises at least two AP2 domains and at least one of the following amino acid sequences:

a) the amino acid sequence set forth in SEQ ID NO: 67 or an amino acid sequence that differs from the amino acid sequence set forth in SEQ ID NO: 67 by one amino acid; and

b) the amino acid sequence set forth in SEQ ID NO: 68 or an amino acid sequence that differs from the amino acid sequence set forth in SEQ ID NO: 68 by one amino acid.

19. The polynucleotide construct of claim 17, wherein the CP_D has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 55, 57, 58, 60, 74, 76, 78, 80, 82, 84, 86, 87, 88, 90, 92, 94, 96, 98, 99, or 101;

b) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 55, 57, 58, 60, 74, 76, 78, 80, 82, 84, 86, 87, 88, 90, 92, 94, 96, 98, 99, or 101;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 56, 59, 75, 77, 79, 81, 83, 85, 89, 91, 93, 95, 97, 100, or 102; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 70% sequence identity to the amino acid sequence set forth in SEQ ID NO: 56, 59, 75, 77, 79, 81, 83, 85, 89, 91, 93, 95, 97, 100, or 102.

20. The polynucleotide construct of claim 17, wherein the polynucleotide encoding a WUSCHEL polypeptide has a nucleotide sequence selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 103, 105, 107, or 109; and

b) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 103, 105, 107, or 109;

c) a nucleotide sequence encoding a polypeptide having the amino acid sequence set forth in SEQ ID NO: 104, 106, 108, or 110; and

d) a nucleotide sequence encoding a polypeptide having an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 104, 106, 108, or 110.

21. The polynucleotide construct of claim 20, wherein the polynucleotide encoding a WUSCHEL polypeptide is operably linked to a maize In2-2 promoter or a nopaline synthase promoter.

22. The polynucleotide construct of claim 16, wherein the excision cassette futher comprises a promoter D (P_D) operably linked to the CP_D.

23. The polynucleotide construct of claim 22, wherein the P_D is a constitutive promoter.

24. The polynucleotide construct of claim 23, wherein the P_D is a ubiquitin promoter or an oleosin promoter.

25. The polynucleotide construct of claim 16, wherein the excision cassette comprises at least a first coding polynucleotide D (CP_{D1}) encoding a babyboom polypeptide and a second coding polynucleotide D (CP_{D2}) encoding a WUSCHEL polypeptide.

26. The polynucleotide construct of claim 1, wherein the polynucleotide construct further comprises a coding polynucleotide E (CP_E) encoding a polypeptide of interest, wherein the CP_E is operably linked to a promoter active in a plant cell.

27. The polynucleotide construct of claim 26, wherein the CP_E is outside of the first and a second recombination sites flanking the excision cassette.

28. A host cell comprising the polynucleotide construct of claim 1.

29. A plant cell comprising the polynucleotide construct of claim 1.

30. A plant or plant part comprising the plant cell of claim 29.

31. The plant or plant part of claim 30, wherein the plant or plant part is a dicot.

32. The plant or plant part of claim 30, wherein the plant or plant part is a monocot.

33. The plant or plant part of claim 32, wherein the monocot is selected from the group consisting of maize, rice, sorghum, barley, millet, oat, rye, triticale, sugarcane, switch grass, and turf/forage grass.

34. The plant or plant part of claim 30, wherein the plant or plant part is recalcitrant to transformation.

35. The plant or plant part of claim 30, wherein the plant part is a seed.

36. A method for producing a transgenic plant or plant part, said method comprising introducing the polynucleotide construct of claim 1 into a plant or plant part.

37. A method for regulating the expression of a herbicide tolerance polynucleotide, wherein the method comprises:

a) providing the host cell of claim 28; and,

b) inducing the expression of the site-specific recombinase, thereby excising the excision cassette from the polynucleotide construct and expressing the herbicide tolerance polynucleotide.

38. A method for selecting a herbicide tolerant plant cell, the method comprising the steps of:

A) providing a population of plant cells, wherein at least one plant cell in the population comprises the polynucleotide construct of claim 1;

B) inducing the expression of the site-specific recombinase; and

C) contacting the population of plant cells with a herbicide to which the herbicide tolerance polypeptide confers tolerance, thereby selecting for a plant cell having tolerance to the herbicide.

39. The method of claim 38, wherein the method further comprises introducing the polynucleotide construct into the at least one plant cell before step A).

40. The method of claim 38, wherein the inducible promoter A (P_A) is induced in response to cold, drought, desiccation, high salinity or a combination thereof.

41. The method of claim 38, wherein the inducing comprises desiccating the population of plant cells.

42. The method of claim 41, wherein the desiccating occurs during the maturation of an immature seed.

43. The method of claim 38, wherein the excision cassette further comprises a coding polynucleotide C (CP_C), wherein the CP_C encodes a selectable marker operably linked to a promoter, and wherein the method further comprises a selection step prior to step B), wherein those plant cells within the population of plant cells that comprise the selectable marker are identified and wherein these selected plant cells comprise the population of plant cells that are induced in step B).

44. A method for increasing the transformation efficiency of a plant tissue, the method comprising the steps of:

a) providing a population of plant cells, wherein at least one plant cell in the population comprises the polynucleotide construct of claim 1;

b) culturing the population of plant cells in the absence of a herbicide to which the herbicide tolerance polypeptide confers herbicide resistance for a period of time sufficient for the population of plant cells to proliferate;

c) inducing the expression of the site-specific recombinase, thereby excising the excision cassette;

d) contacting the population of plant cells from c) with the herbicide to which the herbicide tolerance polypeptide confers tolerance; and

e) selecting for a plant cell having tolerance to the herbicide, wherein the transformation frequency is increased compared to a comparable plant cell not comprising the excision cassette and selected directly by herbicide selection.

45. The method of claim 44, wherein the inducing comprises desiccating the population of plant cells.

46. The method of claim 44, wherein the population of plant cells is cultured in the absence of the herbicide to which the herbicide tolerance polypeptide confers herbicide resistance for about 1 hour to about 6 weeks prior to excision.

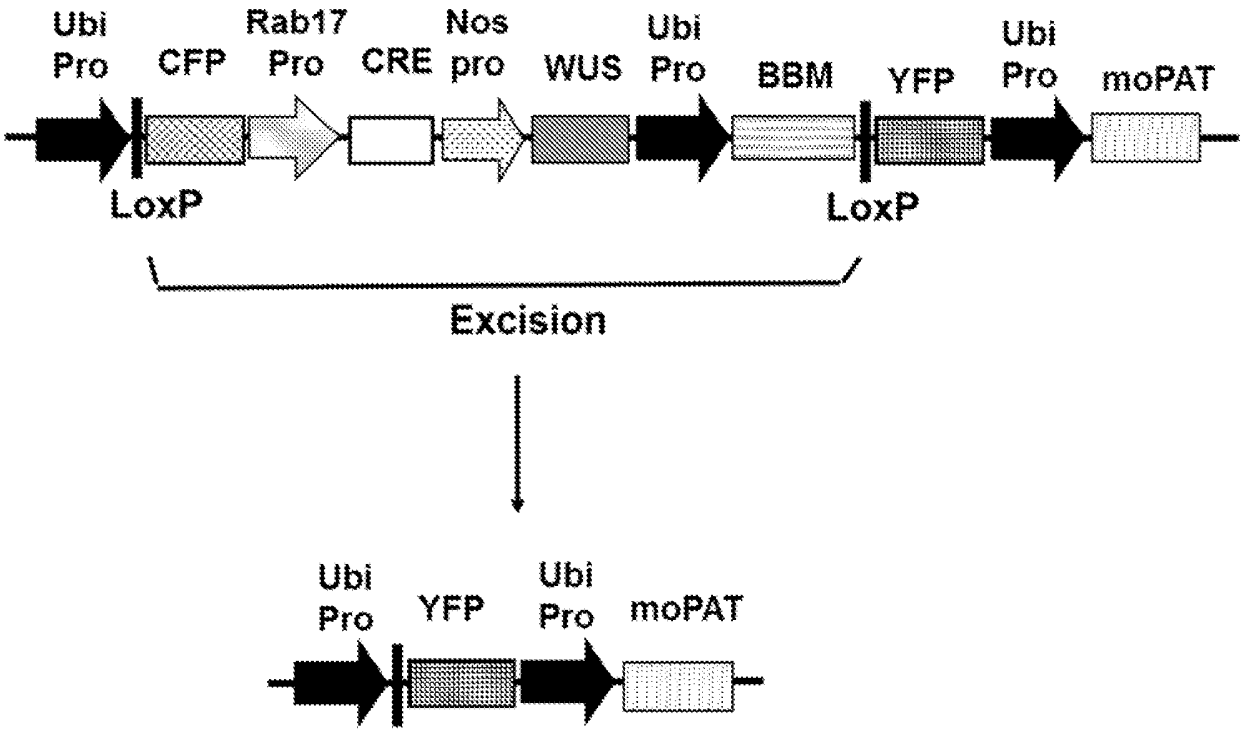
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FIG. 1

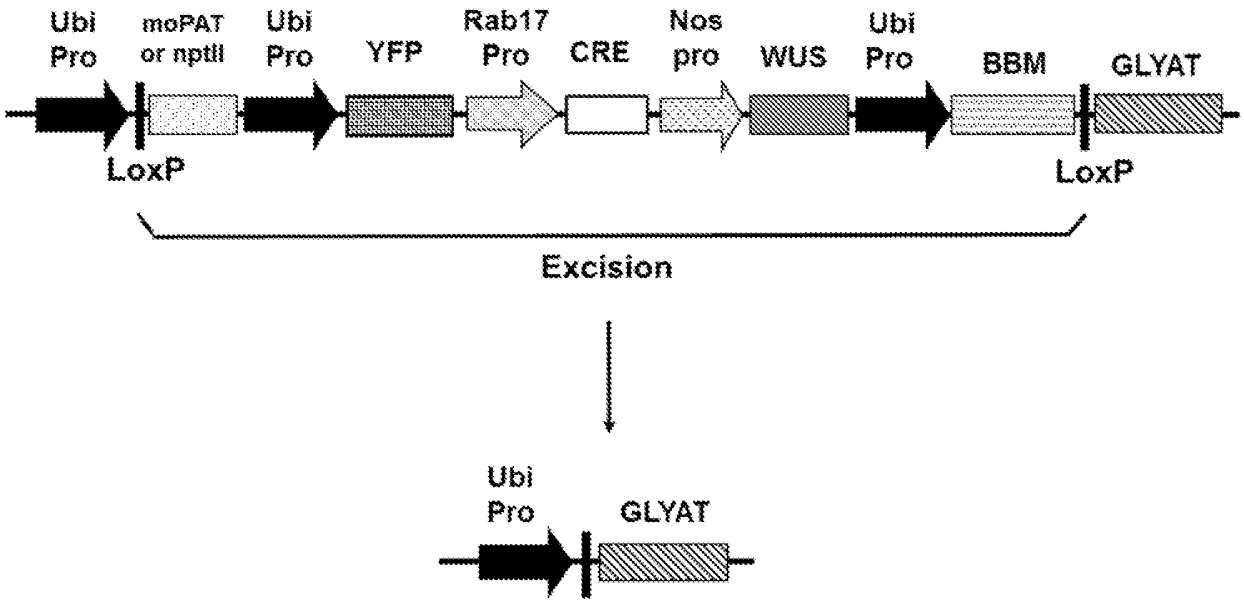
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FIG. 2

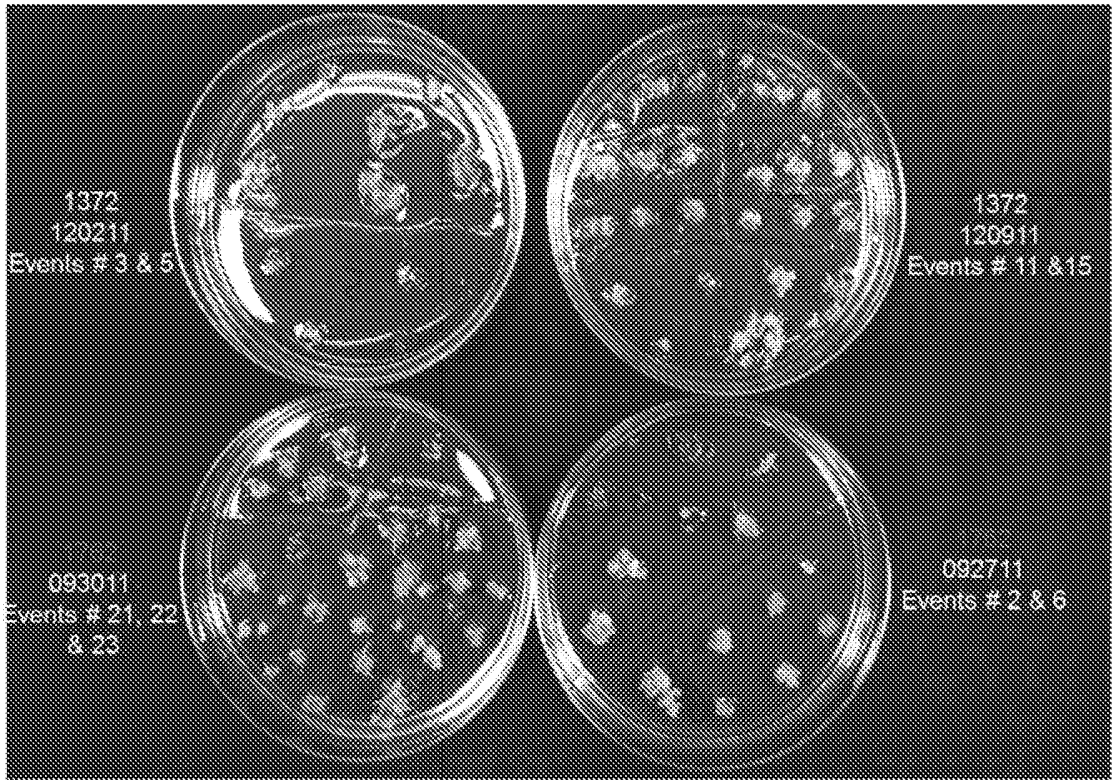
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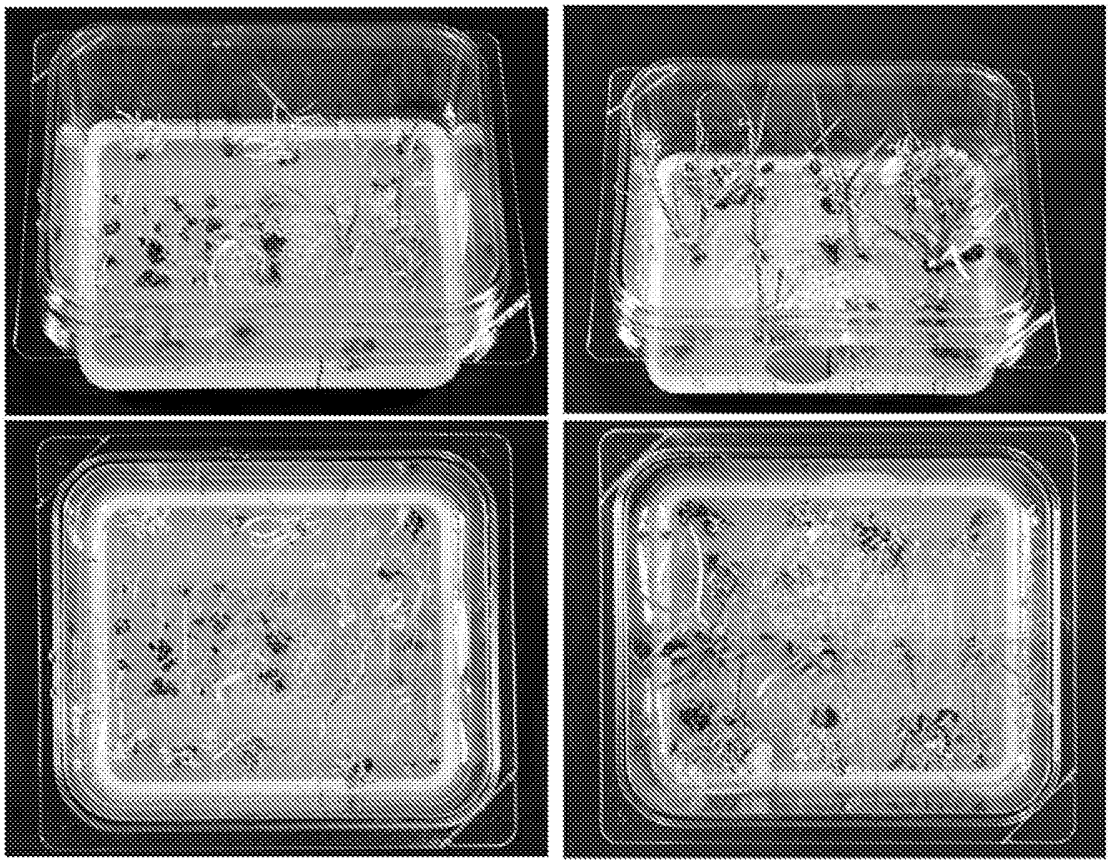
FIG. 3



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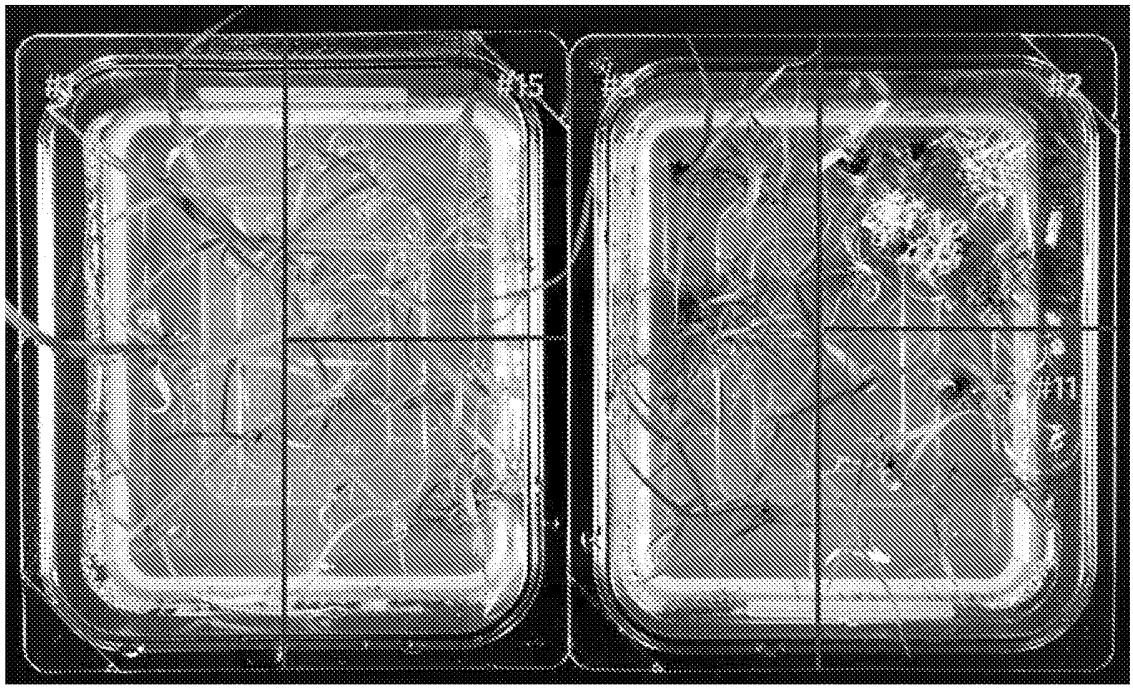
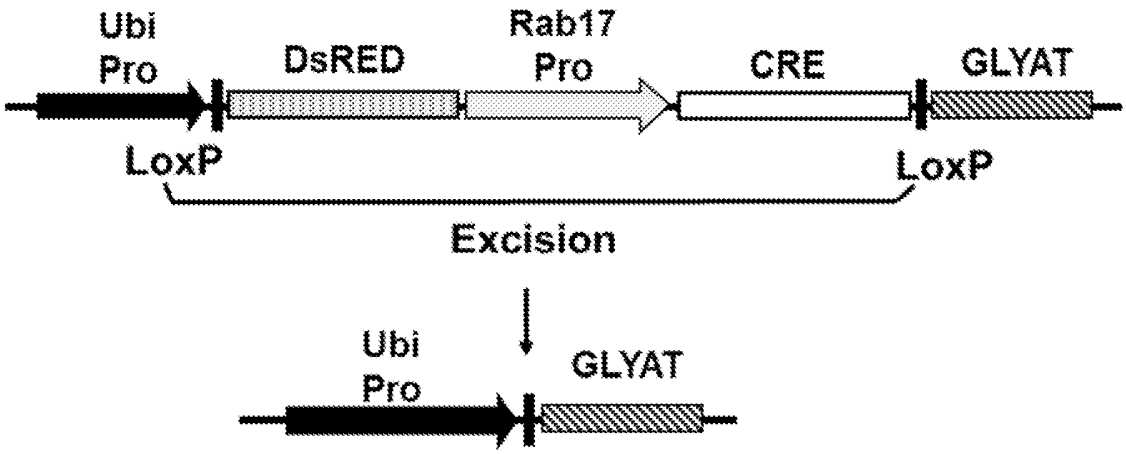


FIG. 5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Pro
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FIG. 6

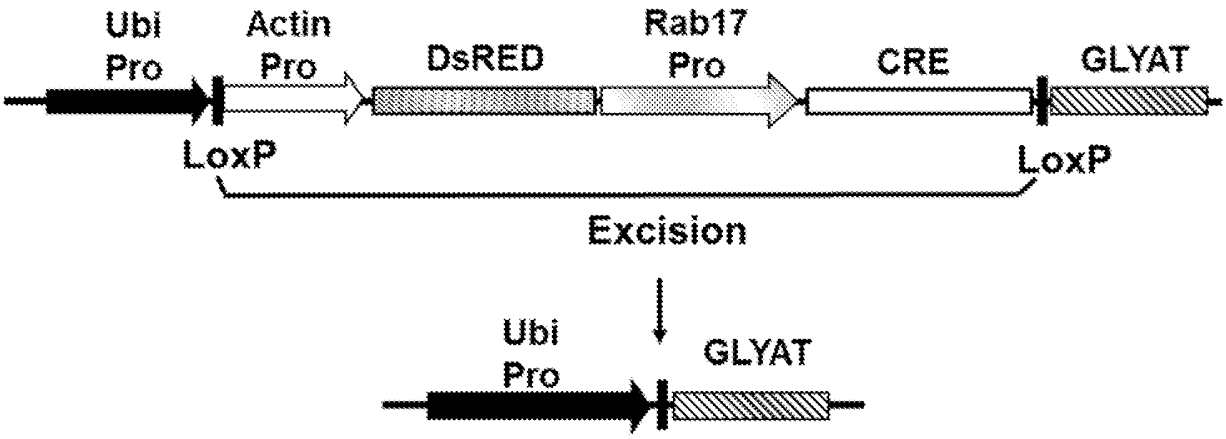
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FIG. 7

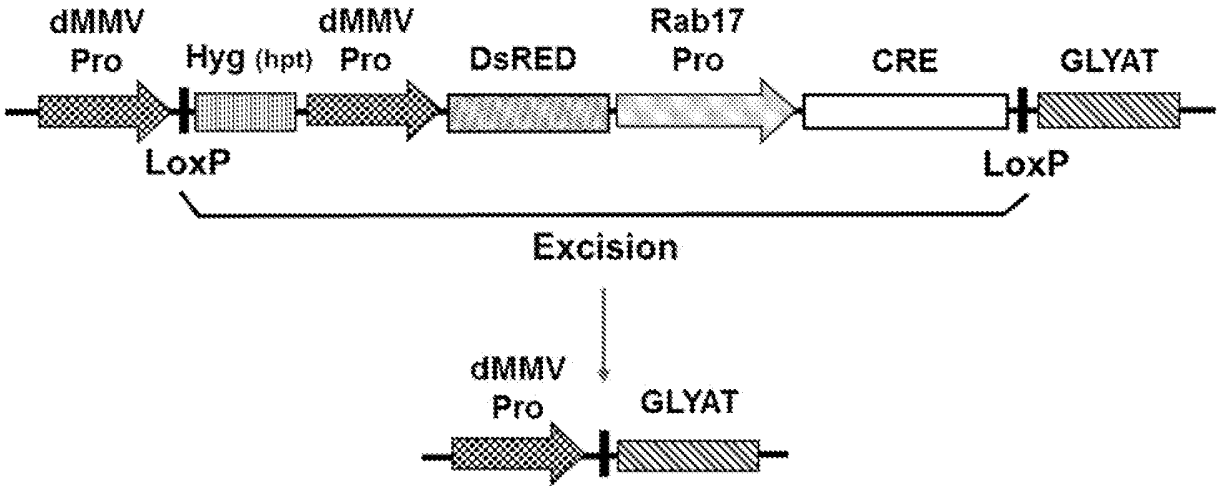
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FIG. 8

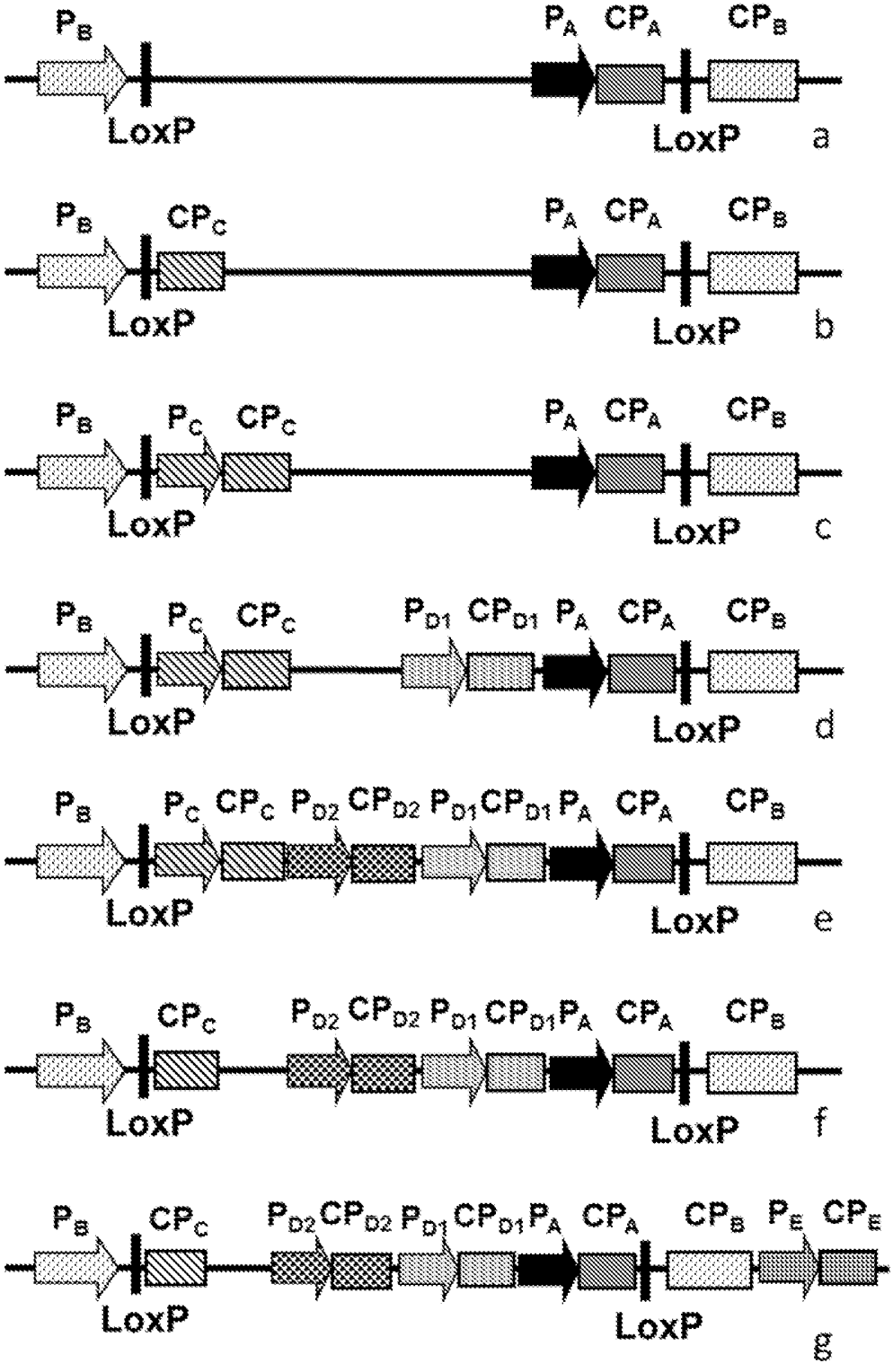
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FIG. 9

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Cho, Myeong-Je
Gordon-Kamm, William James
Ellis, Samuel R.
Zhoa, Zuo-Yu

<120> Methods and Compositions for Producing
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<223> Synthesized

<220>
<223> Plasmid linker sequence

<400> 28
tcaccaccgg cgagccacat cgagaacacg atcgagcaca caagcacgaa gactcgttta 60
ggagaaacca caaaccacca agccgtgcaa gcaccaagct tggtcacccg gtccgggcct 120
agaaggccag ctt 133

<210> 29
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> Plasmid linker sequence

<400> 29
tcgaaggaga tagaaccaat tctctaagga aatacttaac catggtcgac tggatccaac 60
a 61

<210> 30
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> Plasmid linker sequence

<400> 30
tcgaaggaga tagaaccgat ccacc 25

<210> 31
<211> 665
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> Promoter construct comprising Zea mays Rab17
promoter and attB1 site

<400> 31
ctatagtatt ttaaaattgc attaacaaac atgtcctaat tggtactcct gagatactat 60
accctcctgt tttaaaatag ttggcattat cgaattatca ttttacttt taatgtttc 120
tcttctttta atatatttta tgaattttaa tgtattttaa aatgttatgc agttcgctct 180

```
ggacttttct gctgcgccta cacttgggtg tactgggcct aaattcagcc tgaccgaccg 240
cctgcattga ataatggatg agcaccggta aaatccgcgt acccaacttt cgagaagaac 300
cgagacgtgg cgggccgggc caccgacgca cggcaccagc gactgcacac gtcccgccgg 360
cgtacgtgta cgtgctgttc cctcactggc cgcccaatcc actcatgcat gcccacgtac 420
acccctgccg tggcgcgccc agatcctaat cctttcgccg ttctgcactt ctgctgccta 480
taaatggcgg catcgaccgt cacctgcttc accaccggcg agccacatcg agaacacgat 540
cgagcacaca agcacgaaga ctcgtttagg agaaaccaca aaccaccaag ccgtgcaagc 600
accaagcttg gtcacccggt ccgggcctag aaggccagct tcaagtttgt acaaaaaagc 660
aggct                                                             665

<210> 32
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> tet operator

<400> 32
actctatcag tgatagagt                                               19

<210> 33
<211> 1272
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> Maize optimized FLP

<220>
<221> CDS
<222> (1)...(1272)

<400> 33
atg ccc cag ttc gac atc ctc tgc aag acc ccc ccc aag gtg ctc gtg   48
Met Pro Gln Phe Asp Ile Leu Cys Lys Thr Pro Pro Lys Val Leu Val
 1               5                  10                  15

agg cag ttc gtg gag agg ttc gag agg ccc tcc ggc gag aag atc gcc   96
Arg Gln Phe Val Glu Arg Phe Glu Arg Pro Ser Gly Glu Lys Ile Ala
            20                  25                  30

ctc tgc gcc gcc gag ctc acc tac ctc tgc tgg atg atc acc cac aac   144
Leu Cys Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met Ile Thr His Asn
        35                  40                  45

ggc acc gcc att aag agg gcc acc ttc atg tca tac aac acc atc atc   192
Gly Thr Ala Ile Lys Arg Ala Thr Phe Met Ser Tyr Asn Thr Ile Ile
    50                  55                  60

tcc aac tcc ctc tcc ttc gac atc gtg aac aag tcc ctc cag ttc aaa   240
Ser Asn Ser Leu Ser Phe Asp Ile Val Asn Lys Ser Leu Gln Phe Lys
65                  70                  75                  80

tac aag acc cag aag gcc acc atc ctc gag gcc tcc ctc aag aag ctc   288
Tyr Lys Thr Gln Lys Ala Thr Ile Leu Glu Ala Ser Leu Lys Lys Leu
                85                  90                  95

atc ccc gcc tgg gag ttc acc atc atc ccc tac tac ggc cag aag cac   336
Ile Pro Ala Trp Glu Phe Thr Ile Ile Pro Tyr Tyr Gly Gln Lys His
            100                 105                 110
```

```
cag tcc gac atc acc gac atc gtg tca tcc ctc cag ctt cag ttc gag   384
Gln Ser Asp Ile Thr Asp Ile Val Ser Ser Leu Gln Leu Gln Phe Glu
        115                 120                 125

tcc tcc gag gag gct gac aag ggc aac tcc cac tcc aag aag atg ctg   432
Ser Ser Glu Glu Ala Asp Lys Gly Asn Ser His Ser Lys Lys Met Leu
    130                 135                 140

aag gcc ctc ctc tcc gag ggc gag tcc atc tgg gag atc acc gag aag   480
Lys Ala Leu Leu Ser Glu Gly Glu Ser Ile Trp Glu Ile Thr Glu Lys
145                 150                 155                 160

atc ctc aac tcc ttc gag tac acc tcc agg ttc act aag acc aag acc   528
Ile Leu Asn Ser Phe Glu Tyr Thr Ser Arg Phe Thr Lys Thr Lys Thr
                165                 170                 175

ctc tac cag ttc ctc ttc ctc gcc acc ttc atc aac tgc ggc agg ttc   576
Leu Tyr Gln Phe Leu Phe Leu Ala Thr Phe Ile Asn Cys Gly Arg Phe
            180                 185                 190

tca gac atc aag aac gtg gac ccc aag tcc ttc aag ctc gtg cag aac   624
Ser Asp Ile Lys Asn Val Asp Pro Lys Ser Phe Lys Leu Val Gln Asn
        195                 200                 205

aag tac ctc ggc gtg atc atc cag tgc ctc gtg acc gag acc aag acc   672
Lys Tyr Leu Gly Val Ile Ile Gln Cys Leu Val Thr Glu Thr Lys Thr
    210                 215                 220

tcc gtg tcc agg cac atc tac ttc ttc tcc gct cgc ggc agg atc gac   720
Ser Val Ser Arg His Ile Tyr Phe Phe Ser Ala Arg Gly Arg Ile Asp
225                 230                 235                 240

ccc ctc gtg tac ctc gac gag ttc ctc agg aac tca gag ccc gtg ctc   768
Pro Leu Val Tyr Leu Asp Glu Phe Leu Arg Asn Ser Glu Pro Val Leu
                245                 250                 255

aag agg gtg aac agg acc ggc aac tcc tcc tcc aac aag cag gag tac   816
Lys Arg Val Asn Arg Thr Gly Asn Ser Ser Ser Asn Lys Gln Glu Tyr
            260                 265                 270

cag ctc ctc aag gac aac ctc gtg agg tcc tac aac aag gcc ctc aag   864
Gln Leu Leu Lys Asp Asn Leu Val Arg Ser Tyr Asn Lys Ala Leu Lys
        275                 280                 285

aag aac gcc ccc tac tcc atc ttc gcc atc aag aac ggc ccc aag tcc   912
Lys Asn Ala Pro Tyr Ser Ile Phe Ala Ile Lys Asn Gly Pro Lys Ser
    290                 295                 300

cac atc ggt agg cac ctc atg acc tcc ttc ctc tca atg aag ggc ctc   960
His Ile Gly Arg His Leu Met Thr Ser Phe Leu Ser Met Lys Gly Leu
305                 310                 315                 320

acc gag ctc acc aac gtg gtg ggc aac tgg tcc gac aag agg gcc tcc   1008
Thr Glu Leu Thr Asn Val Val Gly Asn Trp Ser Asp Lys Arg Ala Ser
                325                 330                 335

gcc gtg gcc agg acc acc tac acc cac cag atc acc gcc atc ccc gac   1056
Ala Val Ala Arg Thr Thr Tyr Thr His Gln Ile Thr Ala Ile Pro Asp
            340                 345                 350

cac tac ttc gcc ctc gtg tca agg tac tac gcc tac gac ccc atc tcc   1104
His Tyr Phe Ala Leu Val Ser Arg Tyr Tyr Ala Tyr Asp Pro Ile Ser
        355                 360                 365

aag gag atg atc gcc ctc aag gac gag act aac ccc atc gag gag tgg   1152
Lys Glu Met Ile Ala Leu Lys Asp Glu Thr Asn Pro Ile Glu Glu Trp
    370                 375                 380
```

```
cag cac atc gag cag ctc aag ggc tcc gcc gag ggc tcc atc agg tac   1200
Gln His Ile Glu Gln Leu Lys Gly Ser Ala Glu Gly Ser Ile Arg Tyr
385                 390                 395                 400

ccc gcc tgg aac ggc atc atc tcc cag gag gtg ctc gac tac ctc tcc   1248
Pro Ala Trp Asn Gly Ile Ile Ser Gln Glu Val Leu Asp Tyr Leu Ser
                405                 410                 415

tcc tac atc aac agg agg atc tga                                   1272
Ser Tyr Ile Asn Arg Arg Ile
            420
```

<210> 34
<211> 423
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> FLPm

<400> 34

```
Met Pro Gln Phe Asp Ile Leu Cys Lys Thr Pro Pro Lys Val Leu Val
 1               5                  10                  15
Arg Gln Phe Val Glu Arg Phe Glu Arg Pro Ser Gly Glu Lys Ile Ala
            20                  25                  30
Leu Cys Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met Ile Thr His Asn
        35                  40                  45
Gly Thr Ala Ile Lys Arg Ala Thr Phe Met Ser Tyr Asn Thr Ile Ile
    50                  55                  60
Ser Asn Ser Leu Ser Phe Asp Ile Val Asn Lys Ser Leu Gln Phe Lys
65                  70                  75                  80
Tyr Lys Thr Gln Lys Ala Thr Ile Leu Glu Ala Ser Leu Lys Lys Leu
                85                  90                  95
Ile Pro Ala Trp Glu Phe Thr Ile Ile Pro Tyr Tyr Gly Gln Lys His
            100                 105                 110
Gln Ser Asp Ile Thr Asp Ile Val Ser Ser Leu Gln Leu Gln Phe Glu
        115                 120                 125
Ser Ser Glu Glu Ala Asp Lys Gly Asn Ser His Ser Lys Lys Met Leu
    130                 135                 140
Lys Ala Leu Leu Ser Glu Gly Glu Ser Ile Trp Glu Ile Thr Glu Lys
145                 150                 155                 160
Ile Leu Asn Ser Phe Glu Tyr Thr Ser Arg Phe Thr Lys Thr Lys Thr
                165                 170                 175
Leu Tyr Gln Phe Leu Phe Leu Ala Thr Phe Ile Asn Cys Gly Arg Phe
            180                 185                 190
Ser Asp Ile Lys Asn Val Asp Pro Lys Ser Phe Lys Leu Val Gln Asn
        195                 200                 205
Lys Tyr Leu Gly Val Ile Ile Gln Cys Leu Val Thr Glu Thr Lys Thr
    210                 215                 220
Ser Val Ser Arg His Ile Tyr Phe Phe Ser Ala Arg Gly Arg Ile Asp
225                 230                 235                 240
Pro Leu Val Tyr Leu Asp Glu Phe Leu Arg Asn Ser Glu Pro Val Leu
                245                 250                 255
Lys Arg Val Asn Arg Thr Gly Asn Ser Ser Ser Asn Lys Gln Glu Tyr
            260                 265                 270
Gln Leu Leu Lys Asp Asn Leu Val Arg Ser Tyr Asn Lys Ala Leu Lys
        275                 280                 285
Lys Asn Ala Pro Tyr Ser Ile Phe Ala Ile Lys Asn Gly Pro Lys Ser
    290                 295                 300
His Ile Gly Arg His Leu Met Thr Ser Phe Leu Ser Met Lys Gly Leu
305                 310                 315                 320
Thr Glu Leu Thr Asn Val Val Gly Asn Trp Ser Asp Lys Arg Ala Ser
                325                 330                 335
Ala Val Ala Arg Thr Thr Tyr Thr His Gln Ile Thr Ala Ile Pro Asp
```

```
            340                 345                 350
His Tyr Phe Ala Leu Val Ser Arg Tyr Tyr Ala Tyr Asp Pro Ile Ser
        355                 360                 365
Lys Glu Met Ile Ala Leu Lys Asp Glu Thr Asn Pro Ile Glu Glu Trp
    370                 375                 380
Gln His Ile Glu Gln Leu Lys Gly Ser Ala Glu Gly Ser Ile Arg Tyr
385                 390                 395                 400
Pro Ala Trp Asn Gly Ile Ile Ser Gln Glu Val Leu Asp Tyr Leu Ser
                405                 410                 415
Ser Tyr Ile Asn Arg Arg Ile
            420
```

<210> 35
<211> 1032
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> Maize optimized Cre

<220>
<221> CDS
<222> (1)...(1032)

<400> 35

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atg tcc aac ctg ctc acg gtt cac cag aac ctt ccg gct ctt cca gtg   48
Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val
 1               5                  10                  15

gac gcg acg tcc gat gaa gtc agg aag aac ctc atg gac atg ttc cgc   96
Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg
            20                  25                  30

gac agg caa gcg ttc agc gag cac acc tgg aag atg ctg ctc tcc gtc   144
Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val
        35                  40                  45

tgc cgc tcc tgg gct gca tgg tgc aag ctg aac aac agg aag tgg ttc   192
Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe
    50                  55                  60

ccc gct gag ccc gag gac gtg agg gat tac ctt ctg tac ctg caa gct   240
Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala
65                  70                  75                  80

cgc ggg ctg gca gtg aag acc atc cag caa cac ctt gga caa ctg aac   288
Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn
                85                  90                  95

atg ctt cac agg cgc tcc ggc ctc ccg cgc ccc agc gac tcg aac gcc   336
Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala
            100                 105                 110

gtg agc ctc gtc atg cgc cgc atc agg aag gaa aac gtc gat gcc ggc   384
Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly
        115                 120                 125

gaa agg gca aag cag gcc ctc gcg ttc gag agg acc gat ttc gac cag   432
Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln
    130                 135                 140

gtc cgc agc ctg atg gag aac agc gac agg tgc cag gac att agg aac   480
Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn
145                 150                 155                 160
```

```
ctg gcg ttc ctc gga att gca tac aac acg ctc ctc agg atc gcg gaa   528
Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu
                165                 170                 175

att gcc cgc att cgc gtg aag gac att agc cgc acc gac ggc ggc agg   576
Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg
            180                 185                 190

atg ctt atc cac att ggc agg acc aag acg ctc gtt tcc acc gca ggc   624
Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val Ser Thr Ala Gly
        195                 200                 205

gtc gaa aag gcc ctc agc ctc gga gtg acc aag ctc gtc gaa cgc tgg   672
Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu Val Glu Arg Trp
    210                 215                 220

atc tcc gtg tcc ggc gtc gcg gac gac cca aac aac tac ctc ttc tgc   720
Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn Tyr Leu Phe Cys
225                 230                 235                 240

cgc gtc cgc aag aac ggg gtg gct gcc cct agc gcc acc agc caa ctc   768
Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala Thr Ser Gln Leu
                245                 250                 255

agc acg agg gcc ttg gaa ggt att ttc gag gcc acc cac cgc ctg atc   816
Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr His Arg Leu Ile
            260                 265                 270

tac ggc gcg aag gat gac agc ggt caa cgc tac ctc gca tgg tcc ggg   864
Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser Gly
        275                 280                 285

cac tcc gcc cgc gtt gga gct gct agg gac atg gcc cgc gcc ggt gtt   912
His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val
    290                 295                 300

tcc atc ccc gaa atc atg cag gcg ggt gga tgg acg aac gtg aac att   960
Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile
305                 310                 315                 320

gtc atg aac tac att cgc aac ctt gac agc gag acg ggc gca atg gtt  1008
Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val
                325                 330                 335

cgc ctc ctg gaa gat ggt gac tga                                 1032
Arg Leu Leu Glu Asp Gly Asp
            340
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<210> 36
<211> 343
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> maize-optimized Cre

<400> 36

```
Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val
 1               5                  10                  15
Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg
            20                  25                  30
Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val
        35                  40                  45
```

```
Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe
    50                  55                  60
Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala
65                  70                  75                  80
Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn
                85                  90                  95
Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala
            100                 105                 110
Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly
        115                 120                 125
Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln
    130                 135                 140
Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn
145                 150                 155                 160
Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu
                165                 170                 175
Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg
            180                 185                 190
Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val Ser Thr Ala Gly
        195                 200                 205
Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu Val Glu Arg Trp
    210                 215                 220
Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn Tyr Leu Phe Cys
225                 230                 235                 240
Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala Thr Ser Gln Leu
                245                 250                 255
Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr His Arg Leu Ile
            260                 265                 270
Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser Gly
        275                 280                 285
His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val
    290                 295                 300
Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile
305                 310                 315                 320
Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val
                325                 330                 335
Arg Leu Leu Glu Asp Gly Asp
            340
```

<210> 37
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> FRT1

<400> 37
gaagttccta tactttctag agaataggaa cttc 34

<210> 38
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> FRT5

<400> 38
gaagttccta tactcttttg agaataggaa cttc 34

<210> 39
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> FRT6

<400> 39
gaagttccta tacttttga agaataggaa cttc 34

<210> 40
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> FRT7

<400> 40
gaagttccta tacttattga agaataggaa cttc 34

<210> 41
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> FRT12

<400> 41
agttcctata ctctatgtag aataggaact 30

<210> 42
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> FRT87

<400> 42
gaagttccta tactttctgg agaataggaa cttc 34

<210> 43
<211> 146
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> 13_6D10 Synthetic protein sequence

<400> 43

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Met Ile Glu Val Lys Pro Ile Asn Ala Glu Asp Thr Tyr Glu Ile Arg
 1               5                  10                  15
His Arg Ile Leu Arg Pro Asn Gln Pro Leu Glu Ala Cys Met Tyr Glu
            20                  25                  30
Thr Asp Ser Leu Gly Gly Thr Phe His Leu Gly Gly Tyr Tyr Arg Gly
        35                  40                  45
Lys Leu Ile Ser Ile Ala Ser Phe Asn Gln Ala Glu His Pro Glu Leu
    50                  55                  60
Glu Gly Gln Lys Gln Tyr Gln Leu Arg Gly Met Ala Thr Leu Glu Gly
65                  70                  75                  80
Tyr Arg Glu Gln Lys Ala Gly Ser Thr Leu Ile Arg His Ala Glu Glu
                85                  90                  95
Leu Leu Arg Lys Lys Gly Ala Asp Leu Leu Trp Cys Asn Ala Arg Thr
            100                 105                 110
Ser Ala Ser Gly Tyr Tyr Lys Lys Leu Gly Phe Ser Glu Gln Gly Glu
        115                 120                 125
Val Tyr Asp Thr Pro Pro Val Gly Pro His Ile Leu Met Tyr Lys Lys
    130                 135                 140
Leu Thr
145
```

<210> 44
<211> 146
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> 10_4H4 Synthetic protein sequence

<400> 44

```
Met Leu Glu Val Lys Pro Ile Asn Ala Glu Asp Thr Tyr Glu Leu Arg
 1               5                  10                  15
His Lys Ile Leu Arg Pro Asn Gln Pro Leu Glu Val Cys Met Tyr Glu
            20                  25                  30
Thr Asp Leu Leu Arg Gly Ala Phe His Leu Gly Gly Phe Tyr Arg Gly
        35                  40                  45
Lys Leu Ile Ser Ile Ala Ser Phe His Gln Ala Glu His Ser Glu Leu
    50                  55                  60
Gln Gly Gln Lys Gln Tyr Gln Leu Arg Gly Met Ala Thr Leu Glu Gly
65                  70                  75                  80
Tyr Arg Glu Gln Lys Ala Gly Ser Ser Leu Ile Lys His Ala Glu Glu
                85                  90                  95
Ile Leu Arg Lys Arg Gly Ala Asp Leu Leu Trp Cys Asn Ala Arg Thr
            100                 105                 110
Ser Ala Ser Gly Tyr Tyr Lys Lys Leu Gly Phe Ser Glu Gln Gly Glu
        115                 120                 125
Val Phe Asp Thr Pro Pro Val Gly Pro His Ile Leu Met Tyr Lys Arg
    130                 135                 140
Ile Thr
145
```

<210> 45
<211> 146
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> 0_5D3 Synthetic protein sequence

<400> 45

```
Met Leu Glu Val Lys Pro Ile Asn Ala Glu Asp Thr Tyr Glu Leu Arg
 1               5                  10                  15
His Arg Ile Leu Arg Pro Asn Gln Pro Ile Glu Ala Cys Met Tyr Glu
            20                  25                  30
Ser Asp Leu Leu Arg Gly Ala Phe His Leu Gly Gly Tyr Tyr Arg Gly
        35                  40                  45
Lys Leu Ile Ser Ile Ala Ser Phe His Gln Ala Glu His Ser Glu Leu
    50                  55                  60
Gln Gly Gln Lys Gln Tyr Gln Leu Arg Gly Met Ala Thr Leu Glu Gly
65                  70                  75                  80
Tyr Arg Glu Gln Lys Ala Gly Ser Ser Leu Ile Lys His Ala Glu Glu
                85                  90                  95
Ile Leu Arg Lys Arg Gly Ala Asp Leu Leu Trp Cys Asn Ala Arg Thr
            100                 105                 110
Ser Ala Ser Gly Tyr Tyr Lys Lys Leu Gly Phe Ser Glu Gln Gly Glu
        115                 120                 125
Ile Phe Glu Thr Pro Pro Val Gly Pro His Ile Leu Met Tyr Lys Arg
    130                 135                 140
Ile Thr
145
```

<210> 46
<211> 146
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> R12G2 Synthetic protein sequence

<400> 46

```
Met Ile Glu Val Lys Pro Ile Asn Ala Glu Asp Thr Tyr Asp Leu Arg
 1               5                  10                  15
His Arg Val Leu Arg Pro Asn Gln Pro Ile Glu Ala Cys Met Phe Glu
            20                  25                  30
Ser Asp Leu Thr Arg Ser Ala Phe His Leu Gly Gly Phe Tyr Gly Gly
        35                  40                  45
Lys Leu Ile Ser Val Ala Ser Phe His Gln Ala Glu His Thr Glu Leu
    50                  55                  60
Gln Gly Lys Lys Gln Tyr Gln Leu Arg Gly Val Ala Thr Leu Glu Gly
65                  70                  75                  80
Tyr Arg Glu Gln Lys Ala Gly Ser Ser Leu Val Lys His Ala Glu Glu
                85                  90                  95
Ile Leu Arg Lys Arg Gly Ala Asp Met Ile Trp Cys Asn Ala Arg Thr
            100                 105                 110
Ser Ala Ser Gly Tyr Tyr Arg Lys Leu Gly Phe Ser Glu Gln Gly Glu
        115                 120                 125
Val Phe Asp Thr Pro Pro Val Gly Pro His Ile Leu Met Tyr Lys Arg
    130                 135                 140
Ile Thr
145
```

<210> 47
<211> 442
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> optimized GAT sequence (GAT4601)

<220>

<221> CDS
<222> (2)...(442)

<400> 47
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c atg ata gag gtg aaa ccg att aac gca gag gat acc tat gaa cta agg 49
  Met Ile Glu Val Lys Pro Ile Asn Ala Glu Asp Thr Tyr Glu Leu Arg
   1               5                  10                  15

cat aga ata ctc aga cca aac cag ccg ata gaa gcg tgt atg ttt gaa   97
His Arg Ile Leu Arg Pro Asn Gln Pro Ile Glu Ala Cys Met Phe Glu
            20                  25                  30

agc gat tta ctt cgt ggt gca ttt cac tta ggc ggc ttt tac agg ggc   145
Ser Asp Leu Leu Arg Gly Ala Phe His Leu Gly Gly Phe Tyr Arg Gly
        35                  40                  45

aaa ctg att tcc ata gct tca ttc cac cag gcc gag cac tcg gaa ctc   193
Lys Leu Ile Ser Ile Ala Ser Phe His Gln Ala Glu His Ser Glu Leu
    50                  55                  60

caa ggc cag aaa cag tac cag ctc cga ggt atg gct acc ttg gaa ggt   241
Gln Gly Gln Lys Gln Tyr Gln Leu Arg Gly Met Ala Thr Leu Glu Gly
65                  70                  75                  80

tat cgt gag cag aaa gcg gga tca act cta gtt aaa cac gct gaa gaa   289
Tyr Arg Glu Gln Lys Ala Gly Ser Thr Leu Val Lys His Ala Glu Glu
                85                  90                  95

atc ctt cgt aag agg ggg gcg gac atg ctt tgg tgt aat gcg agg aca   337
Ile Leu Arg Lys Arg Gly Ala Asp Met Leu Trp Cys Asn Ala Arg Thr
            100                 105                 110

tcc gcc tca ggc tac tac aaa aag tta ggc ttc agc gag cag gga gag   385
Ser Ala Ser Gly Tyr Tyr Lys Lys Leu Gly Phe Ser Glu Gln Gly Glu
        115                 120                 125

ata ttt gac acg ccg cca gta gga cct cac atc ctg atg tat aaa agg   433
Ile Phe Asp Thr Pro Pro Val Gly Pro His Ile Leu Met Tyr Lys Arg
    130                 135                 140

atc aca taa                                                      442
Ile Thr
145
```

<210> 48
<211> 146
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> optimized GAT sequence (GAT4601)

<400> 48
```
Met Ile Glu Val Lys Pro Ile Asn Ala Glu Asp Thr Tyr Glu Leu Arg
 1               5                  10                  15
His Arg Ile Leu Arg Pro Asn Gln Pro Ile Glu Ala Cys Met Phe Glu
            20                  25                  30
Ser Asp Leu Leu Arg Gly Ala Phe His Leu Gly Gly Phe Tyr Arg Gly
        35                  40                  45
Lys Leu Ile Ser Ile Ala Ser Phe His Gln Ala Glu His Ser Glu Leu
    50                  55                  60
Gln Gly Gln Lys Gln Tyr Gln Leu Arg Gly Met Ala Thr Leu Glu Gly
65                  70                  75                  80
Tyr Arg Glu Gln Lys Ala Gly Ser Thr Leu Val Lys His Ala Glu Glu
```

```
                  85                  90                  95
Ile Leu Arg Lys Arg Gly Ala Asp Met Leu Trp Cys Asn Ala Arg Thr
             100                 105                 110
Ser Ala Ser Gly Tyr Tyr Lys Lys Leu Gly Phe Ser Glu Gln Gly Glu
         115                 120                 125
Ile Phe Asp Thr Pro Pro Val Gly Pro His Ile Leu Met Tyr Lys Arg
     130                 135                 140
Ile Thr
145
```

<210> 49
<211> 441
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> optimized GAT sequence (GAT4602)

<220>
<221> CDS
<222> (1)...(441)

<400> 49

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atg ata gag gtg aaa ccg att aac gca gag gat acc tat gaa cta agg   48
Met Ile Glu Val Lys Pro Ile Asn Ala Glu Asp Thr Tyr Glu Leu Arg
 1               5                  10                  15

cat aga ata ctc aga cca aac cag ccg ata gaa gcg tgt atg ttt gaa   96
His Arg Ile Leu Arg Pro Asn Gln Pro Ile Glu Ala Cys Met Phe Glu
            20                  25                  30

agc gat tta ctt cgt ggt gca ttt cac tta ggc ggc tat tac ggg ggc   144
Ser Asp Leu Leu Arg Gly Ala Phe His Leu Gly Gly Tyr Tyr Gly Gly
        35                  40                  45

aaa ctg att tcc ata gct tca ttc cac cag gcc gag cac tca gaa ctc   192
Lys Leu Ile Ser Ile Ala Ser Phe His Gln Ala Glu His Ser Glu Leu
    50                  55                  60

caa ggc cag aaa cag tac cag ctc cga ggt atg gct acc ttg gaa ggt   240
Gln Gly Gln Lys Gln Tyr Gln Leu Arg Gly Met Ala Thr Leu Glu Gly
65                  70                  75                  80

tat cgt gag cag aag gcg gga tcg agt cta att aaa cac gct gaa gaa   288
Tyr Arg Glu Gln Lys Ala Gly Ser Ser Leu Ile Lys His Ala Glu Glu
                85                  90                  95

att ctt cgt aag agg ggg gcg gac ttg ctt tgg tgt aat gcg cgg aca   336
Ile Leu Arg Lys Arg Gly Ala Asp Leu Leu Trp Cys Asn Ala Arg Thr
            100                 105                 110

tcc gcc tca ggc tac tac aaa aag tta ggc ttc agc gag cag gga gag   384
Ser Ala Ser Gly Tyr Tyr Lys Lys Leu Gly Phe Ser Glu Gln Gly Glu
        115                 120                 125

gta ttc gac acg ccg cca gta gga cct cac atc ctg atg tat aaa agg   432
Val Phe Asp Thr Pro Pro Val Gly Pro His Ile Leu Met Tyr Lys Arg
    130                 135                 140

atc aca taa                                                      441
Ile Thr
145
```

<210> 50
<211> 146
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> optimized GAT sequence (GAT4602)

<400> 50
Met Ile Glu Val Lys Pro Ile Asn Ala Glu Asp Thr Tyr Glu Leu Arg
 1   5   10   15
His Arg Ile Leu Arg Pro Asn Gln Pro Ile Glu Ala Cys Met Phe Glu
            20   25   30
Ser Asp Leu Leu Arg Gly Ala Phe His Leu Gly Gly Tyr Tyr Gly Gly
        35   40   45
Lys Leu Ile Ser Ile Ala Ser Phe His Gln Ala Glu His Ser Glu Leu
    50   55   60
Gln Gly Gln Lys Gln Tyr Gln Leu Arg Gly Met Ala Thr Leu Glu Gly
65   70   75   80
Tyr Arg Glu Gln Lys Ala Gly Ser Ser Leu Ile Lys His Ala Glu Glu
                85   90   95
Ile Leu Arg Lys Arg Gly Ala Asp Leu Leu Trp Cys Asn Ala Arg Thr
            100   105   110
Ser Ala Ser Gly Tyr Tyr Lys Lys Leu Gly Phe Ser Glu Gln Gly Glu
        115   120   125
Val Phe Asp Thr Pro Pro Val Gly Pro His Ile Leu Met Tyr Lys Arg
    130   135   140
Ile Thr
145

<210> 51
<211> 1968
<212> DNA
<213> Glycine max

<220>
<221> misc_feature
<222> (1)...(1968)
<223> HRA sequence

<400> 51
atgccacaca acacaatggc ggccaccgct tccagaacca cccgattctc ttcttcctct 60
tcacacccca ccttccccaa acgcattact agatccaccc tccctctctc tcatcaaacc 120
ctcaccaaac ccaaccacgc tctcaaaatc aaatgttcca tctccaaacc ccccacggcg 180
gcgcccttca ccaaggaagc gccgaccacg gagcccttcg tgtcacggtt cgcctccggc 240
gaacctcgca agggcgcgga catccttgtg gaggcgctgg agaggcaggg cgtgacgacg 300
gtgttcgcgt accccggcgg tgcgtcgatg gagatccacc aggcgctcac gcgctccgcc 360
gccatccgca acgtgctccc gcgccacgag cagggcggcg tcttcgccgc cgaaggctac 420
gcgcgttcct ccggcctccc cggcgtctgc attgccacct ccggccccgg cgccaccaac 480
ctcgtgagcg gcctcgccga cgctttaatg gacagcgtcc cagtcgtcgc catcaccggc 540
caggtcgccc gccggatgat cggcaccgac gccttccaag aaaccccgat cgtggaggtg 600
agcagatcca tcacgaagca caactacctc atcctcgacg tcgacgacat cccccgcgtc 660
gtcgccgagg ctttcttcgt cgccacctcc ggccgccccg gtccggtcct catcgacatt 720
cccaaagacg ttcagcagca actcgccgtg cctaattggg acgagcccgt taacctcccc 780
ggttacctcg ccaggctgcc caggcccccc gccgaggccc aattggaaca cattgtcaga 840
ctcatcatgg aggcccaaaa gcccgttctc tacgtcggcg gtggcagttt gaattccagt 900
gctgaattga ggcgctttgt tgaactcact ggtattcccg ttgctagcac tttaatgggt 960
cttggaactt ttcctattgg tgatgaatat tcccttcaga tgctgggtat gcatggtact 1020
gttttatgct actatgctgt tgacaatagt gatttgttgc ttgcctttgg ggtaaggttt 1080
gatgaccgtg ttactgggaa gcttgaggct tttgctagta gggctaagat tgttcacatt 1140
gatattgatt ctgccgagat tgggaagaac aagcaggcgc acgtgtcggt ttgcgcggat 1200
ttgaagttgg ccttgaaggg aattaatatg attttggagg agaaaggagt ggagggtaag 1260
tttgatcttg gaggttggag agaagagatt aatgtgcaga aacacaagtt tccattgggt 1320
tacaagacat tccaggacgc gatttctccg cagcatgcta tcgaggttct tgatgagttg 1380

actaatggag atgctattgt tagtactggg gttgggcagc atcaaatgtg ggctgcgcag 1440
ttttacaagt acaagagacc gaggcagtgg ttgacctcag ggggtcttgg agccatgggt 1500
tttggattgc ctgcggctat tggtgctgct gttgctaacc ctggggctgt tgtggttgac 1560
attgatgggg atggtagttt catcatgaat gttcaggagt tggccactat aagagtggag 1620
aatctcccag ttaagatatt gttgttgaac aatcagcatt tgggtatggt ggttcagttg 1680
gaggataggt tctacaagtc caatagagct cacacctatc ttggagatcc gtctagcgag 1740
agcgagatat tcccaaacat gctcaagttt gctgatgctt gtgggatacc ggcagcgcga 1800
gtgacgaaga aggaagagct tagagcggca attcagagaa tgttggacac ccctggcccc 1860
tacctttctg atgtcatttgt gccccatcag gagcatgtgt tgccgatgat tcccagtaat 1920
ggatccttca aggatgtgat aactgagggt gatggtagaa cgaggtac 1968

<210> 52
<211> 1917
<212> DNA
<213> Zea mays

<220>
<221> misc_feature
<222> (1)...(1917)
<223> HRA sequence

<400> 52
cagtacacag tcctgccatc accatccagg atcatatcct tgaaagcccc accactaggg 60
atcataggca acacatgctc ctggtgtggg acgattatat ccaagaggta cggccctgga 120
gtctcgagca tcttctttat cgctgcgcgg acttcgttct tctttgtcac acggaccgct 180
ggaatgttga acccttttggc gatcgtcacg aaatctggat atatctcact ttcatttctct 240
gggttttccca agtatgtgtg cgctctgttg gcctttataga acctgtcctc caactgcacc 300
accatcccca ggtgctggtt gttttagcaca aagacctttca ctgggaggtt ctcaatttcgg 360
atcatagcta gctcctgaac gttcatgaga aagctaccat ctccatcgat gtcaacaaca 420
gtgacacctg ggttttgccac agaagcacca gcagcagccg gcaaaccaaa tcccatagcc 480
ccaagaccag ctgaagacaa ccactgcctt ggccgcttgt aagtgtagta ctgtgccgcc 540
cacatctggt gctgcccaac acctgtgccg atgatggcct cgccttttcgt cagctcatca 600
agaacctgaa tagcatattg tggctggatc tcctcattag atgttttata cccaaggggg 660
aattccctct tctgctgatc caactcatcg ttccatgagc caaagtcaaa gctcttcttt 720
gatgtgcttc cttcaagaag agcattcatg ccctgcaaag caagcttaac atctgcacag 780
atggacacat gtggctgctt gttcttgcca atctcagccg gatcaatatc aacgtgcaca 840
atcttagccc tgcttgcaaa agcctcaatc ttccctgtca cgcgatcatc aaaccgcaca 900
ccaagtgcaa gcaacagatc ggcctatatcc actgcataat ttgcatacac cgtcccatgc 960
atacctagca tgcgcagaga cagtgggtcg tcgctgggga agttgccgag gcccataaga 1020
gtagttgtga ccgggattcc agtcagctcc acaaagcgtc gcaactcctc accagatgct 1080
gcgcagccac cgcccacata aagaacaggg cgccgcgatt caccaacaag acgcagcacc 1140
tgctcaagca actcagtcgc agggggcttg ggaaggcgcg caatgtaccc aggcagactc 1200
atgggcttgt cccagacagg caccgccatc tgctgctgga tgtcctgggg gatgtcgaca 1260
agcaccggcc ctggtcgacc agaggaggcg aggaagaaag cctcctgcac gacgcggggg 1320
atgtcgtcga cgtcgaggac caggtagttg tgcttggtga tggagcgggt gacctcgacg 1380
atgggcgtct cctggaaggc gtcggtgcca atcatgcgtc gcgccacctg tcccgtgatg 1440
gcgaccatgg ggacggaatc gagcagcgcg tcggcgagcg cggagactag gttggtggcg 1500
ccggggccgg aggtggcgat gcagacgccg acgcggcccg aggagcgcgc gtagccggag 1560
gcggcaaagg cctcccctg ctcgtggcgg aagaggtggt tggcgatgac gggggagcgg 1620
gtgagtgcct ggtggatctc catggacgcg ccgccggggt aggcgaagac gtcgcggacg 1680
ccgcagcgct cgagggactc gacgaggatg tcagcaccct tgcggggct cggtggggccc 1740
cacggccgga gcggggtggc cgggggagcc atcggcatgg cgggtgacgc cgctgagcac 1800
ctgatgggcg cggcgagggc gcggcgggtg gccaggaggt gcgcccggcg cctcgcctt g 1860
ggcgcagcgg tagtggcgcc agtgagcgcg gtagacgcgg cggcggcggt ggccatg 1917

<210> 53
<211> 2139
<212> DNA
<213> Arabidopsis

<220>
<221> misc_feature
<222> (1)...(2139)
<223> HRA sequence

<400> 53
aaatacgtac ctacgcaccc tgcgctacca tccctagagc tgcagcttat ttttacaaca 60
attaccaaca acaacaaaca acaaacaaca ttacaattac tatttacaat tacagtcgac 120

ccgggatcca tggcggcggc aacaacaaca acaacaacat cttcttcgat ctccttctcc 180
accaaaccat ctccttcctc ctccaaatca ccattaccaa tctccagatt ctccctccca 240
ttctccctaa accccaacaa atcatcctcc tcctcccgcc gccgcggtat caaatccagc 300
tctccctcct ccatctccgc cgtgctcaac acaaccacca atgtcacaac cactccctct 360
ccaaccaaac ctaccaaacc cgaaacattc atctcccgat tcgctccaga tcaaccccgc 420
aaaggcgctg atatcctcgt cgaagcttta gaacgtcaag gcgtagaaac cgtattcgct 480
taccctggag gtgcatcaat ggagattcac caagccttaa cccgctcttc ctcaatccgt 540
aacgtccttc ctcgtcacga acaaggaggt gtattcgcag cagaaggata cgctcgatcc 600
tcaggtaaac caggtatctg tatagccact tcaggtcccg gagctacaaa tctcgttagc 660
ggattagccg atgcgttgtt agatagtgtt cctcttgtag caatcacagg acaagtcgct 720
cgtcgtatga ttggtacaga tgcgttttcaa gagactccga ttgttgaggt aacgcgttcg 780
attacgaagc ataactatct tgtgatggat gttgaagata tccctaggat tattgaggaa 840
gctttctttt tagctactcc tggtagacct ggacctgttt tggttgatgt tcctaaagat 900
attcaacaac agcttgcgat tcctaattgg gaacaggcta tgagattacc tggttatatg 960
tctaggatgc ctaaacctcc ggaagattct cattttggagc agattgttag gttgatttct 1020
gagtctaaga agcctgtgtt gtatgttggt ggtggttgtt tgaattctag cgatgaattg 1080
ggtaggttg ttgagcttac ggggatccct gttgcgagta cgttgatggg gctgggatct 1140
tatccttgtg atgatgagtt gtcgttacat atgcttggaa tgcatgggac tgtgtatgca 1200
aattacgctg tggagcatag tgatttgttg ttggcgtttg gggtaaggtt tgatgatcgt 1260
gtcacgggta agcttgaggc ttttgctagt agggctaaga ttgttcatat tgatattgac 1320
tcggctgaga ttgggaagaa taagactcct catgtgtctg tgtggtggta tgttaagctg 1380
gctttgcaag ggatgaatat gattcttgag agccgagcgg aggagcttaa gcttgatttt 1440
ggagttgga ggaatgagtt gaacgtacag aaacagaagt ttccgttgag ctttaagacg 1500
tttggggaag ctattcctcc acagtatgcg attaaggtcc ttgatgagtt gactgatgga 1560
aaagccataa taagtactgg tgtcgggcaa catcaaatgt gggcggcgca gttctacaat 1620
tacaagaaac caaggcagtg gctatcatca ggaggccttg gagctatggg attttggactt 1680
cctgctgcga ttggagcgtc tgttgctaac cctgatgcga tagttgtgga tattgacgga 1740
gatggaagct ttataatgaa tgtgcaagag ctagccactа ttcgtgtaga gaatcttcca 1800
gtgaaggtac ttttattaaa caaccagcat cttggcatgg ttatgcaatt ggaagatcgg 1860
ttctacaaag ctaaccgagc tcacacattt ctcggggatc cggctcagga ggacgagata 1920
ttcccgaaca tgttgctgtt tgcagcagct tgcgggattc cagcggcgag ggtgacaaag 1980
aaagcagatc tccgagaagc tattcagaca atgctggata caccaggacc ttacctgttg 2040
gatgtgattt gtccgcacca agaacatgtg ttgccgatga tcccgagtgg tggcacttтc 2100
aacgatgtca taacggaagg agatggccgg attaaatac 2139

<210> 54
<211> 552
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> maize optimized PAT sequence

<400> 54
atgtcccccg agcgccgccc cgtcgagatc cgcccggcca ccgccgccga catggccgcc 60
gtgtgcgaca tcgtgaacca ctacatcgag acctccaccg tgaacttccg caccgagccg 120
cagaccccgc aggagtggat cgacgacctg gagcgcctcc aggaccgcta cccgtggctc 180
gtggccgagg tggagggcgt ggtggccggc atcgcctacg ccggcccgtg gaaggcccgc 240
aacgcctacg actggaccgt ggagtccacc gtgtacgtgt cccaccgcca ccagcgcctc 300
ggcctcggct ccaccctcta cacccacctc ctcaagagca tggaggccca gggcttcaag 360
tccgtggtgg ccgtgatcgg cctcccgaac gacccgtccg tgcgcctcca cgaggccctc 420
ggctacaccg cccgcggcac cctccgcgcc gccggctaca agcacggcgg ctggcacgac 480
gtcggcttct ggcagcgcga cttcgagctg ccggccccgc cgcgcccggt gcgcccggtg 540
acgcagatct ga 552

<210> 55
<211> 2130
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)...(2130)

<400> 55

```
atg gcc act gtg aac aac tgg ctc gct ttc tcc ctc tcc ccg cag gag   48
Met Ala Thr Val Asn Asn Trp Leu Ala Phe Ser Leu Ser Pro Gln Glu
 1               5                  10                  15

ctg ccg ccc tcc cag acg acg gac tcc acg ctc atc tcg gcc gcc acc   96
Leu Pro Pro Ser Gln Thr Thr Asp Ser Thr Leu Ile Ser Ala Ala Thr
            20                  25                  30

gcc gac cat gtc tcc ggc gat gtc tgc ttc aac atc ccc caa gat tgg  144
Ala Asp His Val Ser Gly Asp Val Cys Phe Asn Ile Pro Gln Asp Trp
        35                  40                  45

agc atg agg gga tca gag ctt tcg gcg ctc gtc gcg gag ccg aag ctg  192
Ser Met Arg Gly Ser Glu Leu Ser Ala Leu Val Ala Glu Pro Lys Leu
    50                  55                  60

gag gac ttc ctc ggc ggc atc tcc ttc tcc gag cag cat cac aag tcc  240
Glu Asp Phe Leu Gly Gly Ile Ser Phe Ser Glu Gln His His Lys Ser
65                  70                  75                  80

aac tgc aac ttg ata ccc agc act agc agc aca gtt tgc tac gcg agc  288
Asn Cys Asn Leu Ile Pro Ser Thr Ser Ser Thr Val Cys Tyr Ala Ser
                85                  90                  95

tca gct gct agc acc ggc tac cat cac cag ctg tac cag ccc acc agc  336
Ser Ala Ala Ser Thr Gly Tyr His His Gln Leu Tyr Gln Pro Thr Ser
            100                 105                 110

tcc gcg ctc cac ttc gcg gac tcc gtc atg gtg gcc tcc tcg gcc ggt  384
Ser Ala Leu His Phe Ala Asp Ser Val Met Val Ala Ser Ser Ala Gly
        115                 120                 125

gtc cac gac ggc ggt tcc atg ctc agc gcg gcc gcc gct aac ggt gtc  432
Val His Asp Gly Gly Ser Met Leu Ser Ala Ala Ala Ala Asn Gly Val
    130                 135                 140

gct ggc gct gcc agt gcc aac ggc ggc ggc atc ggg ctg tcc atg atc  480
Ala Gly Ala Ala Ser Ala Asn Gly Gly Gly Ile Gly Leu Ser Met Ile
145                 150                 155                 160

aag aac tgg ctg cgg agc caa ccg gcg ccc atg cag ccg agg gcg gcg  528
Lys Asn Trp Leu Arg Ser Gln Pro Ala Pro Met Gln Pro Arg Ala Ala
                165                 170                 175

gcg gct gag ggc gcg cag ggg ctc tct ttg tcc atg aac atg gcg ggg  576
Ala Ala Glu Gly Ala Gln Gly Leu Ser Leu Ser Met Asn Met Ala Gly
            180                 185                 190

acg acc caa ggc gct gct ggc atg cca ctt ctc gct gga gag cgc gca  624
Thr Thr Gln Gly Ala Ala Gly Met Pro Leu Leu Ala Gly Glu Arg Ala
        195                 200                 205

cgg gcg ccc gag agt gta tcg acg tca gca cag ggt ggt gcc gtc gtc  672
Arg Ala Pro Glu Ser Val Ser Thr Ser Ala Gln Gly Gly Ala Val Val
    210                 215                 220

gtc acg gcg ccg aag gag gat agc ggt ggc agc ggt gtt gcc ggt gct  720
Val Thr Ala Pro Lys Glu Asp Ser Gly Gly Ser Gly Val Ala Gly Ala
225                 230                 235                 240

cta gta gcc gtg agc acg gac acg ggt ggc agc ggc ggc gcg tcg gct  768
Leu Val Ala Val Ser Thr Asp Thr Gly Gly Ser Gly Gly Ala Ser Ala
                245                 250                 255

gac aac acg gca agg aag acg gtg gac acg ttc ggg cag cgc acg tcg  816
Asp Asn Thr Ala Arg Lys Thr Val Asp Thr Phe Gly Gln Arg Thr Ser
            260                 265                 270
```

```
att tac cgt ggc gtg aca agg cat aga tgg act ggg aga tat gag gca    864
Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala
        275                 280                 285

cat ctt tgg gat aac agt tgc aga agg gaa gga caa act cgt aag ggt    912
His Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln Thr Arg Lys Gly
    290                 295                 300

cgt caa gtc tat tta ggt ggc tat gat aaa gag gag aaa gct gct agg    960
Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala Arg
305                 310                 315                 320

gct tat gat ctt gct gct ctg aag tac tgg ggt gcc aca aca aca aca   1008
Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Ala Thr Thr Thr Thr
                325                 330                 335

aat ttt cca gtg agt aac tac gaa aag gag ctc gag gac atg aag cac   1056
Asn Phe Pro Val Ser Asn Tyr Glu Lys Glu Leu Glu Asp Met Lys His
            340                 345                 350

atg aca agg cag gag ttt gta gcg tct ctg aga agg aag agc agt ggt   1104
Met Thr Arg Gln Glu Phe Val Ala Ser Leu Arg Arg Lys Ser Ser Gly
        355                 360                 365

ttc tcc aga ggt gca tcc att tac agg gga gtg act agg cat cac caa   1152
Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His Gln
    370                 375                 380

cat gga aga tgg caa gca cgg att gga cga gtt gca ggg aac aag gat   1200
His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp
385                 390                 395                 400

ctt tac ttg ggc acc ttc agc acc cag gag gag gca gcg gag gcg tac   1248
Leu Tyr Leu Gly Thr Phe Ser Thr Gln Glu Glu Ala Ala Glu Ala Tyr
                405                 410                 415

gac atc gcg gcg atc aag ttc cgc ggc ctc aac gcc gtc acc aac ttc   1296
Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Asn Ala Val Thr Asn Phe
            420                 425                 430

gac atg agc cgc tac gac gtg aag agc atc ctg gac agc agc gcc ctc   1344
Asp Met Ser Arg Tyr Asp Val Lys Ser Ile Leu Asp Ser Ser Ala Leu
        435                 440                 445

ccc atc ggc agc gcc gcc aag cgt ctc aag gag gcc gag gcc gca gcg   1392
Pro Ile Gly Ser Ala Ala Lys Arg Leu Lys Glu Ala Glu Ala Ala Ala
    450                 455                 460

tcc gcg cag cac cac cac gcc ggc gtg gtg agc tac gac gtc ggc cgc   1440
Ser Ala Gln His His His Ala Gly Val Val Ser Tyr Asp Val Gly Arg
465                 470                 475                 480

atc gcc tcg cag ctc ggc gac ggc gga gcc cta gcg gcg gcg tac ggc   1488
Ile Ala Ser Gln Leu Gly Asp Gly Gly Ala Leu Ala Ala Ala Tyr Gly
                485                 490                 495

gcg cac tac cac ggc gcc gcc tgg ccg acc atc gcg ttc cag ccg ggc   1536
Ala His Tyr His Gly Ala Ala Trp Pro Thr Ile Ala Phe Gln Pro Gly
            500                 505                 510

gcc gcc acc aca ggc ctg tac cac ccg tac gcg cag cag cca atg cgc   1584
Ala Ala Thr Thr Gly Leu Tyr His Pro Tyr Ala Gln Gln Pro Met Arg
        515                 520                 525

ggc ggc ggg tgg tgc aag cag gag cag gac cac gcg gtg atc gcg gcc   1632
Gly Gly Gly Trp Cys Lys Gln Glu Gln Asp His Ala Val Ile Ala Ala
    530                 535                 540
```

```
gcg cac agc ctg cag gac ctc cac cac ttg aac ctg ggc gcg gcc ggc   1680
Ala His Ser Leu Gln Asp Leu His His Leu Asn Leu Gly Ala Ala Gly
545                 550                 555                 560

gcg cac gac ttt ttc tcg gca ggg cag cag gcc gcc gcc gca gct gcg   1728
Ala His Asp Phe Phe Ser Ala Gly Gln Gln Ala Ala Ala Ala Ala Ala
                565                 570                 575

atg cac ggc ctg gct agc atc gac agt gcg tcg ctc gag cac agc acc   1776
Met His Gly Leu Ala Ser Ile Asp Ser Ala Ser Leu Glu His Ser Thr
            580                 585                 590

ggc tcc aac tcc gtc gtc tac aac ggc ggg gtc ggc gat agc aac ggc   1824
Gly Ser Asn Ser Val Val Tyr Asn Gly Gly Val Gly Asp Ser Asn Gly
        595                 600                 605

gcc agc gcc gtt ggc agc ggc ggt ggc tac atg atg ccg atg agc gct   1872
Ala Ser Ala Val Gly Ser Gly Gly Gly Tyr Met Met Pro Met Ser Ala
    610                 615                 620

gcc gga gca acc act aca tcg gca atg gtg agc cac gag cag atg cat   1920
Ala Gly Ala Thr Thr Thr Ser Ala Met Val Ser His Glu Gln Met His
625                 630                 635                 640

gca cgg gcc tac gac gaa gcc aag cag gct gct cag atg ggg tac gag   1968
Ala Arg Ala Tyr Asp Glu Ala Lys Gln Ala Ala Gln Met Gly Tyr Glu
                645                 650                 655

agc tac ctg gtg aac gcg gag aac aat ggt ggc gga agg atg tct gca   2016
Ser Tyr Leu Val Asn Ala Glu Asn Asn Gly Gly Gly Arg Met Ser Ala
            660                 665                 670

tgg ggg acc gtc gtc tct gca gcc gcg gcg gca gca gca agc agc aac   2064
Trp Gly Thr Val Val Ser Ala Ala Ala Ala Ala Ala Ala Ser Ser Asn
        675                 680                 685

gac aac att gcc gcc gac gtc ggc cat ggc ggc gcg cag ctc ttc agt   2112
Asp Asn Ile Ala Ala Asp Val Gly His Gly Gly Ala Gln Leu Phe Ser
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Val Trp Asn Asp Thr
705
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Ala Asp His Val Ser Gly Asp Val Cys Phe Asn Ile Pro Gln Asp Trp
        35                  40                  45
Ser Met Arg Gly Ser Glu Leu Ser Ala Leu Val Ala Glu Pro Lys Leu
    50                  55                  60
Glu Asp Phe Leu Gly Gly Ile Ser Phe Ser Glu Gln His His Lys Ser
65                  70                  75                  80
Asn Cys Asn Leu Ile Pro Ser Thr Ser Ser Thr Val Cys Tyr Ala Ser
                85                  90                  95
Ser Ala Ala Ser Thr Gly Tyr His His Gln Leu Tyr Gln Pro Thr Ser
            100                 105                 110
Ser Ala Leu His Phe Ala Asp Ser Val Met Val Ala Ser Ser Ala Gly
        115                 120                 125
Val His Asp Gly Gly Ser Met Leu Ser Ala Ala Ala Ala Asn Gly Val
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Ala Gly Ala Ala Ser Ala Asn Gly Gly Gly Ile Gly Leu Ser Met Ile
145 150 155 160
Lys Asn Trp Leu Arg Ser Gln Pro Ala Pro Met Gln Pro Arg Ala Ala
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Ala Ala Glu Gly Ala Gln Gly Leu Ser Leu Ser Met Asn Met Ala Gly
180 185 190
Thr Thr Gln Gly Ala Ala Gly Met Pro Leu Leu Ala Gly Glu Arg Ala
195 200 205
Arg Ala Pro Glu Ser Val Ser Thr Ser Ala Gln Gly Gly Ala Val Val
210 215 220
Val Thr Ala Pro Lys Glu Asp Ser Gly Gly Ser Gly Val Ala Gly Ala
225 230 235 240
Leu Val Ala Val Ser Thr Asp Thr Gly Gly Ser Gly Gly Ala Ser Ala
245 250 255
Asp Asn Thr Ala Arg Lys Thr Val Asp Thr Phe Gly Gln Arg Thr Ser
260 265 270
Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala
275 280 285
His Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln Thr Arg Lys Gly
290 295 300
Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala Arg
305 310 315 320
Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Ala Thr Thr Thr Thr
325 330 335
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340 345 350
Met Thr Arg Gln Glu Phe Val Ala Ser Leu Arg Arg Lys Ser Ser Gly
355 360 365
Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His Gln
370 375 380
His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp
385 390 395 400
Leu Tyr Leu Gly Thr Phe Ser Thr Gln Glu Glu Ala Ala Glu Ala Tyr
405 410 415
Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Asn Ala Val Thr Asn Phe
420 425 430
Asp Met Ser Arg Tyr Asp Val Lys Ser Ile Leu Asp Ser Ser Ala Leu
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Pro Ile Gly Ser Ala Ala Lys Arg Leu Lys Glu Ala Glu Ala Ala Ala
450 455 460
Ser Ala Gln His His His Ala Gly Val Val Ser Tyr Asp Val Gly Arg
465 470 475 480
Ile Ala Ser Gln Leu Gly Asp Gly Gly Ala Leu Ala Ala Ala Tyr Gly
485 490 495
Ala His Tyr His Gly Ala Ala Trp Pro Thr Ile Ala Phe Gln Pro Gly
500 505 510
Ala Ala Thr Thr Gly Leu Tyr His Pro Tyr Ala Gln Gln Pro Met Arg
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Gly Gly Gly Trp Cys Lys Gln Glu Gln Asp His Ala Val Ile Ala Ala
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545 550 555 560
Ala His Asp Phe Phe Ser Ala Gly Gln Gln Ala Ala Ala Ala Ala Ala
565 570 575
Met His Gly Leu Ala Ser Ile Asp Ser Ala Ser Leu Glu His Ser Thr
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Gly Ser Asn Ser Val Val Tyr Asn Gly Gly Val Gly Asp Ser Asn Gly
595 600 605
Ala Ser Ala Val Gly Ser Gly Gly Gly Tyr Met Met Pro Met Ser Ala
610 615 620
Ala Gly Ala Thr Thr Thr Ser Ala Met Val Ser His Glu Gln Met His
625 630 635 640
Ala Arg Ala Tyr Asp Glu Ala Lys Gln Ala Ala Gln Met Gly Tyr Glu
645 650 655
Ser Tyr Leu Val Asn Ala Glu Asn Asn Gly Gly Gly Arg Met Ser Ala
660 665 670
Trp Gly Thr Val Val Ser Ala Ala Ala Ala Ala Ala Ala Ala Ser Ser Asn

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Val Trp Asn Asp Thr
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ctg ccg ccc tcc cag acg acg gac tcc aca ctc atc tcg gcc gcc acc 96
Leu Pro Pro Ser Gln Thr Thr Asp Ser Thr Leu Ile Ser Ala Ala Thr

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                20                  25                  30

gcc gac cat gtc tcc ggc gat gtc tgc ttc aac atc ccc caa gat tgg   144
Ala Asp His Val Ser Gly Asp Val Cys Phe Asn Ile Pro Gln Asp Trp
            35                  40                  45

agc atg agg gga tca gag ctt tcg gcg ctc gtc gcg gag ccg aag ctg   192
Ser Met Arg Gly Ser Glu Leu Ser Ala Leu Val Ala Glu Pro Lys Leu
        50                  55                  60

gag gac ttc ctc ggc ggc atc tcc ttc tcc gag cag cat cac aag gcc   240
Glu Asp Phe Leu Gly Gly Ile Ser Phe Ser Glu Gln His His Lys Ala
    65                  70                  75                  80

aac tgc aac atg ata ccc agc act agc agc aca gtt tgc tac gcg agc   288
Asn Cys Asn Met Ile Pro Ser Thr Ser Ser Thr Val Cys Tyr Ala Ser
                85                  90                  95

tca ggt gct agc acc ggc tac cat cac cag ctg tac cac cag ccc acc   336
Ser Gly Ala Ser Thr Gly Tyr His His Gln Leu Tyr His Gln Pro Thr
            100                 105                 110

agc tca gcg ctc cac ttc gcg gac tcc gta atg gtg gcc tcc tcg gcc   384
Ser Ser Ala Leu His Phe Ala Asp Ser Val Met Val Ala Ser Ser Ala
        115                 120                 125

ggt gtc cac gac ggc ggt gcc atg ctc agc gcg gcc gcc gct aac ggt   432
Gly Val His Asp Gly Gly Ala Met Leu Ser Ala Ala Ala Ala Asn Gly
    130                 135                 140

gtc gct ggc gct gcc agt gcc aac ggc ggc ggc atc ggg ctg tcc atg   480
Val Ala Gly Ala Ala Ser Ala Asn Gly Gly Gly Ile Gly Leu Ser Met
145                 150                 155                 160

att aag aac tgg ctg cgg agc caa ccg gcg ccc atg cag ccg agg gtg   528
Ile Lys Asn Trp Leu Arg Ser Gln Pro Ala Pro Met Gln Pro Arg Val
                165                 170                 175

gcg gcg gct gag ggc gcg cag ggg ctc tct ttg tcc atg aac atg gcg   576
Ala Ala Ala Glu Gly Ala Gln Gly Leu Ser Leu Ser Met Asn Met Ala
            180                 185                 190

ggg acg acc caa ggc gct gct ggc atg cca ctt ctc gct gga gag cgc   624
Gly Thr Thr Gln Gly Ala Ala Gly Met Pro Leu Leu Ala Gly Glu Arg
        195                 200                 205

gca cgg gcg ccc gag agt gta tcg acg tca gca cag ggt gga gcc gtc   672
Ala Arg Ala Pro Glu Ser Val Ser Thr Ser Ala Gln Gly Gly Ala Val
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gtc gtc acg gcg ccg aag gag gat agc ggt ggc agc ggt gtt gcc ggc   720
Val Val Thr Ala Pro Lys Glu Asp Ser Gly Gly Ser Gly Val Ala Gly
225                 230                 235                 240

gct cta gta gcc gtg agc acg gac acg ggt ggc agc ggc ggc gcg tcg   768
Ala Leu Val Ala Val Ser Thr Asp Thr Gly Gly Ser Gly Gly Ala Ser
                245                 250                 255

gct gac aac acg gca agg aag acg gtg gac acg ttc ggg cag cgc acg   816
Ala Asp Asn Thr Ala Arg Lys Thr Val Asp Thr Phe Gly Gln Arg Thr
            260                 265                 270

tcg att tac cgt ggc gtg aca agg cat aga tgg act ggg aga tat gag   864
Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu
        275                 280                 285

gca cat ctt tgg gat aac agt tgc aga agg gaa ggg caa act cgt aag   912
Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln Thr Arg Lys
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    290                 295                 300

ggt cgt caa gtc tat tta ggt ggc tat gat aaa gag gag aaa gct gct   960
Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala
305                 310                 315                 320

agg gct tat gat ctt gct gct ctg aag tac tgg ggt gcc aca aca aca  1008
Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Ala Thr Thr Thr
                325                 330                 335

aca aat ttt cca gtg agt aac tac gaa aag gag ctc gag gac atg aag  1056
Thr Asn Phe Pro Val Ser Asn Tyr Glu Lys Glu Leu Glu Asp Met Lys
            340                 345                 350

cac atg aca agg cag gag ttt gta gcg tct ctg aga agg aag agc agt  1104
His Met Thr Arg Gln Glu Phe Val Ala Ser Leu Arg Arg Lys Ser Ser
        355                 360                 365

ggt ttc tcc aga ggt gca tcc att tac agg gga gtg act agg cat cac  1152
Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His
    370                 375                 380

caa cat gga aga tgg caa gca cgg att gga cga gtt gca ggg aac aag  1200
Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys
385                 390                 395                 400

gat ctt tac ttg ggc acc ttc agc acc cag gag gag gca gcg gag gcg  1248
Asp Leu Tyr Leu Gly Thr Phe Ser Thr Gln Glu Glu Ala Ala Glu Ala
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tac gac atc gcg gcg atc aag ttc cgc ggc ctc aac gcc gtc acc aac  1296
Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Asn Ala Val Thr Asn
            420                 425                 430

ttc gac atg agc cgc tac gac gtg aag agc atc ctg gac agc agc gcc  1344
Phe Asp Met Ser Arg Tyr Asp Val Lys Ser Ile Leu Asp Ser Ser Ala
        435                 440                 445

ctc ccc atc ggc agc gcc gcc aag cgc ctc aag gag gcc gag gcc gca  1392
Leu Pro Ile Gly Ser Ala Ala Lys Arg Leu Lys Glu Ala Glu Ala Ala
    450                 455                 460

gcg tcc gcg cag cac cac cac gcc ggc gtg gtg agc tac gac gtc ggc  1440
Ala Ser Ala Gln His His His Ala Gly Val Val Ser Tyr Asp Val Gly
465                 470                 475                 480

cgc atc gcc tcg cag ctc ggc gac ggc gga gcc ctg gcg gcg gcg tac  1488
Arg Ile Ala Ser Gln Leu Gly Asp Gly Gly Ala Leu Ala Ala Ala Tyr
                485                 490                 495

ggc gcg cac tac cac ggc gcc gcc tgg ccg acc atc gcg ttc cag ccg  1536
Gly Ala His Tyr His Gly Ala Ala Trp Pro Thr Ile Ala Phe Gln Pro
            500                 505                 510

ggc gcc gcc agc aca ggc ctg tac cac ccg tac gcg cag cag cca atg  1584
Gly Ala Ala Ser Thr Gly Leu Tyr His Pro Tyr Ala Gln Gln Pro Met
        515                 520                 525

cgc ggc ggc ggg tgg tgc aag cag gag cag gac cac gcg gtg atc gcg  1632
Arg Gly Gly Gly Trp Cys Lys Gln Glu Gln Asp His Ala Val Ile Ala
    530                 535                 540

gcc gcg cac agc ctg cag gac ctc cac cac ctg aac ctg ggc gcg gcc  1680
Ala Ala His Ser Leu Gln Asp Leu His His Leu Asn Leu Gly Ala Ala
545                 550                 555                 560

ggc gcg cac gac ttt ttc tcg gca ggg cag cag gcc gcc gcc gct gcg  1728
Gly Ala His Asp Phe Phe Ser Ala Gly Gln Gln Ala Ala Ala Ala Ala
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atg cac ggc ctg ggt agc atc gac agt gcg tcg ctc gag cac agc acc   1776
Met His Gly Leu Gly Ser Ile Asp Ser Ala Ser Leu Glu His Ser Thr
                580                 585                 590

ggc tcc aac tcc gtc gtc tac aac ggc ggg gtc ggc gac agc aac ggc   1824
Gly Ser Asn Ser Val Val Tyr Asn Gly Gly Val Gly Asp Ser Asn Gly
        595                 600                 605

gcc agc gcc gtc ggc ggc agt ggc ggt ggc tac atg atg ccg atg agc   1872
Ala Ser Ala Val Gly Gly Ser Gly Gly Gly Tyr Met Met Pro Met Ser
    610                 615                 620

gct gcc gga gca acc act aca tcg gca atg gtg agc cac gag cag gtg   1920
Ala Ala Gly Ala Thr Thr Thr Ser Ala Met Val Ser His Glu Gln Val
625                 630                 635                 640

cat gca cgg gcc tac gac gaa gcc aag cag gct gct cag atg ggg tac   1968
His Ala Arg Ala Tyr Asp Glu Ala Lys Gln Ala Ala Gln Met Gly Tyr
                645                 650                 655

gag agc tac ctg gtg aac gcg gag aac aat ggt ggc gga agg atg tct   2016
Glu Ser Tyr Leu Val Asn Ala Glu Asn Asn Gly Gly Gly Arg Met Ser
            660                 665                 670

gca tgg ggg act gtc gtg tct gca gcc gcg gcg gca gca gca agc agc   2064
Ala Trp Gly Thr Val Val Ser Ala Ala Ala Ala Ala Ala Ala Ser Ser
        675                 680                 685

aac gac aac atg gcc gcc gac gtc ggc cat ggc ggc gcg cag ctc ttc   2112
Asn Asp Asn Met Ala Ala Asp Val Gly His Gly Gly Ala Gln Leu Phe
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Ser Met Arg Gly Ser Glu Leu Ser Ala Leu Val Ala Glu Pro Lys Leu
    50                  55                  60
Glu Asp Phe Leu Gly Gly Ile Ser Phe Ser Glu Gln His His Lys Ala
65                  70                  75                  80
Asn Cys Asn Met Ile Pro Ser Thr Ser Ser Thr Val Cys Tyr Ala Ser
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Ser Gly Ala Ser Thr Gly Tyr His His Gln Leu Tyr His Gln Pro Thr
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Ser Ser Ala Leu His Phe Ala Asp Ser Val Met Val Ala Ser Ser Ala
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Gly Val His Asp Gly Gly Ala Met Leu Ser Ala Ala Ala Ala Asn Gly
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Val Ala Gly Ala Ala Ser Ala Asn Gly Gly Gly Ile Gly Leu Ser Met
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Ile Lys Asn Trp Leu Arg Ser Gln Pro Ala Pro Met Gln Pro Arg Val
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Val Val Thr Ala Pro Lys Glu Asp Ser Gly Gly Ser Gly Val Ala Gly
225 230 235 240
Ala Leu Val Ala Val Ser Thr Asp Thr Gly Gly Ser Gly Gly Ala Ser
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Ala Asp Asn Thr Ala Arg Lys Thr Val Asp Thr Phe Gly Gln Arg Thr
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Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln Thr Arg Lys
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Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys
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465 470 475 480
Arg Ile Ala Ser Gln Leu Gly Asp Gly Gly Ala Leu Ala Ala Ala Tyr
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Glu Ser Tyr Leu Val Asn Ala Glu Asn Asn Gly Gly Gly Arg Met Ser
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atattctttt tcatctcaaa tctcactcga ctctttttttt ttatcttgta ccattgatag 1440
gtggctatga taaagaggag aaagctgcta gggcttatga tcttgctgct ctgaagtact 1500
ggggtcccac aacaacaaca aatttcccag tatgtatatg tagcatccag ttttacttta 1560
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catgacaagg caggagttTg tagcgtctct gagaaggtcg gtctaacagc attgattaat 1860
cagtaccacc tctactgaat aaaatctgct gctattgttt aaatttttgag cgaggtcaac 1920
tgcatatttg atcttatTag accactgtat atgaatgcag gaagagcagt ggttttctcca 1980
gaggtgcatc catttacagg ggagtgacta ggtatgaatt catatagcta agaacttaac 2040
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gtgtgcatgt tgttttactt gaactcgatc tctgtatttA taggcatcac caacatggaa 2160
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cggagccctg gcggcggcgt acggcgcgca ctaccacggc gccgcctggc cgaccatcgc 3120
gttccagccg ggcgccgcca ccacaggcct gtaccacccg tacgcgcagc agccaatgcg 3180
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cactacatcg gcaatggtga gccacgagca ggtgcatgca cgggcctacg acgaagccaa 3540
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aaggatgtct gcatgggga ctgtcgtgtc tgcagccgcg gcggcagcag caagcagcaa 3660

cgacaacatg gccgccgacg tcggccatgg cggcgcgcag ctcttcagtg tctggaacga 3720
cacttaa 3727

<210> 61
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 4

<220>
<221> VARIANT
<222> 3
<223> Xaa=Leu or Val

<220>
<221> VARIANT
<222> 4
<223> Xaa=Glu or Ala

<220>
<221> VARIANT
<222> 5
<223> Xaa=Asp or Asn

<400> 61
Pro Lys Xaa Xaa Xaa Phe Leu Gly
1 5

<210> 62
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 5

<220>
<221> VARIANT
<222> 6
<223> Xaa=Ile or Val

<220>
<221> VARIANT
<222> 9
<223> Xaa=Ala or Leu

<220>
<221> VARIANT
<222> 11, 12
<223> Xaa=Lys or Arg

<220>
<221> VARIANT
<222> 13
<223> Xaa=Leu or Arg

<400> 62
Ser Ser Thr Leu Pro Xaa Gly Gly Xaa Ala Xaa Xaa Xaa
1 5 10

<210> 63
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 6

<220>
<221> VARIANT
<222> 4
<223> Xaa=Gly or Ser

<400> 63
Asn Trp Leu Xaa Phe Ser Leu Ser Pro
 1   5

<210> 64
<211> 63
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 2

<220>
<221> VARIANT
<222> 2
<223> Xaa=Ile or Met

<220>
<221> VARIANT
<222> 36
<223> Xaa=Gln or Glu

<220>
<221> VARIANT
<222> 45
<223> Xaa=Ile or Val

<220>
<221> VARIANT
<222> 60
<223> Xaa=Asp or Glu

<220>
<221> VARIANT
<222> 61
<223> Xaa=Met or Ile

<220>
<221> VARIANT
<222> (62)...(62)
<223> Xaa=Ser or Asn

<400> 64
Ser Xaa Tyr Arg Gly Val Thr Arg His His Gln His Gly Arg Trp Gln

```
 1               5                  10                  15
Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr
            20                  25                  30
Phe Ser Thr Xaa Glu Glu Ala Ala Glu Ala Tyr Asp Xaa Ala Ala Ile
        35                  40                  45
Lys Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Xaa Xaa Xaa Arg
    50                  55                  60
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<210> 65
<211> 68
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 3

<220>
<221> VARIANT
<222> 2
<223> Xaa=Ile or Gln

<220>
<221> VARIANT
<222> 26
<223> Xaa=Arg or Lys

<220>
<221> VARIANT
<222> 30, 59
<223> Xaa=Ser or Thr

<220>
<221> VARIANT
<222> 33
<223> Xaa=Val or Gly

<220>
<221> VARIANT
<222> 34
<223> Xaa=Tyr or Arg

<220>
<221> VARIANT
<222> (35)...(35)
<223> Xaa=Leu or Gln

<220>
<221> VARIANT
<222> (42)...(42)
<223> Xaa=Glu or Asp

<220>
<221> VARIANT
<222> (58)...(58)
<223> Xaa=Pro or Thr

<220>
<221> VARIANT
<222> (61)...(61)

<223> Xaa=Thr or His

<220>
<221> VARIANT
<222> (62)...(62)
<223> Xaa=Thr or Ile

<220>
<221> VARIANT
<222> (66)...(66)
<223> Xaa=Ile, Val, or Leu

<400> 65
Ser Xaa Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu
1 5 10 15
Ala His Leu Trp Asp Asn Ser Cys Arg Xaa Glu Gly Gln Xaa Arg Lys
20 25 30
Xaa Xaa Xaa Gly Gly Tyr Asp Lys Glu Xaa Lys Ala Ala Arg Ala Tyr
35 40 45
Asp Leu Ala Ala Leu Lys Tyr Trp Gly Xaa Xaa Thr Xaa Xaa Asn Phe
50 55 60
Pro Xaa Ser Asn
65

<210> 66
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 1

<220>
<221> VARIANT
<222> 10
<223> Xaa=His or Asn

<220>
<221> VARIANT
<222> 16
<223> Xaa=Phe or Tyr

<220>
<221> VARIANT
<222> 17
<223> Xaa=Val or Ile

<220>
<221> VARIANT
<222> 19
<223> Xaa=Ser or His

<400> 66
Tyr Glu Lys Glu Leu Glu Glu Met Lys Xaa Met Thr Arg Gln Glu Xaa
1 5 10 15
Xaa Ala Xaa Leu Arg Arg Lys Ser Ser Gly Phe Ser Arg Gly Ala
20 25 30

<210> 67
<211> 10
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 7

<220>
<221> VARIANT
<222> 1
<223> Xaa=Gly or Glu

<220>
<221> VARIANT
<222> 7
<223> Xaa=Thr or Asn

<400> 67
Xaa Leu Ser Met Ile Lys Xaa Trp Leu Arg
 1   5   10

<210> 68
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 10

<220>
<221> VARIANT
<222> 4
<223> Xaa=Gln or Pro

<400> 68
Trp Cys Lys Xaa Glu Gln Asp
 1   5

<210> 69
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 8

<220>
<221> VARIANT
<222> 2, 4, 5
<223> Xaa=any amino acid

<400> 69
Pro Xaa Phe Xaa Xaa Trp Asn Asp
 1   5

<210> 70
<211> 5
<212> PRT

<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 9

<220>
<221> VARIANT
<222> 2
<223> Xaa=Ser, Thr, or Ala

<400> 70
Leu Xaa Leu Ser Met
 1   5

<210> 71
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 14

<400> 71
Trp Pro Thr Ile Ala Phe Gln
 1   5

<210> 72
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 15

<220>
<221> VARIANT
<222> 2
<223> Xaa=Ser or Thr

<400> 72
Ser Xaa Gly Ser Asn Ser Val Val Tyr Asn Gly
 1   5   10

<210> 73
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthesized

<220>
<223> BBM consensus sequence motif 19

<220>
<221> VARIANT

<222> 4
<223> Xaa=Ser or Asn

<400> 73
Gln Asp Trp Xaa Met Arg Gly
 1   5

<210> 74
<211> 1755
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)...(1755)

<400> 74
atg aac tcg atg aat aac tgg tta ggc ttc tct ctc tct cct cat gat 48
Met Asn Ser Met Asn Asn Trp Leu Gly Phe Ser Leu Ser Pro His Asp
 1   5   10   15

caa aat cat cac cgt acg gat gtt gac tcc tcc acc acc aga acc gcc 96
Gln Asn His His Arg Thr Asp Val Asp Ser Ser Thr Thr Arg Thr Ala
            20   25   30

gta gat gtt gcc gga ggg tac tgt ttt gat ctg gcc gct ccc tcc gat 144
Val Asp Val Ala Gly Gly Tyr Cys Phe Asp Leu Ala Ala Pro Ser Asp
        35   40   45

gaa tct tct gcc gtt caa aca tct ttt ctt tct cct ttc ggt gtc acc 192
Glu Ser Ser Ala Val Gln Thr Ser Phe Leu Ser Pro Phe Gly Val Thr
    50   55   60

ctc gaa gct ttc acc aga gac aat aat agt cac tcc cga gat tgg gac 240
Leu Glu Ala Phe Thr Arg Asp Asn Asn Ser His Ser Arg Asp Trp Asp
65   70   75   80

atc aat ggt ggt gca tgc aat aca tta acc aat aac gaa caa aat gga 288
Ile Asn Gly Gly Ala Cys Asn Thr Leu Thr Asn Asn Glu Gln Asn Gly
                85   90   95

cca aag ctt gag aat ttc ctc ggc cgc acc acc acg att tac aat acc 336
Pro Lys Leu Glu Asn Phe Leu Gly Arg Thr Thr Thr Ile Tyr Asn Thr
            100   105   110

aac gag acc gtt gta gat gga aat ggc gat tgt gga gga gga gac ggt 384
Asn Glu Thr Val Val Asp Gly Asn Gly Asp Cys Gly Gly Gly Asp Gly
        115   120   125

ggt ggt ggc ggc tca cta ggc ctt tcg atg ata aaa aca tgg ctg agt 432
Gly Gly Gly Gly Ser Leu Gly Leu Ser Met Ile Lys Thr Trp Leu Ser
    130   135   140

aat cat tcg gtt gct aat gct aat cat caa gac aat ggt aac ggt gca 480
Asn His Ser Val Ala Asn Ala Asn His Gln Asp Asn Gly Asn Gly Ala
145   150   155   160

cga ggc ttg tcc ctc tct atg aat tca tct act agt gat agc aac aac 528
Arg Gly Leu Ser Leu Ser Met Asn Ser Ser Thr Ser Asp Ser Asn Asn
                165   170   175

tac aac aac aat gat gat gtc gtc caa gag aag act att gtt gat gtc 576
Tyr Asn Asn Asn Asp Asp Val Val Gln Glu Lys Thr Ile Val Asp Val
            180   185   190

gta gaa act aca ccg aag aaa act att gag agt ttt gga caa agg acg 624
Val Glu Thr Thr Pro Lys Lys Thr Ile Glu Ser Phe Gly Gln Arg Thr

```
          195                 200                 205

tct ata tac cgc ggt gtt aca agg cat cgg tgg aca ggt aga tac gag   672
Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu
    210                 215                 220

gca cat tta tgg gac aat agt tgc aaa aga gaa ggc cag act cgc aaa   720
Ala His Leu Trp Asp Asn Ser Cys Lys Arg Glu Gly Gln Thr Arg Lys
225                 230                 235                 240

gga aga caa gtt tat ctg gga ggt tat gac aaa gaa gaa aaa gca gct   768
Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala
                245                 250                 255

agg gct tac gat tta gcc gca cta aag tat tgg gga ccc acc act act   816
Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Pro Thr Thr Thr
            260                 265                 270

act aac ttc ccc ttg agt gaa tat gag aaa gag gta gaa gag atg aag   864
Thr Asn Phe Pro Leu Ser Glu Tyr Glu Lys Glu Val Glu Glu Met Lys
        275                 280                 285

cac atg acg agg caa gag tat gtt gcc tct ctg cgc agg aaa agt agt   912
His Met Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser
    290                 295                 300

ggt ttc tct cgt ggt gca tcg att tat cga gga gta aca agg cat cac   960
Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His
305                 310                 315                 320

caa cat gga agg tgg caa gct agg atc gga aga gtc gcc ggt aac aaa  1008
Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys
                325                 330                 335

gac ctc tac ttg gga act ttc ggc aca cag gaa gag gct gct gag gct  1056
Asp Leu Tyr Leu Gly Thr Phe Gly Thr Gln Glu Glu Ala Ala Glu Ala
            340                 345                 350

tat gac att gca gcc att aaa ttc aga gga tta agc gca gtg act aac  1104
Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Ser Ala Val Thr Asn
        355                 360                 365

ttc gac atg aac aga tac aat gtt aaa gca atc ctc gag agc ccg agt  1152
Phe Asp Met Asn Arg Tyr Asn Val Lys Ala Ile Leu Glu Ser Pro Ser
    370                 375                 380

cta cct att ggt agt tct gcg aaa cgt ctc aag gac gtt aac aat ccg  1200
Leu Pro Ile Gly Ser Ser Ala Lys Arg Leu Lys Asp Val Asn Asn Pro
385                 390                 395                 400

gtt cca gct atg atg att agt aat aac gtt tca gag agt gca aat aat  1248
Val Pro Ala Met Met Ile Ser Asn Asn Val Ser Glu Ser Ala Asn Asn
                405                 410                 415

gtt agc ggt tgg caa aac act gcg ttt cag cat cat cag gga atg gat  1296
Val Ser Gly Trp Gln Asn Thr Ala Phe Gln His His Gln Gly Met Asp
            420                 425                 430

ttg agc tta ttg cag caa cag cag gag agg tac gtt ggt tat tac aat  1344
Leu Ser Leu Leu Gln Gln Gln Gln Glu Arg Tyr Val Gly Tyr Tyr Asn
        435                 440                 445

gga gga aac ttg tct acc gag agt act agg gtt tgt ttc aaa caa gag  1392
Gly Gly Asn Leu Ser Thr Glu Ser Thr Arg Val Cys Phe Lys Gln Glu
    450                 455                 460

gag gaa caa caa cac ttc ttg aga aac tcg ccg agt cac atg act aat  1440
Glu Glu Gln Gln His Phe Leu Arg Asn Ser Pro Ser His Met Thr Asn
```

```
465                 470                 475                 480

gtt gat cat cat agc tcg acc tct gat gat tct gtt acc gtt tgt gga   1488
Val Asp His His Ser Ser Thr Ser Asp Asp Ser Val Thr Val Cys Gly
                485                 490                 495

aat gtt gtt agt tat ggt ggt tat caa gga ttc gca atc cct gtt gga   1536
Asn Val Val Ser Tyr Gly Gly Tyr Gln Gly Phe Ala Ile Pro Val Gly
            500                 505                 510

aca tcg gtt aat tac gat ccc ttt act gct gct gag att gct tac aac   1584
Thr Ser Val Asn Tyr Asp Pro Phe Thr Ala Ala Glu Ile Ala Tyr Asn
        515                 520                 525

gca aga aat cat tat tac tat gct cag cat cag caa caa cag cag att   1632
Ala Arg Asn His Tyr Tyr Tyr Ala Gln His Gln Gln Gln Gln Gln Ile
    530                 535                 540

cag cag tcg ccg gga gga gat ttt ccg gtg gcg att tcg aat aac cat   1680
Gln Gln Ser Pro Gly Gly Asp Phe Pro Val Ala Ile Ser Asn Asn His
545                 550                 555                 560

agc tct aac atg tac ttt cac ggg gaa ggt ggt gga gaa ggg gct cca   1728
Ser Ser Asn Met Tyr Phe His Gly Glu Gly Gly Gly Glu Gly Ala Pro
                565                 570                 575

acg ttt tca gtt tgg aac gac act tag                              1755
Thr Phe Ser Val Trp Asn Asp Thr
            580
```

<210> 75
<211> 584
<212> PRT
<213> Arabidopsis thaliana

<400> 75

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Met Asn Ser Met Asn Asn Trp Leu Gly Phe Ser Leu Ser Pro His Asp
 1               5                  10                  15
Gln Asn His His Arg Thr Asp Val Asp Ser Ser Thr Thr Arg Thr Ala
            20                  25                  30
Val Asp Val Ala Gly Gly Tyr Cys Phe Asp Leu Ala Ala Pro Ser Asp
        35                  40                  45
Glu Ser Ser Ala Val Gln Thr Ser Phe Leu Ser Pro Phe Gly Val Thr
    50                  55                  60
Leu Glu Ala Phe Thr Arg Asp Asn Asn Ser His Ser Arg Asp Trp Asp
65                  70                  75                  80
Ile Asn Gly Gly Ala Cys Asn Thr Leu Thr Asn Asn Glu Gln Asn Gly
                85                  90                  95
Pro Lys Leu Glu Asn Phe Leu Gly Arg Thr Thr Thr Ile Tyr Asn Thr
            100                 105                 110
Asn Glu Thr Val Val Asp Gly Asn Gly Asp Cys Gly Gly Gly Asp Gly
        115                 120                 125
Gly Gly Gly Gly Ser Leu Gly Leu Ser Met Ile Lys Thr Trp Leu Ser
    130                 135                 140
Asn His Ser Val Ala Asn Ala Asn His Gln Asp Asn Gly Asn Gly Ala
145                 150                 155                 160
Arg Gly Leu Ser Leu Ser Met Asn Ser Ser Thr Ser Asp Ser Asn Asn
                165                 170                 175
Tyr Asn Asn Asn Asp Asp Val Val Gln Glu Lys Thr Ile Val Asp Val
            180                 185                 190
Val Glu Thr Thr Pro Lys Lys Thr Ile Glu Ser Phe Gly Gln Arg Thr
        195                 200                 205
Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu
    210                 215                 220
Ala His Leu Trp Asp Asn Ser Cys Lys Arg Glu Gly Gln Thr Arg Lys
225                 230                 235                 240
Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala
```

```
                    245                 250                 255
Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Pro Thr Thr Thr
                260                 265                 270
Thr Asn Phe Pro Leu Ser Glu Tyr Glu Lys Glu Val Glu Glu Met Lys
            275                 280                 285
His Met Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser
        290                 295                 300
Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His
305                 310                 315                 320
Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys
                325                 330                 335
Asp Leu Tyr Leu Gly Thr Phe Gly Thr Gln Glu Glu Ala Ala Glu Ala
            340                 345                 350
Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Ser Ala Val Thr Asn
        355                 360                 365
Phe Asp Met Asn Arg Tyr Asn Val Lys Ala Ile Leu Glu Ser Pro Ser
    370                 375                 380
Leu Pro Ile Gly Ser Ser Ala Lys Arg Leu Lys Asp Val Asn Asn Pro
385                 390                 395                 400
Val Pro Ala Met Met Ile Ser Asn Asn Val Ser Glu Ser Ala Asn Asn
                405                 410                 415
Val Ser Gly Trp Gln Asn Thr Ala Phe Gln His His Gln Gly Met Asp
            420                 425                 430
Leu Ser Leu Leu Gln Gln Gln Gln Glu Arg Tyr Val Gly Tyr Tyr Asn
        435                 440                 445
Gly Gly Asn Leu Ser Thr Glu Ser Thr Arg Val Cys Phe Lys Gln Glu
    450                 455                 460
Glu Glu Gln Gln His Phe Leu Arg Asn Ser Pro Ser His Met Thr Asn
465                 470                 475                 480
Val Asp His His Ser Ser Thr Ser Asp Asp Ser Val Thr Val Cys Gly
                485                 490                 495
Asn Val Val Ser Tyr Gly Gly Tyr Gln Gly Phe Ala Ile Pro Val Gly
            500                 505                 510
Thr Ser Val Asn Tyr Asp Pro Phe Thr Ala Ala Glu Ile Ala Tyr Asn
        515                 520                 525
Ala Arg Asn His Tyr Tyr Tyr Ala Gln His Gln Gln Gln Gln Gln Ile
    530                 535                 540
Gln Gln Ser Pro Gly Gly Asp Phe Pro Val Ala Ile Ser Asn Asn His
545                 550                 555                 560
Ser Ser Asn Met Tyr Phe His Gly Glu Gly Gly Gly Glu Gly Ala Pro
                565                 570                 575
Thr Phe Ser Val Trp Asn Asp Thr
            580
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<210> 76
<211> 1740
<212> DNA
<213> Brassica napus

<220>
<221> CDS
<222> (1)...(1740)

<400> 76

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atg aat aat aac tgg tta ggc ttt tct ctc tct cct tat gaa caa aat    48
Met Asn Asn Asn Trp Leu Gly Phe Ser Leu Ser Pro Tyr Glu Gln Asn
 1               5                  10                  15

cac cat cgt aag gac gtc tac tct tcc acc acc aca acc gtc gta gat    96
His His Arg Lys Asp Val Tyr Ser Ser Thr Thr Thr Thr Val Val Asp
            20                  25                  30

gtc gcc gga gag tac tgt tac gat ccg acc gct gcc tcc gat gag tct   144
Val Ala Gly Glu Tyr Cys Tyr Asp Pro Thr Ala Ala Ser Asp Glu Ser
        35                  40                  45

tca gcc atc caa aca tcg ttt cct tct ccc ttt ggt gtc gtc gtc gat   192
```

```
Ser Ala Ile Gln Thr Ser Phe Pro Ser Pro Phe Gly Val Val Val Asp
     50                  55                  60

gct ttc acc aga gac aac aat agt cac tcc cga gat tgg gac atc aat   240
Ala Phe Thr Arg Asp Asn Asn Ser His Ser Arg Asp Trp Asp Ile Asn
 65                  70                  75                  80

ggt tgt gca tgc aat aac atc cac aac gat gag caa gat gga cca aag   288
Gly Cys Ala Cys Asn Asn Ile His Asn Asp Glu Gln Asp Gly Pro Lys
                85                  90                  95

ctt gag aat ttc ctt ggc cgc acc acc acg att tac aac acc aac gaa   336
Leu Glu Asn Phe Leu Gly Arg Thr Thr Thr Ile Tyr Asn Thr Asn Glu
            100                 105                 110

aac gtt gga gat gga agt gga agt ggc tgt tat gga gga gga gac ggt   384
Asn Val Gly Asp Gly Ser Gly Ser Gly Cys Tyr Gly Gly Gly Asp Gly
        115                 120                 125

ggt ggt ggc tca cta gga ctt tcg atg ata aag aca tgg ctg aga aat   432
Gly Gly Gly Ser Leu Gly Leu Ser Met Ile Lys Thr Trp Leu Arg Asn
    130                 135                 140

caa ccc gtg gat aat gtt gat aat caa gaa aat ggc aat gct gca aaa   480
Gln Pro Val Asp Asn Val Asp Asn Gln Glu Asn Gly Asn Ala Ala Lys
145                 150                 155                 160

ggc ctg tcc ctc tca atg aac tca tct act tct tgt gat aac aac aac   528
Gly Leu Ser Leu Ser Met Asn Ser Ser Thr Ser Cys Asp Asn Asn Asn
                165                 170                 175

gac agc aat aac aac gtt gtt gcc caa ggg aag act att gat gat agc   576
Asp Ser Asn Asn Asn Val Val Ala Gln Gly Lys Thr Ile Asp Asp Ser
            180                 185                 190

gtt gaa gct aca ccg aag aaa act att gag agt ttt gga cag agg acg   624
Val Glu Ala Thr Pro Lys Lys Thr Ile Glu Ser Phe Gly Gln Arg Thr
        195                 200                 205

tct ata tac cgc ggt gtt aca agg cat cgg tgg aca gga aga tat gag   672
Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu
    210                 215                 220

gca cat tta tgg gat aat agt tgt aaa aga gaa ggc caa acg cgc aaa   720
Ala His Leu Trp Asp Asn Ser Cys Lys Arg Glu Gly Gln Thr Arg Lys
225                 230                 235                 240

gga aga caa gtt tat ttg gga ggt tat gac aaa gaa gaa aaa gca gct   768
Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala
                245                 250                 255

agg gct tat gat tta gcc gca ctc aag tat tgg gga acc acc act act   816
Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr
            260                 265                 270

act aac ttc ccc atg agc gaa tat gaa aaa gag gta gaa gag atg aag   864
Thr Asn Phe Pro Met Ser Glu Tyr Glu Lys Glu Val Glu Glu Met Lys
        275                 280                 285

cac atg aca agg caa gag tat gtt gcc tca ctg cgc agg aaa agt agt   912
His Met Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser
    290                 295                 300

ggt ttc tct cgt ggt gca tcg att tat cgt gga gta aca aga cat cac   960
Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His
305                 310                 315                 320

caa cat gga aga tgg caa gct agg ata gga aga gtc gcc ggt aac aaa  1008
```

```
Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys
                325                 330                 335

gac ctc tac ttg gga act ttt ggc aca caa gaa gaa gct gca gag gca   1056
Asp Leu Tyr Leu Gly Thr Phe Gly Thr Gln Glu Glu Ala Ala Glu Ala
            340                 345                 350

tac gac att gcg gcc atc aaa ttc aga gga tta acc gca gtg act aac   1104
Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Thr Ala Val Thr Asn
        355                 360                 365

ttc gac atg aac aga tac aac gtt aaa gca atc ctc gaa agc cct agt   1152
Phe Asp Met Asn Arg Tyr Asn Val Lys Ala Ile Leu Glu Ser Pro Ser
    370                 375                 380

ctt cct att ggt agc gcc gca aaa cgt ctc aag gag gct aac cgt ccg   1200
Leu Pro Ile Gly Ser Ala Ala Lys Arg Leu Lys Glu Ala Asn Arg Pro
385                 390                 395                 400

gtt cca agt atg atg atg atc agt aat aac gtt tca gag agt gag aat   1248
Val Pro Ser Met Met Met Ile Ser Asn Asn Val Ser Glu Ser Glu Asn
                405                 410                 415

agt gct agc ggt tgg caa aac gct gcg gtt cag cat cat cag gga gta   1296
Ser Ala Ser Gly Trp Gln Asn Ala Ala Val Gln His His Gln Gly Val
            420                 425                 430

gat ttg agc tta ttg cac caa cat caa gag agg tac aat ggt tat tat   1344
Asp Leu Ser Leu Leu His Gln His Gln Glu Arg Tyr Asn Gly Tyr Tyr
        435                 440                 445

tac aat gga gga aac ttg tct tcg gag agt gct agg gct tgt ttc aaa   1392
Tyr Asn Gly Gly Asn Leu Ser Ser Glu Ser Ala Arg Ala Cys Phe Lys
    450                 455                 460

caa gag gat gat caa cac cat ttc ttg agc aac acg cag agc ctc atg   1440
Gln Glu Asp Asp Gln His His Phe Leu Ser Asn Thr Gln Ser Leu Met
465                 470                 475                 480

act aat atc gat cat caa agt tct gtt tcg gat gat tcg gtt act gtt   1488
Thr Asn Ile Asp His Gln Ser Ser Val Ser Asp Asp Ser Val Thr Val
                485                 490                 495

tgt gga aat gtt gtt ggt tat ggt ggt tat caa gga ttt gca gcc ccg   1536
Cys Gly Asn Val Val Gly Tyr Gly Gly Tyr Gln Gly Phe Ala Ala Pro
            500                 505                 510

gtt aac tgc gat gcc tac gct gct agt gag ttt gat tat aac gca aga   1584
Val Asn Cys Asp Ala Tyr Ala Ala Ser Glu Phe Asp Tyr Asn Ala Arg
        515                 520                 525

aac cat tat tac ttt gct cag cag cag cag acc cag cag tcg cca ggt   1632
Asn His Tyr Tyr Phe Ala Gln Gln Gln Gln Thr Gln Gln Ser Pro Gly
    530                 535                 540

gga gat ttt ccc gcg gca atg acg aat aat gtt ggc tct aat atg tat   1680
Gly Asp Phe Pro Ala Ala Met Thr Asn Asn Val Gly Ser Asn Met Tyr
545                 550                 555                 560

tac cat ggg gaa ggt ggt gga gaa gtt gct cca aca ttt aca gtt tgg   1728
Tyr His Gly Glu Gly Gly Gly Glu Val Ala Pro Thr Phe Thr Val Trp
                565                 570                 575

aac gac aat tag                                                  1740
Asn Asp Asn
```

<210> 77
<211> 579
<212> PRT
<213> Brassica napus

<400> 77

```
Met Asn Asn Asn Trp Leu Gly Phe Ser Leu Ser Pro Tyr Glu Gln Asn
 1               5                  10                  15
His His Arg Lys Asp Val Tyr Ser Ser Thr Thr Thr Thr Val Val Asp
            20                  25                  30
Val Ala Gly Glu Tyr Cys Tyr Asp Pro Thr Ala Ala Ser Asp Glu Ser
        35                  40                  45
Ser Ala Ile Gln Thr Ser Phe Pro Ser Pro Phe Gly Val Val Val Asp
    50                  55                  60
Ala Phe Thr Arg Asp Asn Asn Ser His Ser Arg Asp Trp Asp Ile Asn
65                  70                  75                  80
Gly Cys Ala Cys Asn Asn Ile His Asn Asp Glu Gln Asp Gly Pro Lys
                85                  90                  95
Leu Glu Asn Phe Leu Gly Arg Thr Thr Thr Ile Tyr Asn Thr Asn Glu
            100                 105                 110
Asn Val Gly Asp Gly Ser Gly Ser Gly Cys Tyr Gly Gly Gly Asp Gly
        115                 120                 125
Gly Gly Gly Ser Leu Gly Leu Ser Met Ile Lys Thr Trp Leu Arg Asn
    130                 135                 140
Gln Pro Val Asp Asn Val Asp Asn Gln Glu Asn Gly Asn Ala Ala Lys
145                 150                 155                 160
Gly Leu Ser Leu Ser Met Asn Ser Ser Thr Ser Cys Asp Asn Asn Asn
                165                 170                 175
Asp Ser Asn Asn Asn Val Val Ala Gln Gly Lys Thr Ile Asp Asp Ser
            180                 185                 190
Val Glu Ala Thr Pro Lys Lys Thr Ile Glu Ser Phe Gly Gln Arg Thr
        195                 200                 205
Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu
    210                 215                 220
Ala His Leu Trp Asp Asn Ser Cys Lys Arg Glu Gly Gln Thr Arg Lys
225                 230                 235                 240
Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala
                245                 250                 255
Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr
            260                 265                 270
Thr Asn Phe Pro Met Ser Glu Tyr Glu Lys Glu Val Glu Glu Met Lys
        275                 280                 285
His Met Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser
    290                 295                 300
Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His
305                 310                 315                 320
Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys
                325                 330                 335
Asp Leu Tyr Leu Gly Thr Phe Gly Thr Gln Glu Glu Ala Ala Glu Ala
            340                 345                 350
Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Thr Ala Val Thr Asn
        355                 360                 365
Phe Asp Met Asn Arg Tyr Asn Val Lys Ala Ile Leu Glu Ser Pro Ser
    370                 375                 380
Leu Pro Ile Gly Ser Ala Ala Lys Arg Leu Lys Glu Ala Asn Arg Pro
385                 390                 395                 400
Val Pro Ser Met Met Met Ile Ser Asn Asn Val Ser Glu Ser Glu Asn
                405                 410                 415
Ser Ala Ser Gly Trp Gln Asn Ala Ala Val Gln His His Gln Gly Val
            420                 425                 430
Asp Leu Ser Leu Leu His Gln His Gln Glu Arg Tyr Asn Gly Tyr Tyr
        435                 440                 445
Tyr Asn Gly Gly Asn Leu Ser Ser Glu Ser Ala Arg Ala Cys Phe Lys
    450                 455                 460
Gln Glu Asp Asp Gln His His Phe Leu Ser Asn Thr Gln Ser Leu Met
465                 470                 475                 480
Thr Asn Ile Asp His Gln Ser Ser Val Ser Asp Asp Ser Val Thr Val
                485                 490                 495
```

```
Cys Gly Asn Val Val Gly Tyr Gly Gly Tyr Gln Gly Phe Ala Ala Pro
            500                 505                 510
Val Asn Cys Asp Ala Tyr Ala Ala Ser Glu Phe Asp Tyr Asn Ala Arg
        515                 520                 525
Asn His Tyr Tyr Phe Ala Gln Gln Gln Gln Thr Gln Gln Ser Pro Gly
    530                 535                 540
Gly Asp Phe Pro Ala Ala Met Thr Asn Asn Val Gly Ser Asn Met Tyr
545                 550                 555                 560
Tyr His Gly Glu Gly Gly Gly Glu Val Ala Pro Thr Phe Thr Val Trp
                565                 570                 575
Asn Asp Asn
```

<210> 78
<211> 1740
<212> DNA
<213> Brassica napus

<220>
<221> CDS
<222> (1)...(1740)

<400> 78

```
atg aat aat aac tgg tta ggc ttt tct ctc tct cct tat gaa caa aat   48
Met Asn Asn Asn Trp Leu Gly Phe Ser Leu Ser Pro Tyr Glu Gln Asn
 1               5                  10                  15

cac cat cgt aag gac gtc tgc tct tcc acc acc aca acc gcc gta gat   96
His His Arg Lys Asp Val Cys Ser Ser Thr Thr Thr Thr Ala Val Asp
            20                  25                  30

gtc gcc gga gag tac tgt tac gat ccg acc gct gcc tcc gat gag tct  144
Val Ala Gly Glu Tyr Cys Tyr Asp Pro Thr Ala Ala Ser Asp Glu Ser
        35                  40                  45

tca gcc atc caa aca tcg ttt cct tct ccc ttt ggt gtc gtc ctc gat  192
Ser Ala Ile Gln Thr Ser Phe Pro Ser Pro Phe Gly Val Val Leu Asp
    50                  55                  60

gct ttc acc aga gac aac aat agt cac tcc cga gat tgg gac atc aat  240
Ala Phe Thr Arg Asp Asn Asn Ser His Ser Arg Asp Trp Asp Ile Asn
65                  70                  75                  80

ggt agt gca tgt aat aac atc cac aat gat gag caa gat gga cca aaa  288
Gly Ser Ala Cys Asn Asn Ile His Asn Asp Glu Gln Asp Gly Pro Lys
                85                  90                  95

ctt gag aat ttc ctt ggc cgc acc acc acg att tac aac acc aac gaa  336
Leu Glu Asn Phe Leu Gly Arg Thr Thr Thr Ile Tyr Asn Thr Asn Glu
            100                 105                 110

aac gtt gga gat atc gat gga agt ggg tgt tat gga gga gga gac ggt  384
Asn Val Gly Asp Ile Asp Gly Ser Gly Cys Tyr Gly Gly Gly Asp Gly
        115                 120                 125

ggt ggt ggc tca cta gga ctt tcg atg ata aag aca tgg ctg aga aat  432
Gly Gly Gly Ser Leu Gly Leu Ser Met Ile Lys Thr Trp Leu Arg Asn
    130                 135                 140

caa ccc gtg gat aat gtt gat aat caa gaa aat ggc aat ggt gca aaa  480
Gln Pro Val Asp Asn Val Asp Asn Gln Glu Asn Gly Asn Gly Ala Lys
145                 150                 155                 160

ggc ctg tcc ctc tca atg aac tca tct act tct tgt gat aac aac aac  528
Gly Leu Ser Leu Ser Met Asn Ser Ser Thr Ser Cys Asp Asn Asn Asn
                165                 170                 175
```

```
tac agc agt aac aac ctt gtt gcc caa ggg aag act att gat gat agc   576
Tyr Ser Ser Asn Asn Leu Val Ala Gln Gly Lys Thr Ile Asp Asp Ser
            180                 185                 190

gtt gaa gct aca ccg aag aaa act att gag agt ttt gga cag agg acg   624
Val Glu Ala Thr Pro Lys Lys Thr Ile Glu Ser Phe Gly Gln Arg Thr
        195                 200                 205

tct ata tac cgc ggt gtt aca agg cat cgg tgg aca gga aga tat gag   672
Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu
    210                 215                 220

gca cat tta tgg gat aat agt tgt aaa cga gaa ggc caa acg cgc aaa   720
Ala His Leu Trp Asp Asn Ser Cys Lys Arg Glu Gly Gln Thr Arg Lys
225                 230                 235                 240

gga aga caa gtt tat ttg gga ggt tat gac aaa gaa gaa aaa gca gct   768
Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala
                245                 250                 255

agg gct tat gat tta gcc gca ctc aag tat tgg gga acc acc act act   816
Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr
            260                 265                 270

act aac ttc ccc atg agc gaa tat gag aaa gag ata gaa gag atg aag   864
Thr Asn Phe Pro Met Ser Glu Tyr Glu Lys Glu Ile Glu Glu Met Lys
        275                 280                 285

cac atg aca agg caa gag tat gtt gcc tca ctt cgc agg aaa agt agt   912
His Met Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser
    290                 295                 300

ggt ttc tct cgt ggt gca tcg att tat cgt gga gta aca aga cat cac   960
Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His
305                 310                 315                 320

caa cat gga aga tgg caa gct agg ata gga aga gtc gcc ggt aac aaa  1008
Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys
                325                 330                 335

gac ctc tac ttg gga act ttt ggc aca caa gaa gaa gct gca gag gca  1056
Asp Leu Tyr Leu Gly Thr Phe Gly Thr Gln Glu Glu Ala Ala Glu Ala
            340                 345                 350

tac gac att gcg gcc atc aaa ttc aga gga tta acc gca gtg act aac  1104
Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Thr Ala Val Thr Asn
        355                 360                 365

ttc gac atg aac aga tac aac gtt aaa gca atc ctc gaa agc cct agt  1152
Phe Asp Met Asn Arg Tyr Asn Val Lys Ala Ile Leu Glu Ser Pro Ser
    370                 375                 380

ctt cct att ggt agc gcc gca aaa cgt ctc aag gag gct aac cgt ccg  1200
Leu Pro Ile Gly Ser Ala Ala Lys Arg Leu Lys Glu Ala Asn Arg Pro
385                 390                 395                 400

gtt cca agt atg atg atg atc agt aat aac gtt tca gag agt gag aat  1248
Val Pro Ser Met Met Met Ile Ser Asn Asn Val Ser Glu Ser Glu Asn
                405                 410                 415

aat gct agc ggt tgg caa aac gct gcg gtt cag cat cat cag gga gta  1296
Asn Ala Ser Gly Trp Gln Asn Ala Ala Val Gln His His Gln Gly Val
            420                 425                 430

gat ttg agc tta ttg cag caa cat caa gag agg tac aat ggt tat tat  1344
Asp Leu Ser Leu Leu Gln Gln His Gln Glu Arg Tyr Asn Gly Tyr Tyr
        435                 440                 445
```

```
tac aat gga gga aac ttg tct tcg gag agt gct agg gct tgt ttc aaa   1392
Tyr Asn Gly Gly Asn Leu Ser Ser Glu Ser Ala Arg Ala Cys Phe Lys
    450                 455                 460

caa gag gat gat caa cac cat ttc ttg agc aac acg cag agc ctc atg   1440
Gln Glu Asp Asp Gln His His Phe Leu Ser Asn Thr Gln Ser Leu Met
465                 470                 475                 480

act aat atc gat cat caa agt tct gtt tca gat gat tcg gtt act gtt   1488
Thr Asn Ile Asp His Gln Ser Ser Val Ser Asp Asp Ser Val Thr Val
                485                 490                 495

tgt gga aat gtt gtt ggt tat ggt ggt tat caa gga ttt gca gcc ccg   1536
Cys Gly Asn Val Val Gly Tyr Gly Gly Tyr Gln Gly Phe Ala Ala Pro
            500                 505                 510

gtt aac tgc gat gcc tac gct gct agt gag ttt gac tat aac gca aga   1584
Val Asn Cys Asp Ala Tyr Ala Ala Ser Glu Phe Asp Tyr Asn Ala Arg
        515                 520                 525

aac cat tat tac ttt gct cag cag cag cag acc cag cat tcg cca gga   1632
Asn His Tyr Tyr Phe Ala Gln Gln Gln Gln Thr Gln His Ser Pro Gly
    530                 535                 540

gga gat ttt ccc gcg gca atg acg aat aat gtt ggc tct aat atg tat   1680
Gly Asp Phe Pro Ala Ala Met Thr Asn Asn Val Gly Ser Asn Met Tyr
545                 550                 555                 560

tac cat ggg gaa ggt ggt gga gaa gtt gct cca aca ttt aca gtt tgg   1728
Tyr His Gly Glu Gly Gly Gly Glu Val Ala Pro Thr Phe Thr Val Trp
                565                 570                 575

aac gac aat tag                                                  1740
Asn Asp Asn
```

<210> 79
<211> 579
<212> PRT
<213> Brassica napus

<400> 79

```
Met Asn Asn Asn Trp Leu Gly Phe Ser Leu Ser Pro Tyr Glu Gln Asn
 1               5                  10                  15
His His Arg Lys Asp Val Cys Ser Ser Thr Thr Thr Thr Ala Val Asp
            20                  25                  30
Val Ala Gly Glu Tyr Cys Tyr Asp Pro Thr Ala Ala Ser Asp Glu Ser
        35                  40                  45
Ser Ala Ile Gln Thr Ser Phe Pro Ser Pro Phe Gly Val Val Leu Asp
    50                  55                  60
Ala Phe Thr Arg Asp Asn Asn Ser His Ser Arg Asp Trp Asp Ile Asn
65                  70                  75                  80
Gly Ser Ala Cys Asn Asn Ile His Asn Asp Glu Gln Asp Gly Pro Lys
                85                  90                  95
Leu Glu Asn Phe Leu Gly Arg Thr Thr Thr Ile Tyr Asn Thr Asn Glu
            100                 105                 110
Asn Val Gly Asp Ile Asp Gly Ser Gly Cys Tyr Gly Gly Gly Asp Gly
        115                 120                 125
Gly Gly Gly Ser Leu Gly Leu Ser Met Ile Lys Thr Trp Leu Arg Asn
    130                 135                 140
Gln Pro Val Asp Asn Val Asp Asn Gln Glu Asn Gly Asn Gly Ala Lys
145                 150                 155                 160
Gly Leu Ser Leu Ser Met Asn Ser Ser Thr Ser Cys Asp Asn Asn Asn
                165                 170                 175
Tyr Ser Ser Asn Asn Leu Val Ala Gln Gly Lys Thr Ile Asp Asp Ser
            180                 185                 190
Val Glu Ala Thr Pro Lys Lys Thr Ile Glu Ser Phe Gly Gln Arg Thr
```

```
        195                 200                 205
Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu
    210                 215                 220
Ala His Leu Trp Asp Asn Ser Cys Lys Arg Glu Gly Gln Thr Arg Lys
225                 230                 235                 240
Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala
                245                 250                 255
Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr
            260                 265                 270
Thr Asn Phe Pro Met Ser Glu Tyr Glu Lys Glu Ile Glu Glu Met Lys
        275                 280                 285
His Met Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser
    290                 295                 300
Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His
305                 310                 315                 320
Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys
                325                 330                 335
Asp Leu Tyr Leu Gly Thr Phe Gly Thr Gln Glu Glu Ala Ala Glu Ala
            340                 345                 350
Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Thr Ala Val Thr Asn
        355                 360                 365
Phe Asp Met Asn Arg Tyr Asn Val Lys Ala Ile Leu Glu Ser Pro Ser
    370                 375                 380
Leu Pro Ile Gly Ser Ala Ala Lys Arg Leu Lys Glu Ala Asn Arg Pro
385                 390                 395                 400
Val Pro Ser Met Met Met Ile Ser Asn Asn Val Ser Glu Ser Glu Asn
                405                 410                 415
Asn Ala Ser Gly Trp Gln Asn Ala Ala Val Gln His His Gln Gly Val
            420                 425                 430
Asp Leu Ser Leu Leu Gln Gln His Gln Glu Arg Tyr Asn Gly Tyr Tyr
        435                 440                 445
Tyr Asn Gly Gly Asn Leu Ser Ser Glu Ser Ala Arg Ala Cys Phe Lys
    450                 455                 460
Gln Glu Asp Asp Gln His His Phe Leu Ser Asn Thr Gln Ser Leu Met
465                 470                 475                 480
Thr Asn Ile Asp His Gln Ser Ser Val Ser Asp Asp Ser Val Thr Val
                485                 490                 495
Cys Gly Asn Val Val Gly Tyr Gly Gly Tyr Gln Gly Phe Ala Ala Pro
            500                 505                 510
Val Asn Cys Asp Ala Tyr Ala Ala Ser Glu Phe Asp Tyr Asn Ala Arg
        515                 520                 525
Asn His Tyr Tyr Phe Ala Gln Gln Gln Gln Thr Gln His Ser Pro Gly
    530                 535                 540
Gly Asp Phe Pro Ala Ala Met Thr Asn Asn Val Gly Ser Asn Met Tyr
545                 550                 555                 560
Tyr His Gly Glu Gly Gly Gly Glu Val Ala Pro Thr Phe Thr Val Trp
                565                 570                 575
Asn Asp Asn
```

<210> 80
<211> 2070
<212> DNA
<213> Medicago truncatula

<220>
<221> CDS
<222> (1)...(2070)

<400> 80

```
atg gcc tct atg aac ttg tta ggt ttc tct cta tct cca caa gaa caa   48
Met Ala Ser Met Asn Leu Leu Gly Phe Ser Leu Ser Pro Gln Glu Gln
 1               5                  10                  15

cat cca tca aca caa gat caa acg gtg gct tcc cgt ttt ggg ttc aac   96
His Pro Ser Thr Gln Asp Gln Thr Val Ala Ser Arg Phe Gly Phe Asn
            20                  25                  30
```

```
cct aat gaa atc tca ggc tct gat gtt caa gga gat cac tgc tat gat   144
Pro Asn Glu Ile Ser Gly Ser Asp Val Gln Gly Asp His Cys Tyr Asp
          35                  40                  45

ctc tct tct cac aca act cct cat cat tca ctc aac ctt tct cat cct   192
Leu Ser Ser His Thr Thr Pro His His Ser Leu Asn Leu Ser His Pro
      50                  55                  60

ttt tcc att tat gaa gct ttc cac aca aat aac aac att cac acc act   240
Phe Ser Ile Tyr Glu Ala Phe His Thr Asn Asn Asn Ile His Thr Thr
 65                  70                  75                  80

caa gat tgg aag gag aac tac aac aac caa aac cta cta ttg gga aca   288
Gln Asp Trp Lys Glu Asn Tyr Asn Asn Gln Asn Leu Leu Leu Gly Thr
                 85                  90                  95

tca tgc atg aac caa aat gtg aac aac aac aac caa caa gca caa cca   336
Ser Cys Met Asn Gln Asn Val Asn Asn Asn Asn Gln Gln Ala Gln Pro
            100                 105                 110

aag cta gaa aac ttc ctc ggt gga cac tct ttc acc gac cat caa gaa   384
Lys Leu Glu Asn Phe Leu Gly Gly His Ser Phe Thr Asp His Gln Glu
        115                 120                 125

tac ggt ggt agc aac tca tac tct tca tta cac ctc cca cct cat cag   432
Tyr Gly Gly Ser Asn Ser Tyr Ser Ser Leu His Leu Pro Pro His Gln
    130                 135                 140

ccg gaa gca tcc tgt ggc ggt ggt gat ggt agt aca agt aac aat aac   480
Pro Glu Ala Ser Cys Gly Gly Gly Asp Gly Ser Thr Ser Asn Asn Asn
145                 150                 155                 160

tca ata ggt tta tct atg ata aaa aca tgg ctc aga aac caa cca cca   528
Ser Ile Gly Leu Ser Met Ile Lys Thr Trp Leu Arg Asn Gln Pro Pro
                165                 170                 175

cca cca gaa aac aac aac aat aac aac aat gaa agt ggt gca cgt gtg   576
Pro Pro Glu Asn Asn Asn Asn Asn Asn Asn Glu Ser Gly Ala Arg Val
            180                 185                 190

cag aca cta tca ctt tct atg agt act ggc tca cag tca agt tca tct   624
Gln Thr Leu Ser Leu Ser Met Ser Thr Gly Ser Gln Ser Ser Ser Ser
        195                 200                 205

gtg cct ctt ctc aat gca aat gtg atg agt ggt gag att tcc tca tcg   672
Val Pro Leu Leu Asn Ala Asn Val Met Ser Gly Glu Ile Ser Ser Ser
    210                 215                 220

gaa aac aaa caa cca ccc aca act gca gtt gta ctt gat agc aac caa   720
Glu Asn Lys Gln Pro Pro Thr Thr Ala Val Val Leu Asp Ser Asn Gln
225                 230                 235                 240

aca agt gtc gtt gaa agt gct gtg cct aga aaa tcc gtt gat aca ttt   768
Thr Ser Val Val Glu Ser Ala Val Pro Arg Lys Ser Val Asp Thr Phe
                245                 250                 255

gga caa aga act tcc att tac cgt ggt gta aca agg cat aga tgg aca   816
Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr
            260                 265                 270

ggg aga tat gaa gct cac ctt tgg gat aat agt tgt aga aga gag ggg   864
Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly
        275                 280                 285

cag act cgc aaa gga agg caa gtt tac ttg gga ggt tat gac aaa gaa   912
Gln Thr Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu
    290                 295                 300
```

```
gaa aaa gca gct aga gcc tat gat ttg gca gca cta aaa tat tgg gga   960
Glu Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly
305                 310                 315                 320

aca act act aca aca aat ttt cca att agc cat tat gaa aaa gaa gtg   1008
Thr Thr Thr Thr Thr Asn Phe Pro Ile Ser His Tyr Glu Lys Glu Val
                325                 330                 335

gaa gaa atg aag cat atg aca agg caa gag tac gtt gcg tca ttg aga   1056
Glu Glu Met Lys His Met Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg
            340                 345                 350

agg aaa agt agt ggt ttt tca cga ggt gca tcc att tac cga gga gta   1104
Arg Lys Ser Ser Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val
        355                 360                 365

aca aga cat cat caa cat ggt aga tgg caa gct agg att gga aga gtt   1152
Thr Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val
    370                 375                 380

gca ggc aac aaa gat ctc tac cta gga act ttc agc act caa gaa gag   1200
Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe Ser Thr Gln Glu Glu
385                 390                 395                 400

gca gca gag gca tat gat gtg gca gca ata aaa ttc aga gga ctg agt   1248
Ala Ala Glu Ala Tyr Asp Val Ala Ala Ile Lys Phe Arg Gly Leu Ser
                405                 410                 415

gca gtt aca aac ttt gac atg agc aga tat gat gtc aaa acc ata ctt   1296
Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Thr Ile Leu
            420                 425                 430

gag agc agc aca tta cca att ggt ggt gct gca aag cgt tta aaa gac   1344
Glu Ser Ser Thr Leu Pro Ile Gly Gly Ala Ala Lys Arg Leu Lys Asp
        435                 440                 445

atg gag caa gtt gaa ttg aat cat gtg aat gtt gat att agc cat aga   1392
Met Glu Gln Val Glu Leu Asn His Val Asn Val Asp Ile Ser His Arg
    450                 455                 460

act gaa caa gat cat agc atc atc aac aac act tcc cat tta aca gaa   1440
Thr Glu Gln Asp His Ser Ile Ile Asn Asn Thr Ser His Leu Thr Glu
465                 470                 475                 480

caa gcc atc tat gca gca aca aat gca tct aat tgg cat gca ctt tca   1488
Gln Ala Ile Tyr Ala Ala Thr Asn Ala Ser Asn Trp His Ala Leu Ser
                485                 490                 495

ttc caa cat caa caa cca cat cat cat tac aat gcc aac aac atg cag   1536
Phe Gln His Gln Gln Pro His His His Tyr Asn Ala Asn Asn Met Gln
            500                 505                 510

tta cag aat tat cct tat gga act caa act caa aag ctt tgg tgc aaa   1584
Leu Gln Asn Tyr Pro Tyr Gly Thr Gln Thr Gln Lys Leu Trp Cys Lys
        515                 520                 525

caa gaa caa gat tct gat gat cat agt act tat act act gct act gat   1632
Gln Glu Gln Asp Ser Asp Asp His Ser Thr Tyr Thr Thr Ala Thr Asp
    530                 535                 540

att cat caa cta cag tta ggg aat aat aat aac aat act cac aat ttc   1680
Ile His Gln Leu Gln Leu Gly Asn Asn Asn Asn Asn Thr His Asn Phe
545                 550                 555                 560

ttt ggt tta caa aat atc atg agt atg gat tct gct tcc atg gat aat   1728
Phe Gly Leu Gln Asn Ile Met Ser Met Asp Ser Ala Ser Met Asp Asn
                565                 570                 575
```

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agt tct gga tct aat tct gtt gtt tat ggt ggt gga gat cat ggt ggt    1776
Ser Ser Gly Ser Asn Ser Val Val Tyr Gly Gly Gly Asp His Gly Gly
            580                 585                 590

tat gga gga aat ggt gga tat atg att cca atg gct att gca aat gat    1824
Tyr Gly Gly Asn Gly Gly Tyr Met Ile Pro Met Ala Ile Ala Asn Asp
        595                 600                 605

ggt aac caa aat cca aga agc aac aac aat ttt ggt gag agt gag att    1872
Gly Asn Gln Asn Pro Arg Ser Asn Asn Asn Phe Gly Glu Ser Glu Ile
    610                 615                 620

aaa gga ttt ggt tat gaa aat gtt ttt ggg act act act gat cct tat    1920
Lys Gly Phe Gly Tyr Glu Asn Val Phe Gly Thr Thr Thr Asp Pro Tyr
625                 630                 635                 640

cat gca cag gca gca agg aac ttg tac tat cag cca caa caa tta tct    1968
His Ala Gln Ala Ala Arg Asn Leu Tyr Tyr Gln Pro Gln Gln Leu Ser
                645                 650                 655

gtt gat caa gga tca aat tgg gtt cca act gct att cca aca ctt gct    2016
Val Asp Gln Gly Ser Asn Trp Val Pro Thr Ala Ile Pro Thr Leu Ala
            660                 665                 670

cca agg act acc aat gtc tct cta tgt cct cct ttc act ttg ttg cat    2064
Pro Arg Thr Thr Asn Val Ser Leu Cys Pro Pro Phe Thr Leu Leu His
        675                 680                 685

gaa tag                                                            2070
Glu
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<210> 81
<211> 689
<212> PRT
<213> Medicago truncatula

<400> 81

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Met Ala Ser Met Asn Leu Leu Gly Phe Ser Leu Ser Pro Gln Glu Gln
 1               5                  10                  15
His Pro Ser Thr Gln Asp Gln Thr Val Ala Ser Arg Phe Gly Phe Asn
            20                  25                  30
Pro Asn Glu Ile Ser Gly Ser Asp Val Gln Gly Asp His Cys Tyr Asp
        35                  40                  45
Leu Ser Ser His Thr Thr Pro His His Ser Leu Asn Leu Ser His Pro
    50                  55                  60
Phe Ser Ile Tyr Glu Ala Phe His Thr Asn Asn Asn Ile His Thr Thr
65                  70                  75                  80
Gln Asp Trp Lys Glu Asn Tyr Asn Asn Gln Asn Leu Leu Leu Gly Thr
                85                  90                  95
Ser Cys Met Asn Gln Asn Val Asn Asn Asn Asn Gln Gln Ala Gln Pro
            100                 105                 110
Lys Leu Glu Asn Phe Leu Gly Gly His Ser Phe Thr Asp His Gln Glu
        115                 120                 125
Tyr Gly Gly Ser Asn Ser Tyr Ser Ser Leu His Leu Pro Pro His Gln
    130                 135                 140
Pro Glu Ala Ser Cys Gly Gly Gly Asp Gly Ser Thr Ser Asn Asn Asn
145                 150                 155                 160
Ser Ile Gly Leu Ser Met Ile Lys Thr Trp Leu Arg Asn Gln Pro Pro
                165                 170                 175
Pro Pro Glu Asn Asn Asn Asn Asn Asn Asn Glu Ser Gly Ala Arg Val
            180                 185                 190
Gln Thr Leu Ser Leu Ser Met Ser Thr Gly Ser Gln Ser Ser Ser Ser
        195                 200                 205
Val Pro Leu Leu Asn Ala Asn Val Met Ser Gly Glu Ile Ser Ser Ser
    210                 215                 220
```

Glu Asn Lys Gln Pro Pro Thr Thr Ala Val Val Leu Asp Ser Asn Gln
225 230 235 240
Thr Ser Val Val Glu Ser Ala Val Pro Arg Lys Ser Val Asp Thr Phe
245 250 255
Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr
260 265 270
Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly
275 280 285
Gln Thr Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu
290 295 300
Glu Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly
305 310 315 320
Thr Thr Thr Thr Thr Asn Phe Pro Ile Ser His Tyr Glu Lys Glu Val
325 330 335
Glu Glu Met Lys His Met Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg
340 345 350
Arg Lys Ser Ser Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val
355 360 365
Thr Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val
370 375 380
Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe Ser Thr Gln Glu Glu
385 390 395 400
Ala Ala Glu Ala Tyr Asp Val Ala Ala Ile Lys Phe Arg Gly Leu Ser
405 410 415
Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Thr Ile Leu
420 425 430
Glu Ser Ser Thr Leu Pro Ile Gly Gly Ala Ala Lys Arg Leu Lys Asp
435 440 445
Met Glu Gln Val Glu Leu Asn His Val Asn Val Asp Ile Ser His Arg
450 455 460
Thr Glu Gln Asp His Ser Ile Ile Asn Asn Thr Ser His Leu Thr Glu
465 470 475 480
Gln Ala Ile Tyr Ala Ala Thr Asn Ala Ser Asn Trp His Ala Leu Ser
485 490 495
Phe Gln His Gln Gln Pro His His His Tyr Asn Ala Asn Asn Met Gln
500 505 510
Leu Gln Asn Tyr Pro Tyr Gly Thr Gln Thr Gln Lys Leu Trp Cys Lys
515 520 525
Gln Glu Gln Asp Ser Asp Asp His Ser Thr Tyr Thr Thr Ala Thr Asp
530 535 540
Ile His Gln Leu Gln Leu Gly Asn Asn Asn Asn Asn Thr His Asn Phe
545 550 555 560
Phe Gly Leu Gln Asn Ile Met Ser Met Asp Ser Ala Ser Met Asp Asn
565 570 575
Ser Ser Gly Ser Asn Ser Val Val Tyr Gly Gly Gly Asp His Gly Gly
580 585 590
Tyr Gly Gly Asn Gly Gly Tyr Met Ile Pro Met Ala Ile Ala Asn Asp
595 600 605
Gly Asn Gln Asn Pro Arg Ser Asn Asn Asn Phe Gly Glu Ser Glu Ile
610 615 620
Lys Gly Phe Gly Tyr Glu Asn Val Phe Gly Thr Thr Thr Asp Pro Tyr
625 630 635 640
His Ala Gln Ala Ala Arg Asn Leu Tyr Tyr Gln Pro Gln Gln Leu Ser
645 650 655
Val Asp Gln Gly Ser Asn Trp Val Pro Thr Ala Ile Pro Thr Leu Ala
660 665 670
Pro Arg Thr Thr Asn Val Ser Leu Cys Pro Pro Phe Thr Leu Leu His
675 680 685
Glu

<210> 82
<211> 2133
<212> DNA
<213> Glycine max

<220>

<221> CDS
<222> (1)...(2133)

<400> 82

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atg ggg tct atg aat ttg tta ggt ttt tct ctc tct cct caa gaa cac   48
Met Gly Ser Met Asn Leu Leu Gly Phe Ser Leu Ser Pro Gln Glu His
 1               5                  10                  15

cct tct agt caa gat cac tct caa acg gca cct tct cgt ttt tgc ttc   96
Pro Ser Ser Gln Asp His Ser Gln Thr Ala Pro Ser Arg Phe Cys Phe
            20                  25                  30

aac cct gat gga atc tca agc act gat gta gca gga gac tgc ttt gat   144
Asn Pro Asp Gly Ile Ser Ser Thr Asp Val Ala Gly Asp Cys Phe Asp
        35                  40                  45

ctc act tct gac tca act cct cat tta ctc aac ctt ccc tct tac ggc   192
Leu Thr Ser Asp Ser Thr Pro His Leu Leu Asn Leu Pro Ser Tyr Gly
    50                  55                  60

ata tac gaa gct ttt cat agg agc aac aat att cac acc act caa gat   240
Ile Tyr Glu Ala Phe His Arg Ser Asn Asn Ile His Thr Thr Gln Asp
65                  70                  75                  80

tgg aag gag aac tac aac agc caa aac ttg cta ttg gga act tca tgc   288
Trp Lys Glu Asn Tyr Asn Ser Gln Asn Leu Leu Leu Gly Thr Ser Cys
                85                  90                  95

agc aac caa aac atg aac cac aac cat cag caa caa caa caa caa cag   336
Ser Asn Gln Asn Met Asn His Asn His Gln Gln Gln Gln Gln Gln Gln
            100                 105                 110

cca aag ctt gaa aac ttc ctc ggt gga cac tca ttt ggt gaa cat gag   384
Pro Lys Leu Glu Asn Phe Leu Gly Gly His Ser Phe Gly Glu His Glu
        115                 120                 125

caa ccc tac ggt ggt aac tca gcc tct aca gaa tac atg ttc ccg gct   432
Gln Pro Tyr Gly Gly Asn Ser Ala Ser Thr Glu Tyr Met Phe Pro Ala
    130                 135                 140

cag ccg gta ttg gcc ggt ggc ggc ggc ggt ggt agc aat agc agc aac   480
Gln Pro Val Leu Ala Gly Gly Gly Gly Gly Gly Ser Asn Ser Ser Asn
145                 150                 155                 160

aca agc aac agt agc tcc ata ggg tta tcc atg ata aag aca tgg ttg   528
Thr Ser Asn Ser Ser Ser Ile Gly Leu Ser Met Ile Lys Thr Trp Leu
                165                 170                 175

agg aac caa cca cca cac tca gaa aac aac aat aac aac aac aat gaa   576
Arg Asn Gln Pro Pro His Ser Glu Asn Asn Asn Asn Asn Asn Asn Glu
            180                 185                 190

agt ggt ggc aat agt aga agc agt gtg cag cag act cta tca ctt tcc   624
Ser Gly Gly Asn Ser Arg Ser Ser Val Gln Gln Thr Leu Ser Leu Ser
        195                 200                 205

atg agt act ggt tca caa tca agc aca tca cta ccc ctt ctc act gct   672
Met Ser Thr Gly Ser Gln Ser Ser Thr Ser Leu Pro Leu Leu Thr Ala
    210                 215                 220

agt gtg gat aat gga gag agt tct tct gat aac aaa caa cca cat acc   720
Ser Val Asp Asn Gly Glu Ser Ser Ser Asp Asn Lys Gln Pro His Thr
225                 230                 235                 240

acg gct gca ctt gat aca acc caa acc gga gcc att gaa act gca ccc   768
Thr Ala Ala Leu Asp Thr Thr Gln Thr Gly Ala Ile Glu Thr Ala Pro
                245                 250                 255
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aga aag tcc att gac act ttt gga cag aga act tct atc tac cgt ggt    816
Arg Lys Ser Ile Asp Thr Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly
            260                 265                 270

gta aca agg cat agg tgg acg ggg agg tat gag gct cac ctg tgg gat    864
Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His Leu Trp Asp
        275                 280                 285

aat agt tgt aga aga gag gga caa act cgc aaa gga agg caa gtt tac    912
Asn Ser Cys Arg Arg Glu Gly Gln Thr Arg Lys Gly Arg Gln Val Tyr
    290                 295                 300

ttg gga ggt tat gac aaa gaa gaa aag gca gct aga gcc tac gat ttg    960
Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala Arg Ala Tyr Asp Leu
305                 310                 315                 320

gca gca cta aaa tac tgg gga aca act acg aca aca aat ttt cca att   1008
Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr Thr Asn Phe Pro Ile
                325                 330                 335

agc cac tat gag aaa gag ttg gaa gaa atg aag cac atg act agg caa   1056
Ser His Tyr Glu Lys Glu Leu Glu Glu Met Lys His Met Thr Arg Gln
            340                 345                 350

gag tac gtt gcg tca ttg aga agg aag agt agt ggg ttt tct cgc ggg   1104
Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser Gly Phe Ser Arg Gly
        355                 360                 365

gca tcc att tat cga ggt gtg acg aga cac cat caa cat gga aga tgg   1152
Ala Ser Ile Tyr Arg Gly Val Thr Arg His His Gln His Gly Arg Trp
    370                 375                 380

caa gcg agg att gga aga gtt gct ggc aac aag gat ctc tac ttg gga   1200
Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly
385                 390                 395                 400

act ttc agc acc caa gag gag gca gca gaa gca tat gat gta gca gca   1248
Thr Phe Ser Thr Gln Glu Glu Ala Ala Glu Ala Tyr Asp Val Ala Ala
                405                 410                 415

atc aaa ttc aga gga cta agt gct gtt aca aac ttt gac atg agc aga   1296
Ile Lys Phe Arg Gly Leu Ser Ala Val Thr Asn Phe Asp Met Ser Arg
            420                 425                 430

tat gac gtg aaa agc ata ctt gag agc acc act ttg cca att ggt ggt   1344
Tyr Asp Val Lys Ser Ile Leu Glu Ser Thr Thr Leu Pro Ile Gly Gly
        435                 440                 445

gct gca aag cgt ttg aag gat atg gag cag gtg gaa ctg agg gtg gag   1392
Ala Ala Lys Arg Leu Lys Asp Met Glu Gln Val Glu Leu Arg Val Glu
    450                 455                 460

aat gtt cat aga gca gat caa gaa gat cat agt agc atc atg aac tct   1440
Asn Val His Arg Ala Asp Gln Glu Asp His Ser Ser Ile Met Asn Ser
465                 470                 475                 480

cac tta act caa gga atc att aac aac tat gca gca gga gga aca aca   1488
His Leu Thr Gln Gly Ile Ile Asn Asn Tyr Ala Ala Gly Gly Thr Thr
                485                 490                 495

gcg act cat cat cat aac tgg cac aat gct ctt gca ttc cac caa cct   1536
Ala Thr His His His Asn Trp His Asn Ala Leu Ala Phe His Gln Pro
            500                 505                 510

caa cct tgc acc acc ata cac tac cct tat gga caa aga att aat tgg   1584
Gln Pro Cys Thr Thr Ile His Tyr Pro Tyr Gly Gln Arg Ile Asn Trp
        515                 520                 525
```

```
tgc aag caa gaa caa gac aac tct gat gcc tct cac tct ttg tct tat    1632
Cys Lys Gln Glu Gln Asp Asn Ser Asp Ala Ser His Ser Leu Ser Tyr
    530                 535                 540

tca gat att cat caa cta cag cta ggg aac aat ggc aca cac aac ttc    1680
Ser Asp Ile His Gln Leu Gln Leu Gly Asn Asn Gly Thr His Asn Phe
545                 550                 555                 560

ttt cac aca aat tca ggg ttg cac cct atg tta agc atg gat tct gct    1728
Phe His Thr Asn Ser Gly Leu His Pro Met Leu Ser Met Asp Ser Ala
                565                 570                 575

tcc att gac aat agc tct tca tct aac tct gtt gtt tat gat ggt tat    1776
Ser Ile Asp Asn Ser Ser Ser Ser Asn Ser Val Val Tyr Asp Gly Tyr
            580                 585                 590

gga ggt ggt ggg ggc tat aat gtg att cct atg ggg act act act act    1824
Gly Gly Gly Gly Gly Tyr Asn Val Ile Pro Met Gly Thr Thr Thr Thr
        595                 600                 605

gtt gtt gca aat gat ggt gat caa aat cca aga agc aat cat ggt ttt    1872
Val Val Ala Asn Asp Gly Asp Gln Asn Pro Arg Ser Asn His Gly Phe
    610                 615                 620

ggt gat aat gag ata aag gca ctt ggt tat gaa agt gtg tat ggt tct    1920
Gly Asp Asn Glu Ile Lys Ala Leu Gly Tyr Glu Ser Val Tyr Gly Ser
625                 630                 635                 640

aca act gat cct tat cat gca cat gca agg aac ttg tat tat ctt act    1968
Thr Thr Asp Pro Tyr His Ala His Ala Arg Asn Leu Tyr Tyr Leu Thr
                645                 650                 655

caa cag caa cca tct tct gtt gat gca gtg aag gct agt gca tat gat    2016
Gln Gln Gln Pro Ser Ser Val Asp Ala Val Lys Ala Ser Ala Tyr Asp
            660                 665                 670

caa gga tct gca tgc aat act tgg gtt cca act gct att cca act cat    2064
Gln Gly Ser Ala Cys Asn Thr Trp Val Pro Thr Ala Ile Pro Thr His
        675                 680                 685

gca cca agg tct agt act agt atg gct ctc tgc cat ggt gct acg ccc    2112
Ala Pro Arg Ser Ser Thr Ser Met Ala Leu Cys His Gly Ala Thr Pro
    690                 695                 700

ttc tct tta ttg cat gaa tag                                       2133
Phe Ser Leu Leu His Glu
705                 710
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<210> 83
<211> 710
<212> PRT
<213> Glycine max

<400> 83

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Met Gly Ser Met Asn Leu Leu Gly Phe Ser Leu Ser Pro Gln Glu His
 1               5                  10                  15
Pro Ser Ser Gln Asp His Ser Gln Thr Ala Pro Ser Arg Phe Cys Phe
            20                  25                  30
Asn Pro Asp Gly Ile Ser Ser Thr Asp Val Ala Gly Asp Cys Phe Asp
        35                  40                  45
Leu Thr Ser Asp Ser Thr Pro His Leu Leu Asn Leu Pro Ser Tyr Gly
    50                  55                  60
Ile Tyr Glu Ala Phe His Arg Ser Asn Asn Ile His Thr Thr Gln Asp
65                  70                  75                  80
Trp Lys Glu Asn Tyr Asn Ser Gln Asn Leu Leu Leu Gly Thr Ser Cys
                85                  90                  95
Ser Asn Gln Asn Met Asn His Asn His Gln Gln Gln Gln Gln Gln Gln
```

```
                100                 105                 110
Pro Lys Leu Glu Asn Phe Leu Gly Gly His Ser Phe Gly Glu His Glu
            115                 120                 125
Gln Pro Tyr Gly Gly Asn Ser Ala Ser Thr Glu Tyr Met Phe Pro Ala
        130                 135                 140
Gln Pro Val Leu Ala Gly Gly Gly Gly Gly Gly Ser Asn Ser Ser Asn
145                 150                 155                 160
Thr Ser Asn Ser Ser Ser Ile Gly Leu Ser Met Ile Lys Thr Trp Leu
                165                 170                 175
Arg Asn Gln Pro Pro His Ser Glu Asn Asn Asn Asn Asn Asn Asn Glu
            180                 185                 190
Ser Gly Gly Asn Ser Arg Ser Ser Val Gln Gln Thr Leu Ser Leu Ser
            195                 200                 205
Met Ser Thr Gly Ser Gln Ser Ser Thr Ser Leu Pro Leu Leu Thr Ala
        210                 215                 220
Ser Val Asp Asn Gly Glu Ser Ser Ser Asp Asn Lys Gln Pro His Thr
225                 230                 235                 240
Thr Ala Ala Leu Asp Thr Thr Gln Thr Gly Ala Ile Glu Thr Ala Pro
                245                 250                 255
Arg Lys Ser Ile Asp Thr Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly
            260                 265                 270
Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His Leu Trp Asp
            275                 280                 285
Asn Ser Cys Arg Arg Glu Gly Gln Thr Arg Lys Gly Arg Gln Val Tyr
        290                 295                 300
Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala Arg Ala Tyr Asp Leu
305                 310                 315                 320
Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr Thr Asn Phe Pro Ile
                325                 330                 335
Ser His Tyr Glu Lys Glu Leu Glu Glu Met Lys His Met Thr Arg Gln
            340                 345                 350
Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser Gly Phe Ser Arg Gly
            355                 360                 365
Ala Ser Ile Tyr Arg Gly Val Thr Arg His His Gln His Gly Arg Trp
        370                 375                 380
Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly
385                 390                 395                 400
Thr Phe Ser Thr Gln Glu Glu Ala Ala Glu Ala Tyr Asp Val Ala Ala
                405                 410                 415
Ile Lys Phe Arg Gly Leu Ser Ala Val Thr Asn Phe Asp Met Ser Arg
            420                 425                 430
Tyr Asp Val Lys Ser Ile Leu Glu Ser Thr Thr Leu Pro Ile Gly Gly
            435                 440                 445
Ala Ala Lys Arg Leu Lys Asp Met Glu Gln Val Glu Leu Arg Val Glu
        450                 455                 460
Asn Val His Arg Ala Asp Gln Glu Asp His Ser Ser Ile Met Asn Ser
465                 470                 475                 480
His Leu Thr Gln Gly Ile Ile Asn Asn Tyr Ala Ala Gly Gly Thr Thr
                485                 490                 495
Ala Thr His His His Asn Trp His Asn Ala Leu Ala Phe His Gln Pro
            500                 505                 510
Gln Pro Cys Thr Thr Ile His Tyr Pro Tyr Gly Gln Arg Ile Asn Trp
            515                 520                 525
Cys Lys Gln Glu Gln Asp Asn Ser Asp Ala Ser His Ser Leu Ser Tyr
        530                 535                 540
Ser Asp Ile His Gln Leu Gln Leu Gly Asn Asn Gly Thr His Asn Phe
545                 550                 555                 560
Phe His Thr Asn Ser Gly Leu His Pro Met Leu Ser Met Asp Ser Ala
                565                 570                 575
Ser Ile Asp Asn Ser Ser Ser Ser Asn Ser Val Val Tyr Asp Gly Tyr
            580                 585                 590
Gly Gly Gly Gly Gly Tyr Asn Val Ile Pro Met Gly Thr Thr Thr Thr
            595                 600                 605
Val Val Ala Asn Asp Gly Asp Gln Asn Pro Arg Ser Asn His Gly Phe
        610                 615                 620
Gly Asp Asn Glu Ile Lys Ala Leu Gly Tyr Glu Ser Val Tyr Gly Ser
625                 630                 635                 640
Thr Thr Asp Pro Tyr His Ala His Ala Arg Asn Leu Tyr Tyr Leu Thr
```

```
                    645                 650                 655
Gln Gln Gln Pro Ser Ser Val Asp Ala Val Lys Ala Ser Ala Tyr Asp
                660                 665                 670
Gln Gly Ser Ala Cys Asn Thr Trp Val Pro Thr Ala Ile Pro Thr His
            675                 680                 685
Ala Pro Arg Ser Ser Thr Ser Met Ala Leu Cys His Gly Ala Thr Pro
    690                 695                 700
Phe Ser Leu Leu His Glu
705                 710


<210> 84
<211> 1932
<212> DNA
<213> Vitis vinifera

<220>
<221> CDS
<222> (1)...(1932)

<400> 84
atg gct tcc atg aac aac tgg ttg ggt ttc tct ttg tcc cct cga gaa   48
Met Ala Ser Met Asn Asn Trp Leu Gly Phe Ser Leu Ser Pro Arg Glu
 1               5                  10                  15

ctt cca cca cag cct gaa aat cac tca cag aac agt gtc tct aga ctt   96
Leu Pro Pro Gln Pro Glu Asn His Ser Gln Asn Ser Val Ser Arg Leu
            20                  25                  30

ggt ttc aac tct gat gaa atc tct ggg act gat gtg tca ggt gag tgt   144
Gly Phe Asn Ser Asp Glu Ile Ser Gly Thr Asp Val Ser Gly Glu Cys
        35                  40                  45

ttt gat ctc act tca gat tcc act gct ccc tct ctc aac ctc cct ccc   192
Phe Asp Leu Thr Ser Asp Ser Thr Ala Pro Ser Leu Asn Leu Pro Pro
    50                  55                  60

cct ttt ggg ata ctt gaa gca ttc aac agg aat aat cag ccc caa gat   240
Pro Phe Gly Ile Leu Glu Ala Phe Asn Arg Asn Asn Gln Pro Gln Asp
65                  70                  75                  80

act aac tac aaa acc acc act tct gag ctc tcc atg ctc atg ggt agt   288
Thr Asn Tyr Lys Thr Thr Thr Ser Glu Leu Ser Met Leu Met Gly Ser
                85                  90                  95

tca tgc agt agt cat cat aac ctc gaa aac caa gaa ccc aaa ctt gaa   336
Ser Cys Ser Ser His His Asn Leu Glu Asn Gln Glu Pro Lys Leu Glu
            100                 105                 110

aat ttc ctg ggc tgc cgc tct ttt gct gat cat gag cag aaa ctt caa   384
Asn Phe Leu Gly Cys Arg Ser Phe Ala Asp His Glu Gln Lys Leu Gln
        115                 120                 125

ggg tac tac att tcc att ggt tta tcc atg atc aag aca tgg ctg cgg   432
Gly Tyr Tyr Ile Ser Ile Gly Leu Ser Met Ile Lys Thr Trp Leu Arg
    130                 135                 140

aac caa cct gca ccc acc cat cag gat aac aac aag agt act gat act   480
Asn Gln Pro Ala Pro Thr His Gln Asp Asn Asn Lys Ser Thr Asp Thr
145                 150                 155                 160

ggg cct gtc ggt gga gcc gcc gct ggg aac cta ccc aat gca cag acc   528
Gly Pro Val Gly Gly Ala Ala Ala Gly Asn Leu Pro Asn Ala Gln Thr
                165                 170                 175

tta tcg ttg tcc atg agc acc ggc tcg cac cag acc ggt gcc att gaa   576
Leu Ser Leu Ser Met Ser Thr Gly Ser His Gln Thr Gly Ala Ile Glu
            180                 185                 190
```

```
acg gtg cca agg aag tcc att gat aca ttt gga cag agg aca tcc ata   624
Thr Val Pro Arg Lys Ser Ile Asp Thr Phe Gly Gln Arg Thr Ser Ile
        195                 200                 205

tac cgt ggt gta aca agg cat aga tgg acg ggt aga tat gag gct cat   672
Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His
    210                 215                 220

cta tgg gac aac agt tgc aga aga gaa gga caa act cga aag gga agg   720
Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln Thr Arg Lys Gly Arg
225                 230                 235                 240

caa gtt tat tta ggt ggt tat gac aaa gaa gaa aag gca gct agg gct   768
Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala Arg Ala
                245                 250                 255

tac gat tta gca gca ctg aag tat tgg ggt acc acc acc aca aca aat   816
Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr Thr Asn
            260                 265                 270

ttc cct att agc aac tat gaa aaa gag ata gag gag atg aag cac atg   864
Phe Pro Ile Ser Asn Tyr Glu Lys Glu Ile Glu Glu Met Lys His Met
        275                 280                 285

aca agg cag gag tac gta gca tct ctg cga agg aag agt agc ggg ttt   912
Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser Gly Phe
    290                 295                 300

tct cgt gga gca tcc ata tat aga gga gtg acc aga cac cat cag cat   960
Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His Gln His
305                 310                 315                 320

ggg aga tgg cag gca agg att gga aga gtc gca ggc aac aaa gat ctt  1008
Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu
                325                 330                 335

tac ttg gga act ttc agc acc caa gag gaa gca gca gag gcc tat gac  1056
Tyr Leu Gly Thr Phe Ser Thr Gln Glu Glu Ala Ala Glu Ala Tyr Asp
            340                 345                 350

att gct gcc att aag ttt cga gga ttg aat gcg gtg acc aac ttt gat  1104
Ile Ala Ala Ile Lys Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Asp
        355                 360                 365

atg agt aga tat gat gtt aat agc att cta gag agc agt acc ttg ccg  1152
Met Ser Arg Tyr Asp Val Asn Ser Ile Leu Glu Ser Ser Thr Leu Pro
    370                 375                 380

att ggt gga gct gca aag cgg ttg aaa gat gct gag cag gct gaa atg  1200
Ile Gly Gly Ala Ala Lys Arg Leu Lys Asp Ala Glu Gln Ala Glu Met
385                 390                 395                 400

act ata gat gga cag agg aca gac gat gag atg agc tca cag ctg act  1248
Thr Ile Asp Gly Gln Arg Thr Asp Asp Glu Met Ser Ser Gln Leu Thr
                405                 410                 415

gat gga atc aac aac tat gga gca cac cac cat ggc tgg cct act gtt  1296
Asp Gly Ile Asn Asn Tyr Gly Ala His His His Gly Trp Pro Thr Val
            420                 425                 430

gca ttc caa caa gct cag cca ttt agc atg cac tac cct tat ggc cat  1344
Ala Phe Gln Gln Ala Gln Pro Phe Ser Met His Tyr Pro Tyr Gly His
        435                 440                 445

cag cag agg gct gtt tgg tgt aag caa gag caa gac cct gat ggc aca  1392
Gln Gln Arg Ala Val Trp Cys Lys Gln Glu Gln Asp Pro Asp Gly Thr
    450                 455                 460
```

```
cac aac ttt caa gat ctt cac caa cta caa ttg gga aac act cac aac   1440
His Asn Phe Gln Asp Leu His Gln Leu Gln Leu Gly Asn Thr His Asn
465                 470                 475                 480

ttc ttc cag cct aat gtt ctg cac aac ctc atg agc atg gac tct tct   1488
Phe Phe Gln Pro Asn Val Leu His Asn Leu Met Ser Met Asp Ser Ser
                485                 490                 495

tca atg gac cat agc tca ggc tcc aat tca gtc atc tat agc ggt ggt   1536
Ser Met Asp His Ser Ser Gly Ser Asn Ser Val Ile Tyr Ser Gly Gly
            500                 505                 510

gga gcc gct gat ggc agc gct gca act ggc ggc agt ggc agt ggg agc   1584
Gly Ala Ala Asp Gly Ser Ala Ala Thr Gly Gly Ser Gly Ser Gly Ser
        515                 520                 525

ttc caa ggg gta ggt tat ggg aac aac att ggc ttt gtg atg ccc ata   1632
Phe Gln Gly Val Gly Tyr Gly Asn Asn Ile Gly Phe Val Met Pro Ile
    530                 535                 540

agc acc gtc atc gct cat gaa ggc ggc cat ggc cag gga aat ggt ggc   1680
Ser Thr Val Ile Ala His Glu Gly Gly His Gly Gln Gly Asn Gly Gly
545                 550                 555                 560

ttt gga gat agc gaa gtg aag gcg att ggt tac gac aac atg ttt gga   1728
Phe Gly Asp Ser Glu Val Lys Ala Ile Gly Tyr Asp Asn Met Phe Gly
                565                 570                 575

tcg aca gat cct tac cat gct agg agc ttg tac tat ctt tca cag caa   1776
Ser Thr Asp Pro Tyr His Ala Arg Ser Leu Tyr Tyr Leu Ser Gln Gln
            580                 585                 590

tca tct gca ggc atg gtg aag ggc agt agt gca tat gat cag ggg tca   1824
Ser Ser Ala Gly Met Val Lys Gly Ser Ser Ala Tyr Asp Gln Gly Ser
        595                 600                 605

ggg tgt aac aac tgg gtt cca act gca gtt cca acc cta gct cca agg   1872
Gly Cys Asn Asn Trp Val Pro Thr Ala Val Pro Thr Leu Ala Pro Arg
    610                 615                 620

act aac agc ttg gca gta tgc cat gga aca cct aca ttc aca gta tgg   1920
Thr Asn Ser Leu Ala Val Cys His Gly Thr Pro Thr Phe Thr Val Trp
625                 630                 635                 640

aat gat aca taa                                                  1932
Asn Asp Thr
```

<210> 85
<211> 643
<212> PRT
<213> Vitis vinifera

<400> 85

```
Met Ala Ser Met Asn Asn Trp Leu Gly Phe Ser Leu Ser Pro Arg Glu
 1               5                  10                  15
Leu Pro Pro Gln Pro Glu Asn His Ser Gln Asn Ser Val Ser Arg Leu
            20                  25                  30
Gly Phe Asn Ser Asp Glu Ile Ser Gly Thr Asp Val Ser Gly Glu Cys
        35                  40                  45
Phe Asp Leu Thr Ser Asp Ser Thr Ala Pro Ser Leu Asn Leu Pro Pro
    50                  55                  60
Pro Phe Gly Ile Leu Glu Ala Phe Asn Arg Asn Asn Gln Pro Gln Asp
65                  70                  75                  80
Thr Asn Tyr Lys Thr Thr Thr Ser Glu Leu Ser Met Leu Met Gly Ser
                85                  90                  95
```

Ser Cys Ser Ser His His Asn Leu Glu Asn Gln Glu Pro Lys Leu Glu
100 105 110
Asn Phe Leu Gly Cys Arg Ser Phe Ala Asp His Glu Gln Lys Leu Gln
115 120 125
Gly Tyr Tyr Ile Ser Ile Gly Leu Ser Met Ile Lys Thr Trp Leu Arg
130 135 140
Asn Gln Pro Ala Pro Thr His Gln Asp Asn Asn Lys Ser Thr Asp Thr
145 150 155 160
Gly Pro Val Gly Gly Ala Ala Ala Gly Asn Leu Pro Asn Ala Gln Thr
165 170 175
Leu Ser Leu Ser Met Ser Thr Gly Ser His Gln Thr Gly Ala Ile Glu
180 185 190
Thr Val Pro Arg Lys Ser Ile Asp Thr Phe Gly Gln Arg Thr Ser Ile
195 200 205
Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His
210 215 220
Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln Thr Arg Lys Gly Arg
225 230 235 240
Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu Lys Ala Ala Arg Ala
245 250 255
Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr Thr Asn
260 265 270
Phe Pro Ile Ser Asn Tyr Glu Lys Glu Ile Glu Glu Met Lys His Met
275 280 285
Thr Arg Gln Glu Tyr Val Ala Ser Leu Arg Arg Lys Ser Ser Gly Phe
290 295 300
Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr Arg His His Gln His
305 310 315 320
Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu
325 330 335
Tyr Leu Gly Thr Phe Ser Thr Gln Glu Glu Ala Ala Glu Ala Tyr Asp
340 345 350
Ile Ala Ala Ile Lys Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Asp
355 360 365
Met Ser Arg Tyr Asp Val Asn Ser Ile Leu Glu Ser Ser Thr Leu Pro
370 375 380
Ile Gly Gly Ala Ala Lys Arg Leu Lys Asp Ala Glu Gln Ala Glu Met
385 390 395 400
Thr Ile Asp Gly Gln Arg Thr Asp Asp Glu Met Ser Ser Gln Leu Thr
405 410 415
Asp Gly Ile Asn Asn Tyr Gly Ala His His His Gly Trp Pro Thr Val
420 425 430
Ala Phe Gln Gln Ala Gln Pro Phe Ser Met His Tyr Pro Tyr Gly His
435 440 445
Gln Gln Arg Ala Val Trp Cys Lys Gln Glu Gln Asp Pro Asp Gly Thr
450 455 460
His Asn Phe Gln Asp Leu His Gln Leu Gln Leu Gly Asn Thr His Asn
465 470 475 480
Phe Phe Gln Pro Asn Val Leu His Asn Leu Met Ser Met Asp Ser Ser
485 490 495
Ser Met Asp His Ser Ser Gly Ser Asn Ser Val Ile Tyr Ser Gly Gly
500 505 510
Gly Ala Ala Asp Gly Ser Ala Ala Thr Gly Gly Ser Gly Ser Gly Ser
515 520 525
Phe Gln Gly Val Gly Tyr Gly Asn Asn Ile Gly Phe Val Met Pro Ile
530 535 540
Ser Thr Val Ile Ala His Glu Gly Gly His Gly Gln Gly Asn Gly Gly
545 550 555 560
Phe Gly Asp Ser Glu Val Lys Ala Ile Gly Tyr Asp Asn Met Phe Gly
565 570 575
Ser Thr Asp Pro Tyr His Ala Arg Ser Leu Tyr Tyr Leu Ser Gln Gln
580 585 590
Ser Ser Ala Gly Met Val Lys Gly Ser Ser Ala Tyr Asp Gln Gly Ser
595 600 605
Gly Cys Asn Asn Trp Val Pro Thr Ala Val Pro Thr Leu Ala Pro Arg
610 615 620
Thr Asn Ser Leu Ala Val Cys His Gly Thr Pro Thr Phe Thr Val Trp
625 630 635 640

Asn Asp Thr

<210> 86
<211> 2088
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (1)...(2088)

<400> 86

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atg gcc acc atg aac aac tgg ctg gcc ttc tcc ctc tcc ccg cag gat    48
Met Ala Thr Met Asn Asn Trp Leu Ala Phe Ser Leu Ser Pro Gln Asp
 1               5                  10                  15

cag ctc ccg ccg tct cag acc aac tcc act ctc atc tcc gcc gcc gcc    96
Gln Leu Pro Pro Ser Gln Thr Asn Ser Thr Leu Ile Ser Ala Ala Ala
            20                  25                  30

acc acc acc acc gcc ggc gac tcc tcc acc ggc gac gtc tgc ttc aac   144
Thr Thr Thr Thr Ala Gly Asp Ser Ser Thr Gly Asp Val Cys Phe Asn
        35                  40                  45

atc ccc caa gat tgg agc atg agg gga tcg gag ctc tcg gcg ctc gtc   192
Ile Pro Gln Asp Trp Ser Met Arg Gly Ser Glu Leu Ser Ala Leu Val
    50                  55                  60

gcc gag ccg aag ctg gag gac ttc ctc ggc ggc atc tcc ttc tcg gag   240
Ala Glu Pro Lys Leu Glu Asp Phe Leu Gly Gly Ile Ser Phe Ser Glu
65                  70                  75                  80

cag cag cat cat cac ggc ggc aag ggc ggc gtg atc ccg agc agc gcc   288
Gln Gln His His His Gly Gly Lys Gly Gly Val Ile Pro Ser Ser Ala
                85                  90                  95

gcc gct tgc tac gcg agc tcc ggc agc agc gtc ggc tac ctg tac cct   336
Ala Ala Cys Tyr Ala Ser Ser Gly Ser Ser Val Gly Tyr Leu Tyr Pro
            100                 105                 110

cct cca agc tca tcc tcg ctc cag ttc gcc gac tcc gtc atg gtg gcc   384
Pro Pro Ser Ser Ser Ser Leu Gln Phe Ala Asp Ser Val Met Val Ala
        115                 120                 125

acc tcc tcg ccc gtc gtc gcc cac gac ggc gtc agc ggc ggc ggc atg   432
Thr Ser Ser Pro Val Val Ala His Asp Gly Val Ser Gly Gly Gly Met
    130                 135                 140

gtg agc gcc gcc gcc gcc gcg gcg gcc agt ggc aac ggc ggc att ggc   480
Val Ser Ala Ala Ala Ala Ala Ala Ala Ser Gly Asn Gly Gly Ile Gly
145                 150                 155                 160

ctg tcc atg atc aag aac tgg ctc cgg agc cag ccg gcg ccg cag ccg   528
Leu Ser Met Ile Lys Asn Trp Leu Arg Ser Gln Pro Ala Pro Gln Pro
                165                 170                 175

gcg cag gcg ctg tct ctg tcc atg aac atg gcg ggg acg acg acg gcg   576
Ala Gln Ala Leu Ser Leu Ser Met Asn Met Ala Gly Thr Thr Thr Ala
            180                 185                 190

cag ggc ggc ggc gcc atg gcg ctc ctc gcc ggc gca ggg gag cga ggc   624
Gln Gly Gly Gly Ala Met Ala Leu Leu Ala Gly Ala Gly Glu Arg Gly
        195                 200                 205

cgg acg acg ccc gcg tca gag agc ctg tcc acg tcg gcg cac gga gcg   672
Arg Thr Thr Pro Ala Ser Glu Ser Leu Ser Thr Ser Ala His Gly Ala
```

```
    210                 215                 220

acg acg gcg acg atg gct ggt ggt cgc aag gag att aac gag gaa ggc   720
Thr Thr Ala Thr Met Ala Gly Gly Arg Lys Glu Ile Asn Glu Glu Gly
225                 230                 235                 240

agc ggc agc gcc ggc gcc gtg gtt gcc gtc ggc tcg gag tca ggc ggc   768
Ser Gly Ser Ala Gly Ala Val Val Ala Val Gly Ser Glu Ser Gly Gly
                245                 250                 255

agc ggc gcc gtg gtg gag gcc ggc gcg gcg gcg gcg gcg gcg agg aag   816
Ser Gly Ala Val Val Glu Ala Gly Ala Ala Ala Ala Ala Ala Arg Lys
            260                 265                 270

tcc gtc gac acg ttc ggc cag aga aca tcg atc tac cgc ggc gtg aca   864
Ser Val Asp Thr Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr
        275                 280                 285

agg cat aga tgg aca ggg agg tat gag gct cat ctt tgg gac aac agc   912
Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser
    290                 295                 300

tgc aga aga gag ggc caa act cgc aag ggt cgt caa gtc tat cta ggt   960
Cys Arg Arg Glu Gly Gln Thr Arg Lys Gly Arg Gln Val Tyr Leu Gly
305                 310                 315                 320

ggt tat gac aaa gag gaa aaa gct gct aga gct tat gat ttg gct gct  1008
Gly Tyr Asp Lys Glu Glu Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala
                325                 330                 335

ctc aaa tac tgg ggc ccg acg acg acg aca aat ttt ccg gta aat aac  1056
Leu Lys Tyr Trp Gly Pro Thr Thr Thr Thr Asn Phe Pro Val Asn Asn
            340                 345                 350

tat gaa aag gag ctg gag gag atg aag cac atg aca agg cag gag ttc  1104
Tyr Glu Lys Glu Leu Glu Glu Met Lys His Met Thr Arg Gln Glu Phe
        355                 360                 365

gta gcc tct ttg aga agg aag agc agt ggt ttc tcc aga ggt gca tcc  1152
Val Ala Ser Leu Arg Arg Lys Ser Ser Gly Phe Ser Arg Gly Ala Ser
    370                 375                 380

att tac cgt gga gta act agg cat cac cag cat ggg aga tgg caa gca  1200
Ile Tyr Arg Gly Val Thr Arg His His Gln His Gly Arg Trp Gln Ala
385                 390                 395                 400

agg ata gga aga gtt gca ggg aac aag gac ctc tac ttg ggc acc ttc  1248
Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe
                405                 410                 415

agc acg cag gag gag gcg gcg gag gcg tac gac atc gcg gcg atc aag  1296
Ser Thr Gln Glu Glu Ala Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys
            420                 425                 430

ttc cgg ggg ctc aac gcc gtc acc aac ttc gac atg agc cgc tac gac  1344
Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp
        435                 440                 445

gtc aag agc atc ctc gac agc gct gcc ctc ccc gtc ggc acc gcc gcc  1392
Val Lys Ser Ile Leu Asp Ser Ala Ala Leu Pro Val Gly Thr Ala Ala
    450                 455                 460

aag cgc ctc aag gac gcc gag gcc gcc gcc gcc tac gac gtc ggc cgc  1440
Lys Arg Leu Lys Asp Ala Glu Ala Ala Ala Ala Tyr Asp Val Gly Arg
465                 470                 475                 480

atc gcc tcg cac ctc ggc ggc gac ggc gcc tac gcc gcg cat tac ggc  1488
Ile Ala Ser His Leu Gly Gly Asp Gly Ala Tyr Ala Ala His Tyr Gly
```

```
                    485                 490                 495

cac cac cac cac tcg gcc gcc gcc gcc tgg ccg acc atc gcg ttc cag   1536
His His His His Ser Ala Ala Ala Ala Trp Pro Thr Ile Ala Phe Gln
                500                 505                 510

gcg gcg gcg gcg ccg ccg ccg cac gcc gcc ggg ctt tac cac ccg tac   1584
Ala Ala Ala Ala Pro Pro Pro His Ala Ala Gly Leu Tyr His Pro Tyr
        515                 520                 525

gcg cag ccg ctg cgt ggg tgg tgc aag cag gag cag gac cac gcc gtg   1632
Ala Gln Pro Leu Arg Gly Trp Cys Lys Gln Glu Gln Asp His Ala Val
    530                 535                 540

atc gcg gcg gcg cac agc ctg cag gat ctc cac cac ctc aac ctc ggc   1680
Ile Ala Ala Ala His Ser Leu Gln Asp Leu His His Leu Asn Leu Gly
545                 550                 555                 560

gcc gcc gcc gcc gcg cat gac ttc ttc tcg cag gcg atg cag cag cag   1728
Ala Ala Ala Ala Ala His Asp Phe Phe Ser Gln Ala Met Gln Gln Gln
                565                 570                 575

cac ggc ctc ggc agc atc gac aac gcg tcg ctc gag cac agc acc ggc   1776
His Gly Leu Gly Ser Ile Asp Asn Ala Ser Leu Glu His Ser Thr Gly
            580                 585                 590

tcc aac tcc gtc gtc tac aac ggc gac aat ggc ggc gga ggc ggc ggc   1824
Ser Asn Ser Val Val Tyr Asn Gly Asp Asn Gly Gly Gly Gly Gly Gly
        595                 600                 605

tac atc atg gcg ccg atg agc gcc gtg tcg gcc acg gcc acc gcg gtg   1872
Tyr Ile Met Ala Pro Met Ser Ala Val Ser Ala Thr Ala Thr Ala Val
    610                 615                 620

gcg agc agc cac gat cac ggc ggc gac ggc ggg aag cag gtg cag atg   1920
Ala Ser Ser His Asp His Gly Gly Asp Gly Gly Lys Gln Val Gln Met
625                 630                 635                 640

ggg tac gac agc tac ctc gtc ggc gca gac gcc tac ggc ggc ggc ggc   1968
Gly Tyr Asp Ser Tyr Leu Val Gly Ala Asp Ala Tyr Gly Gly Gly Gly
                645                 650                 655

gcc ggg agg atg cca tcc tgg gcg atg acg ccg gcg tcg gcg ccg gcc   2016
Ala Gly Arg Met Pro Ser Trp Ala Met Thr Pro Ala Ser Ala Pro Ala
            660                 665                 670

gcc acg agc agc agc gac atg acc gga gtc tgc cat ggc gca cag ctc   2064
Ala Thr Ser Ser Ser Asp Met Thr Gly Val Cys His Gly Ala Gln Leu
        675                 680                 685

ttc agc gtc tgg aac gac aca taa                                  2088
Phe Ser Val Trp Asn Asp Thr
    690                 695
```

<210> 87
<211> 2088
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (1)...(2088)

<400> 87

```
atg gcc act atg aac aac tgg ctc gcc ttc tcg ctc tcg ccg cag gac   48
Met Ala Thr Met Asn Asn Trp Leu Ala Phe Ser Leu Ser Pro Gln Asp
 1               5                  10                  15
```

```
caa ctc cca ccg tcg cag acc aat agc act ctc atc tcc gct gct gca   96
Gln Leu Pro Pro Ser Gln Thr Asn Ser Thr Leu Ile Ser Ala Ala Ala
                20                  25                  30

acc acc aca acc gca ggc gat tcg tca acg ggc gac gtc tgc ttc aac  144
Thr Thr Thr Thr Ala Gly Asp Ser Ser Thr Gly Asp Val Cys Phe Asn
            35                  40                  45

atc cct caa gac tgg tcc atg cgc gga agc gag ctt agc gct ctc gtc  192
Ile Pro Gln Asp Trp Ser Met Arg Gly Ser Glu Leu Ser Ala Leu Val
        50                  55                  60

gcg gag ccc aag ttg gag gat ttc ttg gga ggc atc tcc ttc tcg gag  240
Ala Glu Pro Lys Leu Glu Asp Phe Leu Gly Gly Ile Ser Phe Ser Glu
    65                  70                  75                  80

caa cag cat cat cac ggc gga aag ggc ggt gtt atc cca agc tct gct  288
Gln Gln His His His Gly Gly Lys Gly Gly Val Ile Pro Ser Ser Ala
                85                  90                  95

gcc gca tgc tat gca agc tcc ggc tcc agc gtg ggc tac ctc tac cct  336
Ala Ala Cys Tyr Ala Ser Ser Gly Ser Ser Val Gly Tyr Leu Tyr Pro
            100                 105                 110

ccg cct tca tcc tcg tca ctt cag ttt gca gac agc gtg atg gtc gca  384
Pro Pro Ser Ser Ser Ser Leu Gln Phe Ala Asp Ser Val Met Val Ala
        115                 120                 125

acc tca tct cca gtg gtt gcg cac gat ggc gtg agc ggt ggc ggt atg  432
Thr Ser Ser Pro Val Val Ala His Asp Gly Val Ser Gly Gly Gly Met
    130                 135                 140

gtc tca gca gca gcg gct gca gca gct tcg ggt aat ggc ggg att ggc  480
Val Ser Ala Ala Ala Ala Ala Ala Ala Ser Gly Asn Gly Gly Ile Gly
145                 150                 155                 160

ctc tcc atg atc aag aac tgg ctc agg agc caa ccg gct ccg caa cct  528
Leu Ser Met Ile Lys Asn Trp Leu Arg Ser Gln Pro Ala Pro Gln Pro
                165                 170                 175

gcg caa gca ctc agc ctg tcg atg aac atg gct ggt act act acc gct  576
Ala Gln Ala Leu Ser Leu Ser Met Asn Met Ala Gly Thr Thr Thr Ala
            180                 185                 190

caa ggt gga ggc gca atg gca ctt ctc gca ggc gct ggc gaa aga gga  624
Gln Gly Gly Gly Ala Met Ala Leu Leu Ala Gly Ala Gly Glu Arg Gly
        195                 200                 205

agg acc aca cca gca tcc gag agc ctc tct act tcc gcg cac gga gcc  672
Arg Thr Thr Pro Ala Ser Glu Ser Leu Ser Thr Ser Ala His Gly Ala
    210                 215                 220

acc acg gct aca atg gct ggc ggg agg aaa gag atc aac gag gaa gga  720
Thr Thr Ala Thr Met Ala Gly Gly Arg Lys Glu Ile Asn Glu Glu Gly
225                 230                 235                 240

tct gga tcc gct ggt gcc gtg gtt gca gtt ggc tca gaa tca ggt gga  768
Ser Gly Ser Ala Gly Ala Val Val Ala Val Gly Ser Glu Ser Gly Gly
                245                 250                 255

tcc ggc gct gtt gtt gaa gct ggt gcc gct gcg gca gcg gct cgg aag  816
Ser Gly Ala Val Val Glu Ala Gly Ala Ala Ala Ala Ala Ala Arg Lys
            260                 265                 270

agc gtt gat act ttc ggc caa aga acg agc atc tac aga ggc gtt act  864
Ser Val Asp Thr Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr
        275                 280                 285
```

```
cgg cac cgc tgg acc ggc agg tac gag gca cac ttg tgg gac aac agc   912
Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser
    290                 295                 300

tgt cgc cgc gag ggc caa act agg aag gga aga cag gtc tat cta gga   960
Cys Arg Arg Glu Gly Gln Thr Arg Lys Gly Arg Gln Val Tyr Leu Gly
305                 310                 315                 320

gga tat gac aaa gag gag aag gct gcc aga gcg tac gac ctg gcc gcg  1008
Gly Tyr Asp Lys Glu Glu Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala
                325                 330                 335

ttg aag tac tgg ggt cca aca acg acg acc aac ttc ccg gtg aac aac  1056
Leu Lys Tyr Trp Gly Pro Thr Thr Thr Thr Asn Phe Pro Val Asn Asn
            340                 345                 350

tac gag aag gag ctg gaa gag atg aag cac atg acg cgg cag gag ttc  1104
Tyr Glu Lys Glu Leu Glu Glu Met Lys His Met Thr Arg Gln Glu Phe
        355                 360                 365

gtc gct tct ctc agg cgc aag tca tct ggt ttc tcc aga ggt gcg tcg  1152
Val Ala Ser Leu Arg Arg Lys Ser Ser Gly Phe Ser Arg Gly Ala Ser
    370                 375                 380

atc tat aga gga gtt acc cgc cac cac cag cac gga agg tgg cag gca  1200
Ile Tyr Arg Gly Val Thr Arg His His Gln His Gly Arg Trp Gln Ala
385                 390                 395                 400

aga atc ggg aga gtc gcc ggt aac aag gac ctg tac ttg gga acc ttc  1248
Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe
                405                 410                 415

tcg act cag gag gag gca gcg gaa gcg tat gac att gcg gcg atc aag  1296
Ser Thr Gln Glu Glu Ala Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys
            420                 425                 430

ttc cgc ggt ctc aat gcc gtg acc aac ttc gac atg tca cgc tat gat  1344
Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp
        435                 440                 445

gtc aag tcg att ctg gat agc gct gcg ttg cct gtg gga acc gct gcc  1392
Val Lys Ser Ile Leu Asp Ser Ala Ala Leu Pro Val Gly Thr Ala Ala
    450                 455                 460

aaa cgc ctc aag gac gcg gaa gca gct gcc gcg tac gat gtt ggc agg  1440
Lys Arg Leu Lys Asp Ala Glu Ala Ala Ala Ala Tyr Asp Val Gly Arg
465                 470                 475                 480

att gcc tca cat ctc ggt gga gat gga gct tac gct gcc cac tac ggg  1488
Ile Ala Ser His Leu Gly Gly Asp Gly Ala Tyr Ala Ala His Tyr Gly
                485                 490                 495

cat cat cac cac tct gca gcc gca gct tgg cct aca ata gca ttc caa  1536
His His His His Ser Ala Ala Ala Ala Trp Pro Thr Ile Ala Phe Gln
            500                 505                 510

gcg gca gcg gct cct cct cca cac gct gct ggt ctt tac cat ccg tac  1584
Ala Ala Ala Ala Pro Pro Pro His Ala Ala Gly Leu Tyr His Pro Tyr
        515                 520                 525

gcg caa cct ctc cgc ggt tgg tgt aag cag gaa caa gat cat gcg gtg  1632
Ala Gln Pro Leu Arg Gly Trp Cys Lys Gln Glu Gln Asp His Ala Val
    530                 535                 540

att gcg gct gca cac agc ttg caa gat ctg cat cac ctc aat ctg gga  1680
Ile Ala Ala Ala His Ser Leu Gln Asp Leu His His Leu Asn Leu Gly
545                 550                 555                 560
```

```
gcc gca gca gct gcc cat gac ttc ttc tca caa gcc atg cag cag cag   1728
Ala Ala Ala Ala Ala His Asp Phe Phe Ser Gln Ala Met Gln Gln Gln
                565                 570                 575

cat ggc ctg ggc agc ata gac aat gcg tct ctg gag cac tcc acc gga   1776
His Gly Leu Gly Ser Ile Asp Asn Ala Ser Leu Glu His Ser Thr Gly
            580                 585                 590

tcg aac tcg gtg gtg tac aat gga gac aac ggc gga gga ggt gga ggt   1824
Ser Asn Ser Val Val Tyr Asn Gly Asp Asn Gly Gly Gly Gly Gly Gly
        595                 600                 605

tac atc atg gca cct atg tca gcg gtc tct gct acc gct acg gcg gtg   1872
Tyr Ile Met Ala Pro Met Ser Ala Val Ser Ala Thr Ala Thr Ala Val
    610                 615                 620

gcc tca tcc cac gac cac ggt gga gac ggc ggc aag cag gtc caa atg   1920
Ala Ser Ser His Asp His Gly Gly Asp Gly Gly Lys Gln Val Gln Met
625                 630                 635                 640

ggc tac gac tcc tac ctt gtg gga gct gac gct tac ggc gga gga gga   1968
Gly Tyr Asp Ser Tyr Leu Val Gly Ala Asp Ala Tyr Gly Gly Gly Gly
                645                 650                 655

gct ggt cgc atg cct agc tgg gcc atg acg cct gct tct gct cct gcg   2016
Ala Gly Arg Met Pro Ser Trp Ala Met Thr Pro Ala Ser Ala Pro Ala
            660                 665                 670

gct acg agc tcg tcg gat atg aca gga gtg tgt cat ggc gcc caa ctg   2064
Ala Thr Ser Ser Ser Asp Met Thr Gly Val Cys His Gly Ala Gln Leu
        675                 680                 685

ttc tcg gtg tgg aat gat aca tag                                  2088
Phe Ser Val Trp Asn Asp Thr
    690                 695
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<210> 88
<211> 4325
<212> DNA
<213> Oryza sativa

<400> 88

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<210> 89
<211> 695
<212> PRT
<213> Oryza sativa

<400> 89

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Gln Leu Pro Pro Ser Gln Thr Asn Ser Thr Leu Ile Ser Ala Ala Ala
            20                  25                  30
Thr Thr Thr Thr Ala Gly Asp Ser Ser Thr Gly Asp Val Cys Phe Asn
        35                  40                  45
Ile Pro Gln Asp Trp Ser Met Arg Gly Ser Glu Leu Ser Ala Leu Val
    50                  55                  60
Ala Glu Pro Lys Leu Glu Asp Phe Leu Gly Gly Ile Ser Phe Ser Glu
65                  70                  75                  80
Gln Gln His His His Gly Gly Lys Gly Gly Val Ile Pro Ser Ser Ala
                85                  90                  95
```

Ala Ala Cys Tyr Ala Ser Ser Gly Ser Ser Val Gly Tyr Leu Tyr Pro
100 105 110
Pro Pro Ser Ser Ser Ser Leu Gln Phe Ala Asp Ser Val Met Val Ala
115 120 125
Thr Ser Ser Pro Val Val Ala His Asp Gly Val Ser Gly Gly Gly Met
130 135 140
Val Ser Ala Ala Ala Ala Ala Ala Ala Ser Gly Asn Gly Gly Ile Gly
145 150 155 160
Leu Ser Met Ile Lys Asn Trp Leu Arg Ser Gln Pro Ala Pro Gln Pro
165 170 175
Ala Gln Ala Leu Ser Leu Ser Met Asn Met Ala Gly Thr Thr Thr Ala
180 185 190
Gln Gly Gly Gly Ala Met Ala Leu Leu Ala Gly Ala Gly Glu Arg Gly
195 200 205
Arg Thr Thr Pro Ala Ser Glu Ser Leu Ser Thr Ser Ala His Gly Ala
210 215 220
Thr Thr Ala Thr Met Ala Gly Gly Arg Lys Glu Ile Asn Glu Glu Gly
225 230 235 240
Ser Gly Ser Ala Gly Ala Val Val Ala Val Gly Ser Glu Ser Gly Gly
245 250 255
Ser Gly Ala Val Val Glu Ala Gly Ala Ala Ala Ala Ala Ala Arg Lys
260 265 270
Ser Val Asp Thr Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr
275 280 285
Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser
290 295 300
Cys Arg Arg Glu Gly Gln Thr Arg Lys Gly Arg Gln Val Tyr Leu Gly
305 310 315 320
Gly Tyr Asp Lys Glu Glu Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala
325 330 335
Leu Lys Tyr Trp Gly Pro Thr Thr Thr Thr Asn Phe Pro Val Asn Asn
340 345 350
Tyr Glu Lys Glu Leu Glu Glu Met Lys His Met Thr Arg Gln Glu Phe
355 360 365
Val Ala Ser Leu Arg Arg Lys Ser Ser Gly Phe Ser Arg Gly Ala Ser
370 375 380
Ile Tyr Arg Gly Val Thr Arg His His Gln His Gly Arg Trp Gln Ala
385 390 395 400
Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe
405 410 415
Ser Thr Gln Glu Glu Ala Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys
420 425 430
Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp
435 440 445
Val Lys Ser Ile Leu Asp Ser Ala Ala Leu Pro Val Gly Thr Ala Ala
450 455 460
Lys Arg Leu Lys Asp Ala Glu Ala Ala Ala Ala Tyr Asp Val Gly Arg
465 470 475 480
Ile Ala Ser His Leu Gly Gly Asp Gly Ala Tyr Ala Ala His Tyr Gly
485 490 495
His His His His Ser Ala Ala Ala Ala Trp Pro Thr Ile Ala Phe Gln
500 505 510
Ala Ala Ala Ala Pro Pro Pro His Ala Ala Gly Leu Tyr His Pro Tyr
515 520 525
Ala Gln Pro Leu Arg Gly Trp Cys Lys Gln Glu Gln Asp His Ala Val
530 535 540
Ile Ala Ala Ala His Ser Leu Gln Asp Leu His His Leu Asn Leu Gly
545 550 555 560
Ala Ala Ala Ala Ala His Asp Phe Phe Ser Gln Ala Met Gln Gln Gln
565 570 575
His Gly Leu Gly Ser Ile Asp Asn Ala Ser Leu Glu His Ser Thr Gly
580 585 590
Ser Asn Ser Val Val Tyr Asn Gly Asp Asn Gly Gly Gly Gly Gly Gly
595 600 605
Tyr Ile Met Ala Pro Met Ser Ala Val Ser Ala Thr Ala Thr Ala Val
610 615 620
Ala Ser Ser His Asp His Gly Gly Asp Gly Gly Lys Gln Val Gln Met
625 630 635 640

```
Gly Tyr Asp Ser Tyr Leu Val Gly Ala Asp Ala Tyr Gly Gly Gly Gly
                645                 650                 655
Ala Gly Arg Met Pro Ser Trp Ala Met Thr Pro Ala Ser Ala Pro Ala
            660                 665                 670
Ala Thr Ser Ser Ser Asp Met Thr Gly Val Cys His Gly Ala Gln Leu
        675                 680                 685
Phe Ser Val Trp Asn Asp Thr
    690                 695


<210> 90
<211> 1680
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (1)...(1680)

<400> 90
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Met Ala Ser Ile Thr Asn Trp Leu Gly Phe Ser Ser Ser Ser Phe Ser
 1               5                  10                  15

ggc gcc ggc gcc gac ccc gtc ctg ccc cac ccg ccg ctg caa gag tgg   96
Gly Ala Gly Ala Asp Pro Val Leu Pro His Pro Pro Leu Gln Glu Trp
            20                  25                  30

ggg agc gct tat gag ggc ggc ggc acg gtg gcg gcc gcc ggc ggg gag   144
Gly Ser Ala Tyr Glu Gly Gly Gly Thr Val Ala Ala Ala Gly Gly Glu
        35                  40                  45

gag acg gcg gcg ccg aag ctg gag gac ttc ctc ggc atg cag gtg cag   192
Glu Thr Ala Ala Pro Lys Leu Glu Asp Phe Leu Gly Met Gln Val Gln
    50                  55                  60

cag gag acg gcc gcc gcg gcg gcg ggg cac ggc cgt gga ggc agc tcg   240
Gln Glu Thr Ala Ala Ala Ala Ala Gly His Gly Arg Gly Gly Ser Ser
65                  70                  75                  80

tcg gtc gtt ggg ctg tcc atg atc aag aac tgg cta cgc agc cag ccg   288
Ser Val Val Gly Leu Ser Met Ile Lys Asn Trp Leu Arg Ser Gln Pro
                85                  90                  95

ccg ccc gcg gtg gtt ggg gga gaa gac gct atg atg gcg ctc gcg gtg   336
Pro Pro Ala Val Val Gly Gly Glu Asp Ala Met Met Ala Leu Ala Val
            100                 105                 110

tcg acg tcg gcg tcg ccg ccg gtg gac gcg acg gtg ccg gcc tgc att   384
Ser Thr Ser Ala Ser Pro Pro Val Asp Ala Thr Val Pro Ala Cys Ile
        115                 120                 125

tcg ccg gat ggg atg ggg tcg aag gcg gcc gac ggc ggc ggc gcg gcc   432
Ser Pro Asp Gly Met Gly Ser Lys Ala Ala Asp Gly Gly Gly Ala Ala
    130                 135                 140

gag gcg gcg gcg gcg gcg gcg gcg cag agg atg aag gcg gcc atg gac   480
Glu Ala Ala Ala Ala Ala Ala Ala Gln Arg Met Lys Ala Ala Met Asp
145                 150                 155                 160

acg ttc ggg cag cgg acg tcc atc tac cgg ggt gtc acc aag cac agg   528
Thr Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Lys His Arg
                165                 170                 175

tgg aca gga agg tat gaa gcc cat ctt tgg gat aac agc tgc aga aga   576
Trp Thr Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg
            180                 185                 190
```

```
gaa ggt cag act cgc aaa ggc aga caa gta tat ctt gga gga tat gat   624
Glu Gly Gln Thr Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp
        195                 200                 205

aag gaa gaa aaa gct gct agg gct tat gat ttg gct gcc ctt aaa tac   672
Lys Glu Glu Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr
    210                 215                 220

tgg ggc act aca acg acg acg aat ttt ccg gta agc aac tac gaa aaa   720
Trp Gly Thr Thr Thr Thr Thr Asn Phe Pro Val Ser Asn Tyr Glu Lys
225                 230                 235                 240

gag ttg gat gaa atg aag cac atg aat agg cag gaa ttt gtt gca tcc   768
Glu Leu Asp Glu Met Lys His Met Asn Arg Gln Glu Phe Val Ala Ser
                245                 250                 255

ctt aga aga aaa agc agt gga ttt tca cgt ggt gct tcc ata tat cgt   816
Leu Arg Arg Lys Ser Ser Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg
            260                 265                 270

ggt gtt aca aga cac cat cag cat gga agg tgg caa gca agg ata gga   864
Gly Val Thr Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly
        275                 280                 285

cgg gtg gca gga aac aag gat ctg tat ttg ggc aca ttt ggc acc caa   912
Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe Gly Thr Gln
    290                 295                 300

gag gaa gct gca gag gca tat gat atc gct gca atc aaa ttc cgt ggt   960
Glu Glu Ala Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly
305                 310                 315                 320

ctc aat gct gtg aca aac ttt gac atg agc cgg tac gat gtc aag agc  1008
Leu Asn Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Ser
                325                 330                 335

atc att gaa agc agc aat ctc cca att ggt act gga acc acc cgg cga  1056
Ile Ile Glu Ser Ser Asn Leu Pro Ile Gly Thr Gly Thr Thr Arg Arg
            340                 345                 350

ttg aag gac tcc tct gat cac act gat aat gtc atg gac atc aat gtc  1104
Leu Lys Asp Ser Ser Asp His Thr Asp Asn Val Met Asp Ile Asn Val
        355                 360                 365

aat acc gaa ccc aat aat gtg gta tca tcc cac ttc acc aat ggg gtt  1152
Asn Thr Glu Pro Asn Asn Val Val Ser Ser His Phe Thr Asn Gly Val
    370                 375                 380

ggc aac tat ggt tcg cag cat tat ggt tac aat gga tgg tcg cca att  1200
Gly Asn Tyr Gly Ser Gln His Tyr Gly Tyr Asn Gly Trp Ser Pro Ile
385                 390                 395                 400

agc atg cag ccg atc ccc tcg cag tac gcc aac ggc cag ccc agg gca  1248
Ser Met Gln Pro Ile Pro Ser Gln Tyr Ala Asn Gly Gln Pro Arg Ala
                405                 410                 415

tgg ttg aaa caa gag cag gac agc tct gtg gtt aca gcg gcg cag aac  1296
Trp Leu Lys Gln Glu Gln Asp Ser Ser Val Val Thr Ala Ala Gln Asn
            420                 425                 430

ctg cac aat cta cat cat ttt agt tcc ttg ggc tac acc cac aac ttc  1344
Leu His Asn Leu His His Phe Ser Ser Leu Gly Tyr Thr His Asn Phe
        435                 440                 445

ttc cag caa tct gat gtt cca gac gtc aca ggt ttc gtt gat gcg cct  1392
Phe Gln Gln Ser Asp Val Pro Asp Val Thr Gly Phe Val Asp Ala Pro
    450                 455                 460
```

```
tcg agg tcc agt gac tca tac tcc ttc agg tac aat gga aca aat ggc   1440
Ser Arg Ser Ser Asp Ser Tyr Ser Phe Arg Tyr Asn Gly Thr Asn Gly
465                 470                 475                 480

ttt cat ggt ctc ccg ggt gga atc agc tat gct atg ccg gtt gcg aca   1488
Phe His Gly Leu Pro Gly Gly Ile Ser Tyr Ala Met Pro Val Ala Thr
                485                 490                 495

gcg gtg gac caa ggt cag ggc atc cat ggc tat gga gaa gat ggt gtg   1536
Ala Val Asp Gln Gly Gln Gly Ile His Gly Tyr Gly Glu Asp Gly Val
            500                 505                 510

gca ggc att gac acc aca cat gac ctg tat ggc agc cgt aat gtg tac   1584
Ala Gly Ile Asp Thr Thr His Asp Leu Tyr Gly Ser Arg Asn Val Tyr
        515                 520                 525

tac ctt tcc gag ggt tcg ctt ctt gcc gat gtc gaa aaa gaa ggc gac   1632
Tyr Leu Ser Glu Gly Ser Leu Leu Ala Asp Val Glu Lys Glu Gly Asp
    530                 535                 540

tat ggc caa tct gtg ggg ggc aac agc tgg gtt ttg ccg aca ccg tag   1680
Tyr Gly Gln Ser Val Gly Gly Asn Ser Trp Val Leu Pro Thr Pro
545                 550                 555
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<210> 91
<211> 559
<212> PRT
<213> Oryza sativa

<400> 91

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Met Ala Ser Ile Thr Asn Trp Leu Gly Phe Ser Ser Ser Ser Phe Ser
 1               5                  10                  15
Gly Ala Gly Ala Asp Pro Val Leu Pro His Pro Pro Leu Gln Glu Trp
            20                  25                  30
Gly Ser Ala Tyr Glu Gly Gly Gly Thr Val Ala Ala Ala Gly Gly Glu
        35                  40                  45
Glu Thr Ala Ala Pro Lys Leu Glu Asp Phe Leu Gly Met Gln Val Gln
    50                  55                  60
Gln Glu Thr Ala Ala Ala Ala Ala Gly His Gly Arg Gly Gly Ser Ser
65                  70                  75                  80
Ser Val Val Gly Leu Ser Met Ile Lys Asn Trp Leu Arg Ser Gln Pro
                85                  90                  95
Pro Pro Ala Val Val Gly Gly Glu Asp Ala Met Met Ala Leu Ala Val
            100                 105                 110
Ser Thr Ser Ala Ser Pro Pro Val Asp Ala Thr Val Pro Ala Cys Ile
        115                 120                 125
Ser Pro Asp Gly Met Gly Ser Lys Ala Ala Asp Gly Gly Gly Ala Ala
    130                 135                 140
Glu Ala Ala Ala Ala Ala Ala Ala Gln Arg Met Lys Ala Ala Met Asp
145                 150                 155                 160
Thr Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Lys His Arg
                165                 170                 175
Trp Thr Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg
            180                 185                 190
Glu Gly Gln Thr Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp
        195                 200                 205
Lys Glu Glu Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr
    210                 215                 220
Trp Gly Thr Thr Thr Thr Thr Asn Phe Pro Val Ser Asn Tyr Glu Lys
225                 230                 235                 240
Glu Leu Asp Glu Met Lys His Met Asn Arg Gln Glu Phe Val Ala Ser
                245                 250                 255
Leu Arg Arg Lys Ser Ser Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg
            260                 265                 270
Gly Val Thr Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly
        275                 280                 285
```

```
Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe Gly Thr Gln
    290                 295                 300
Glu Glu Ala Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly
305                 310                 315                 320
Leu Asn Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Ser
                325                 330                 335
Ile Ile Glu Ser Ser Asn Leu Pro Ile Gly Thr Gly Thr Thr Arg Arg
            340                 345                 350
Leu Lys Asp Ser Ser Asp His Thr Asp Asn Val Met Asp Ile Asn Val
        355                 360                 365
Asn Thr Glu Pro Asn Asn Val Val Ser Ser His Phe Thr Asn Gly Val
    370                 375                 380
Gly Asn Tyr Gly Ser Gln His Tyr Gly Tyr Asn Gly Trp Ser Pro Ile
385                 390                 395                 400
Ser Met Gln Pro Ile Pro Ser Gln Tyr Ala Asn Gly Gln Pro Arg Ala
                405                 410                 415
Trp Leu Lys Gln Glu Gln Asp Ser Ser Val Val Thr Ala Ala Gln Asn
            420                 425                 430
Leu His Asn Leu His His Phe Ser Ser Leu Gly Tyr Thr His Asn Phe
        435                 440                 445
Phe Gln Gln Ser Asp Val Pro Asp Val Thr Gly Phe Val Asp Ala Pro
    450                 455                 460
Ser Arg Ser Ser Asp Ser Tyr Ser Phe Arg Tyr Asn Gly Thr Asn Gly
465                 470                 475                 480
Phe His Gly Leu Pro Gly Gly Ile Ser Tyr Ala Met Pro Val Ala Thr
                485                 490                 495
Ala Val Asp Gln Gly Gln Gly Ile His Gly Tyr Gly Glu Asp Gly Val
            500                 505                 510
Ala Gly Ile Asp Thr Thr His Asp Leu Tyr Gly Ser Arg Asn Val Tyr
        515                 520                 525
Tyr Leu Ser Glu Gly Ser Leu Leu Ala Asp Val Glu Lys Glu Gly Asp
    530                 535                 540
Tyr Gly Gln Ser Val Gly Gly Asn Ser Trp Val Leu Pro Thr Pro
545                 550                 555
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<210> 92
<211> 2112
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (1)...(2112)

<400> 92

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Met Ala Ser Ala Asn Asn Trp Leu Gly Phe Ser Leu Ser Gly Gln Glu
 1               5                  10                  15

aat ccg cag cct cac cag gat agc tcg cct ccg gca gcc atc gac gtc   96
Asn Pro Gln Pro His Gln Asp Ser Ser Pro Pro Ala Ala Ile Asp Val
            20                  25                  30

tcc ggc gcc ggc gac ttc tat ggc ctg ccg acg tcg cag ccg acg gcg  144
Ser Gly Ala Gly Asp Phe Tyr Gly Leu Pro Thr Ser Gln Pro Thr Ala
        35                  40                  45

gcc gac gcg cac ctc ggc gtg gcg ggg cat cat cac aac gcc tcg tat  192
Ala Asp Ala His Leu Gly Val Ala Gly His His His Asn Ala Ser Tyr
    50                  55                  60

ggc atc atg gag gcc ttc aat agg gga gct caa gag gca caa gat tgg  240
Gly Ile Met Glu Ala Phe Asn Arg Gly Ala Gln Glu Ala Gln Asp Trp
65                  70                  75                  80

aac atg agg ggg ctg gac tac aac ggc ggc gcc tcg gag ctg tcg atg  288
Asn Met Arg Gly Leu Asp Tyr Asn Gly Gly Ala Ser Glu Leu Ser Met
```

```
                    85                  90                  95

ctc gtc ggc tcc agc ggc ggc aag agg gcg gcg gcg gtg gag gag acc   336
Leu Val Gly Ser Ser Gly Gly Lys Arg Ala Ala Ala Val Glu Glu Thr
                100                 105                 110

gag ccg aag ctg gag gac ttc ctc ggc ggc aac tcg ttc gtc tcc gag   384
Glu Pro Lys Leu Glu Asp Phe Leu Gly Gly Asn Ser Phe Val Ser Glu
        115                 120                 125

caa gat cat cac gcg gcg ggg ggc ttc ctc ttc tcc ggc gtc ccg atg   432
Gln Asp His His Ala Ala Gly Gly Phe Leu Phe Ser Gly Val Pro Met
    130                 135                 140

gcc agc agc acc aac agc aac agc ggg agc aac act atg gag ctc tcc   480
Ala Ser Ser Thr Asn Ser Asn Ser Gly Ser Asn Thr Met Glu Leu Ser
145                 150                 155                 160

atg atc aag acc tgg ctc cgg aac aac ggc cag gtg ccc gcc ggc cac   528
Met Ile Lys Thr Trp Leu Arg Asn Asn Gly Gln Val Pro Ala Gly His
                165                 170                 175

cag ccg cag cag cag cag ccg gcg gcc gcg gcc gcc gcc gcg cag cag   576
Gln Pro Gln Gln Gln Gln Pro Ala Ala Ala Ala Ala Ala Ala Gln Gln
            180                 185                 190

cag gcg cac gag gcg gcg gag atg agc acc gac gcg agc gcg agc agc   624
Gln Ala His Glu Ala Ala Glu Met Ser Thr Asp Ala Ser Ala Ser Ser
        195                 200                 205

ttc ggg tgc tcc tcc gac gcg atg ggg agg agt aac aac ggc ggc gcg   672
Phe Gly Cys Ser Ser Asp Ala Met Gly Arg Ser Asn Asn Gly Gly Ala
    210                 215                 220

gtc tcg gcg gcg gcc ggc ggg acg agc tcg cag agc ctg gcg ctc tcg   720
Val Ser Ala Ala Ala Gly Gly Thr Ser Ser Gln Ser Leu Ala Leu Ser
225                 230                 235                 240

atg agc acg ggc tcg cac tcg cac ctg cct atc gtc gtc gcc ggc ggc   768
Met Ser Thr Gly Ser His Ser His Leu Pro Ile Val Val Ala Gly Gly
                245                 250                 255

ggg aac gcc agc ggc gga gcg gcc gag agc aca tcg tcg gag aac aag   816
Gly Asn Ala Ser Gly Gly Ala Ala Glu Ser Thr Ser Ser Glu Asn Lys
            260                 265                 270

cgg gcc agc ggc gcc atg gat tcg ccg ggc ggt ggc gcg ata gag gcc   864
Arg Ala Ser Gly Ala Met Asp Ser Pro Gly Gly Gly Ala Ile Glu Ala
        275                 280                 285

gtg ccg agg aag tcc atc gac acg ttc ggg caa agg acc tcg ata tat   912
Val Pro Arg Lys Ser Ile Asp Thr Phe Gly Gln Arg Thr Ser Ile Tyr
    290                 295                 300

cga ggt gta aca agg cat aga tgg aca ggg cga tat gag gct cat ctc   960
Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His Leu
305                 310                 315                 320

tgg gat aat agc tgt aga aga gaa ggg cag agt cgc aag ggt agg caa  1008
Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln Ser Arg Lys Gly Arg Gln
                325                 330                 335

gtt tat ctt ggt ggc tat gac aag gag gat aaa gca gcg aga gct tat  1056
Val Tyr Leu Gly Gly Tyr Asp Lys Glu Asp Lys Ala Ala Arg Ala Tyr
            340                 345                 350

gat ttg gca gct ctg aag tat tgg ggc aca aca aca aca aca aat ttc  1104
Asp Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr Thr Asn Phe
```

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          355                 360                 365

cca ata agt aac tat gaa aaa gag cta gat gaa atg aaa cat atg acc   1152
Pro Ile Ser Asn Tyr Glu Lys Glu Leu Asp Glu Met Lys His Met Thr
    370                 375                 380

agg cag gag tat att gca tac cta aga agg aat agc agt gga ttt tct   1200
Arg Gln Glu Tyr Ile Ala Tyr Leu Arg Arg Asn Ser Ser Gly Phe Ser
385                 390                 395                 400

cgt ggt gca tcg aaa tat cgt ggt gta acc agg cac cat cag cat ggg   1248
Arg Gly Ala Ser Lys Tyr Arg Gly Val Thr Arg His His Gln His Gly
                405                 410                 415

aga tgg caa gca agg ata ggg agg gtt gca gga aac aag gac ctc tac   1296
Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr
            420                 425                 430

tta ggc acc ttc agc acc gag gag gag gcg gcg gag gcg tac gac atc   1344
Leu Gly Thr Phe Ser Thr Glu Glu Glu Ala Ala Glu Ala Tyr Asp Ile
        435                 440                 445

gcg gcg atc aag ttc cgg ggg ctc aac gcc gtc acc aac ttt gac atg   1392
Ala Ala Ile Lys Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Asp Met
    450                 455                 460

agc cgc tac gac gtc aag agc atc ctg gag agc agc acg ctg ccg gtg   1440
Ser Arg Tyr Asp Val Lys Ser Ile Leu Glu Ser Ser Thr Leu Pro Val
465                 470                 475                 480

ggc ggc gcg gcg agg cgg ctg aag gag gcg gcg gac cac gcg gag gcg   1488
Gly Gly Ala Ala Arg Arg Leu Lys Glu Ala Ala Asp His Ala Glu Ala
                485                 490                 495

gcc ggc gcc acc atc tgg cgc gcc gcc gac atg gac ggc gcc ggc gtc   1536
Ala Gly Ala Thr Ile Trp Arg Ala Ala Asp Met Asp Gly Ala Gly Val
            500                 505                 510

atc tcc ggc ctg gcc gac gtc ggg atg ggc gcc tac gcc gcc tcg tac   1584
Ile Ser Gly Leu Ala Asp Val Gly Met Gly Ala Tyr Ala Ala Ser Tyr
        515                 520                 525

cac cac cac cac cac cac ggc tgg ccg acc atc gcg ttc cag cag ccg   1632
His His His His His His Gly Trp Pro Thr Ile Ala Phe Gln Gln Pro
    530                 535                 540

ccg ccg ctc gcc gtg cac tac ccg tac ggc cag gcg ccg gcg gcg ccg   1680
Pro Pro Leu Ala Val His Tyr Pro Tyr Gly Gln Ala Pro Ala Ala Pro
545                 550                 555                 560

tcg cgc ggg tgg tgc aag ccc gag cag gac gcc gcc gtc gct gcc gcc   1728
Ser Arg Gly Trp Cys Lys Pro Glu Gln Asp Ala Ala Val Ala Ala Ala
                565                 570                 575

gcg cac agc ctc cag gac ctc cag cag ctg cac ctc ggc agc gcc gcc   1776
Ala His Ser Leu Gln Asp Leu Gln Gln Leu His Leu Gly Ser Ala Ala
            580                 585                 590

gcc cac aac ttc ttc cag gcg tcg tcg agc tcg acg gtc tac aac ggc   1824
Ala His Asn Phe Phe Gln Ala Ser Ser Ser Ser Thr Val Tyr Asn Gly
        595                 600                 605

ggc ggc ggc ggg tac cag ggc ctc ggt ggc aac gcc ttc ttg atg ccg   1872
Gly Gly Gly Gly Tyr Gln Gly Leu Gly Gly Asn Ala Phe Leu Met Pro
    610                 615                 620

gcg agc acc gtc gtg gcc gac cag ggg cac agc agc acg gcc acc aac   1920
Ala Ser Thr Val Val Ala Asp Gln Gly His Ser Ser Thr Ala Thr Asn
```

625 630 635 640

cat gga aac acc tgc agc tac ggc aac gag gag cag ggg aag ctc atc 1968
His Gly Asn Thr Cys Ser Tyr Gly Asn Glu Glu Gln Gly Lys Leu Ile
645 650 655

ggg tac gac gcc atg gcg atg gcg agc ggc gcc gcc ggc ggc ggg tac 2016
Gly Tyr Asp Ala Met Ala Met Ala Ser Gly Ala Ala Gly Gly Gly Tyr
660 665 670

cag ctg tcg cag ggc tcg gcg tcg acg gtg agc atc gcg agg gcg aac 2064
Gln Leu Ser Gln Gly Ser Ala Ser Thr Val Ser Ile Ala Arg Ala Asn
675 680 685

ggc tac tcg gcc aac tgg agc tcg cct ttc aat ggc gcc atg gga tga 2112
Gly Tyr Ser Ala Asn Trp Ser Ser Pro Phe Asn Gly Ala Met Gly
690 695 700

<210> 93
<211> 703
<212> PRT
<213> Oryza sativa

<400> 93
Met Ala Ser Ala Asn Asn Trp Leu Gly Phe Ser Leu Ser Gly Gln Glu
1 5 10 15
Asn Pro Gln Pro His Gln Asp Ser Ser Pro Pro Ala Ala Ile Asp Val
20 25 30
Ser Gly Ala Gly Asp Phe Tyr Gly Leu Pro Thr Ser Gln Pro Thr Ala
35 40 45
Ala Asp Ala His Leu Gly Val Ala Gly His His His Asn Ala Ser Tyr
50 55 60
Gly Ile Met Glu Ala Phe Asn Arg Gly Ala Gln Glu Ala Gln Asp Trp
65 70 75 80
Asn Met Arg Gly Leu Asp Tyr Asn Gly Gly Ala Ser Glu Leu Ser Met
85 90 95
Leu Val Gly Ser Ser Gly Gly Lys Arg Ala Ala Ala Val Glu Glu Thr
100 105 110
Glu Pro Lys Leu Glu Asp Phe Leu Gly Gly Asn Ser Phe Val Ser Glu
115 120 125
Gln Asp His His Ala Ala Gly Gly Phe Leu Phe Ser Gly Val Pro Met
130 135 140
Ala Ser Ser Thr Asn Ser Asn Ser Gly Ser Asn Thr Met Glu Leu Ser
145 150 155 160
Met Ile Lys Thr Trp Leu Arg Asn Asn Gly Gln Val Pro Ala Gly His
165 170 175
Gln Pro Gln Gln Gln Gln Pro Ala Ala Ala Ala Ala Ala Ala Gln Gln
180 185 190
Gln Ala His Glu Ala Ala Glu Met Ser Thr Asp Ala Ser Ala Ser Ser
195 200 205
Phe Gly Cys Ser Ser Asp Ala Met Gly Arg Ser Asn Asn Gly Gly Ala
210 215 220
Val Ser Ala Ala Ala Gly Gly Thr Ser Ser Gln Ser Leu Ala Leu Ser
225 230 235 240
Met Ser Thr Gly Ser His Ser His Leu Pro Ile Val Val Ala Gly Gly
245 250 255
Gly Asn Ala Ser Gly Gly Ala Ala Glu Ser Thr Ser Ser Glu Asn Lys
260 265 270
Arg Ala Ser Gly Ala Met Asp Ser Pro Gly Gly Gly Ala Ile Glu Ala
275 280 285
Val Pro Arg Lys Ser Ile Asp Thr Phe Gly Gln Arg Thr Ser Ile Tyr
290 295 300
Arg Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His Leu
305 310 315 320
Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln Ser Arg Lys Gly Arg Gln
325 330 335

```
Val Tyr Leu Gly Gly Tyr Asp Lys Glu Asp Lys Ala Ala Arg Ala Tyr
            340                 345                 350
Asp Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr Thr Asn Phe
        355                 360                 365
Pro Ile Ser Asn Tyr Glu Lys Glu Leu Asp Glu Met Lys His Met Thr
    370                 375                 380
Arg Gln Glu Tyr Ile Ala Tyr Leu Arg Arg Asn Ser Ser Gly Phe Ser
385                 390                 395                 400
Arg Gly Ala Ser Lys Tyr Arg Gly Val Thr Arg His His Gln His Gly
                405                 410                 415
Arg Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr
            420                 425                 430
Leu Gly Thr Phe Ser Thr Glu Glu Glu Ala Ala Glu Ala Tyr Asp Ile
        435                 440                 445
Ala Ala Ile Lys Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Asp Met
    450                 455                 460
Ser Arg Tyr Asp Val Lys Ser Ile Leu Glu Ser Ser Thr Leu Pro Val
465                 470                 475                 480
Gly Gly Ala Ala Arg Arg Leu Lys Glu Ala Ala Asp His Ala Glu Ala
                485                 490                 495
Ala Gly Ala Thr Ile Trp Arg Ala Ala Asp Met Asp Gly Ala Gly Val
            500                 505                 510
Ile Ser Gly Leu Ala Asp Val Gly Met Gly Ala Tyr Ala Ala Ser Tyr
        515                 520                 525
His His His His His His Gly Trp Pro Thr Ile Ala Phe Gln Gln Pro
    530                 535                 540
Pro Pro Leu Ala Val His Tyr Pro Tyr Gly Gln Ala Pro Ala Ala Pro
545                 550                 555                 560
Ser Arg Gly Trp Cys Lys Pro Glu Gln Asp Ala Ala Val Ala Ala Ala
                565                 570                 575
Ala His Ser Leu Gln Asp Leu Gln Gln Leu His Leu Gly Ser Ala Ala
            580                 585                 590
Ala His Asn Phe Phe Gln Ala Ser Ser Ser Ser Thr Val Tyr Asn Gly
        595                 600                 605
Gly Gly Gly Gly Tyr Gln Gly Leu Gly Gly Asn Ala Phe Leu Met Pro
    610                 615                 620
Ala Ser Thr Val Val Ala Asp Gln Gly His Ser Ser Thr Ala Thr Asn
625                 630                 635                 640
His Gly Asn Thr Cys Ser Tyr Gly Asn Glu Glu Gln Gly Lys Leu Ile
                645                 650                 655
Gly Tyr Asp Ala Met Ala Met Ala Ser Gly Ala Ala Gly Gly Gly Tyr
            660                 665                 670
Gln Leu Ser Gln Gly Ser Ala Ser Thr Val Ser Ile Ala Arg Ala Asn
        675                 680                 685
Gly Tyr Ser Ala Asn Trp Ser Ser Pro Phe Asn Gly Ala Met Gly
    690                 695                 700
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<210> 94
<211> 1977
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (1)...(1977)

<400> 94

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atg gct tct gca gat aac tgg cta ggc ttc tcg ctc tcc ggc caa ggc   48
Met Ala Ser Ala Asp Asn Trp Leu Gly Phe Ser Leu Ser Gly Gln Gly
 1               5                  10                  15

aac cca cag cat cac cag aac ggc tcg ccg tct gcc gcc ggc gac gcc   96
Asn Pro Gln His His Gln Asn Gly Ser Pro Ser Ala Ala Gly Asp Ala
            20                  25                  30

gcc atc gac atc tcc ggc tca ggc gac ttc tat ggt ctg cca acg ccg  144
Ala Ile Asp Ile Ser Gly Ser Gly Asp Phe Tyr Gly Leu Pro Thr Pro
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          35                  40                  45

gac gca cac cac atc ggc atg gcg ggc gaa gac gcg ccc tat ggc gtc   192
Asp Ala His His Ile Gly Met Ala Gly Glu Asp Ala Pro Tyr Gly Val
     50                  55                  60

atg gat gct ttc aac aga ggc acc cat gaa acc caa gat tgg gcg atg   240
Met Asp Ala Phe Asn Arg Gly Thr His Glu Thr Gln Asp Trp Ala Met
 65                  70                  75                  80

agg ggt ttg gac tac ggc ggc ggc tcc tcc gac ctc tcg atg ctc gtc   288
Arg Gly Leu Asp Tyr Gly Gly Gly Ser Ser Asp Leu Ser Met Leu Val
                 85                  90                  95

ggc tcg agc ggc ggc ggg agg agg acg gtg gcc ggc gac ggc gtc ggc   336
Gly Ser Ser Gly Gly Gly Arg Arg Thr Val Ala Gly Asp Gly Val Gly
            100                 105                 110

gag gcg ccg aag ctg gag aac ttc ctc gac ggc aac tca ttc tcc gac   384
Glu Ala Pro Lys Leu Glu Asn Phe Leu Asp Gly Asn Ser Phe Ser Asp
        115                 120                 125

gtg cac ggc caa gcc gcc ggc ggg tac ctc tac tcc gga agc gct gtc   432
Val His Gly Gln Ala Ala Gly Gly Tyr Leu Tyr Ser Gly Ser Ala Val
    130                 135                 140

ggc ggc gcc ggt ggt tac agt aac ggc gga tgc ggc ggc gga acc ata   480
Gly Gly Ala Gly Gly Tyr Ser Asn Gly Gly Cys Gly Gly Gly Thr Ile
145                 150                 155                 160

gag ctg tcc atg atc aag acg tgg ctc cgg agc aac cag tcg cag cag   528
Glu Leu Ser Met Ile Lys Thr Trp Leu Arg Ser Asn Gln Ser Gln Gln
                165                 170                 175

cag cca tcg ccg ccg cag cac gct gat cag ggc atg agc acc gac gcc   576
Gln Pro Ser Pro Pro Gln His Ala Asp Gln Gly Met Ser Thr Asp Ala
            180                 185                 190

agc gcg agc agc tac gcg tgc tcc gac gtg ctg gtg ggg agc tgc ggc   624
Ser Ala Ser Ser Tyr Ala Cys Ser Asp Val Leu Val Gly Ser Cys Gly
        195                 200                 205

ggc ggc ggc gcc ggg ggc acg gcg agc tcg cat ggg cag ggc ctg gcg   672
Gly Gly Gly Ala Gly Gly Thr Ala Ser Ser His Gly Gln Gly Leu Ala
    210                 215                 220

ctg tcg atg agc acg ggg tcg gtg gcc gcc gcc gga ggg ggc ggc gcc   720
Leu Ser Met Ser Thr Gly Ser Val Ala Ala Ala Gly Gly Gly Gly Ala
225                 230                 235                 240

gtc gtc gcg gcc gag agc tcg tcg tcg gag aac aag cgg gtg gat tcg   768
Val Val Ala Ala Glu Ser Ser Ser Ser Glu Asn Lys Arg Val Asp Ser
                245                 250                 255

ccg ggc ggc gcc gtg gac ggc gcc gtc ccg agg aaa tcc atc gac acc   816
Pro Gly Gly Ala Val Asp Gly Ala Val Pro Arg Lys Ser Ile Asp Thr
            260                 265                 270

ttc ggg caa agg acg tct ata tac cga ggt gta aca agg cat aga tgg   864
Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp
        275                 280                 285

aca gga aga tat gaa gct cat ctg tgg gat aat agc tgt agg aga gaa   912
Thr Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu
    290                 295                 300

ggc caa agt cgc aag ggg aga cag gtt tat ttg ggc ggt tat gac aaa   960
Gly Gln Ser Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys
```

```
305                 310                 315                 320

gaa gat aag gcg gct cgg gct tat gat ttg gca gct cta aaa tac tgg   1008
Glu Asp Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp
                325                 330                 335

ggc acg acc aca aca aca aat ttc cca atg agt aat tat gaa aag gag   1056
Gly Thr Thr Thr Thr Thr Asn Phe Pro Met Ser Asn Tyr Glu Lys Glu
            340                 345                 350

cta gag gaa atg aaa cac atg acc agg cag gag tac att gca cat ctt   1104
Leu Glu Glu Met Lys His Met Thr Arg Gln Glu Tyr Ile Ala His Leu
        355                 360                 365

aga agg aat agc agt gga ttt tct cgt ggt gca tcc aaa tat cgt ggt   1152
Arg Arg Asn Ser Ser Gly Phe Ser Arg Gly Ala Ser Lys Tyr Arg Gly
    370                 375                 380

gtt act agg cat cat cag cat ggg aga tgg cag gca agg ata ggg cga   1200
Val Thr Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg
385                 390                 395                 400

gtt gca ggc aac aag gat atc tac cta ggc acc ttc agc acc gag gag   1248
Val Ala Gly Asn Lys Asp Ile Tyr Leu Gly Thr Phe Ser Thr Glu Glu
                405                 410                 415

gag gcc gcc gag gcg tac gac atc gcc gcc atc aag ttc cgc ggg ctc   1296
Glu Ala Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu
            420                 425                 430

aac gcc gtc acc aac ttc gac atg agc cgg tac gac gtc aag agc atc   1344
Asn Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Ser Ile
        435                 440                 445

ctg gac agc agc acg ctg ccg gtc ggc ggc gcg gcg cgg cgg ctc aag   1392
Leu Asp Ser Ser Thr Leu Pro Val Gly Gly Ala Ala Arg Arg Leu Lys
    450                 455                 460

gag gcg gag gtc gcc gcc gcc gcc gcg ggc ggc ggc gtg atc gtc tcc   1440
Glu Ala Glu Val Ala Ala Ala Ala Ala Gly Gly Gly Val Ile Val Ser
465                 470                 475                 480

cac ctg gcc gac ggc ggt gtg ggt ggg tac tac tac ggg tgc ggc ccg   1488
His Leu Ala Asp Gly Gly Val Gly Gly Tyr Tyr Tyr Gly Cys Gly Pro
                485                 490                 495

acc atc gcg ttc ggc ggc ggc ggc cag cag ccg gcg ccg ctc gcc gtg   1536
Thr Ile Ala Phe Gly Gly Gly Gly Gln Gln Pro Ala Pro Leu Ala Val
            500                 505                 510

cac tac ccg tcg tac ggc cag gcc agc ggg tgg tgc aag ccg gag cag   1584
His Tyr Pro Ser Tyr Gly Gln Ala Ser Gly Trp Cys Lys Pro Glu Gln
        515                 520                 525

gac gcg gtg atc gcg gcc ggg cac tgc gcg acg gac ctc cag cac ctg   1632
Asp Ala Val Ile Ala Ala Gly His Cys Ala Thr Asp Leu Gln His Leu
    530                 535                 540

cac ctc ggg agc ggc ggc gcc gcc gcc acc cac aac ttc ttc cag cag   1680
His Leu Gly Ser Gly Gly Ala Ala Ala Thr His Asn Phe Phe Gln Gln
545                 550                 555                 560

ccg gcg tca agc tcg gcc gtc tac ggc aac ggc ggc ggc ggc ggc ggc   1728
Pro Ala Ser Ser Ser Ala Val Tyr Gly Asn Gly Gly Gly Gly Gly Gly
                565                 570                 575

aac gcg ttc atg atg ccg atg ggc gcc gtg gtg gcc gcc gcc gat cac   1776
Asn Ala Phe Met Met Pro Met Gly Ala Val Val Ala Ala Ala Asp His
```

580 585 590

ggc ggg cag agc agc gcc tac ggc ggt ggc gac gag agc ggg agg ctc 1824
Gly Gly Gln Ser Ser Ala Tyr Gly Gly Gly Asp Glu Ser Gly Arg Leu
595 600 605

gtc gtg ggg tac gac ggc gtc gtc gac ccg tac gcg gcc atg aga agc 1872
Val Val Gly Tyr Asp Gly Val Val Asp Pro Tyr Ala Ala Met Arg Ser
610 615 620

gcg tac gag ctc tcg cag ggc tcg tcg tcg tcg tcg gtg agc gtc gcg 1920
Ala Tyr Glu Leu Ser Gln Gly Ser Ser Ser Ser Ser Val Ser Val Ala
625 630 635 640

aag gcg gcg aac ggg tac ccg gac aac tgg agc tcg ccg ttc aac ggc 1968
Lys Ala Ala Asn Gly Tyr Pro Asp Asn Trp Ser Ser Pro Phe Asn Gly
645 650 655

atg gga tga 1977
Met Gly

<210> 95
<211> 658
<212> PRT
<213> Oryza sativa

<400> 95
Met Ala Ser Ala Asp Asn Trp Leu Gly Phe Ser Leu Ser Gly Gln Gly
1 5 10 15
Asn Pro Gln His His Gln Asn Gly Ser Pro Ser Ala Ala Gly Asp Ala
20 25 30
Ala Ile Asp Ile Ser Gly Ser Gly Asp Phe Tyr Gly Leu Pro Thr Pro
35 40 45
Asp Ala His His Ile Gly Met Ala Gly Glu Asp Ala Pro Tyr Gly Val
50 55 60
Met Asp Ala Phe Asn Arg Gly Thr His Glu Thr Gln Asp Trp Ala Met
65 70 75 80
Arg Gly Leu Asp Tyr Gly Gly Gly Ser Ser Asp Leu Ser Met Leu Val
85 90 95
Gly Ser Ser Gly Gly Gly Arg Arg Thr Val Ala Gly Asp Gly Val Gly
100 105 110
Glu Ala Pro Lys Leu Glu Asn Phe Leu Asp Gly Asn Ser Phe Ser Asp
115 120 125
Val His Gly Gln Ala Ala Gly Gly Tyr Leu Tyr Ser Gly Ser Ala Val
130 135 140
Gly Gly Ala Gly Gly Tyr Ser Asn Gly Gly Cys Gly Gly Gly Thr Ile
145 150 155 160
Glu Leu Ser Met Ile Lys Thr Trp Leu Arg Ser Asn Gln Ser Gln Gln
165 170 175
Gln Pro Ser Pro Pro Gln His Ala Asp Gln Gly Met Ser Thr Asp Ala
180 185 190
Ser Ala Ser Ser Tyr Ala Cys Ser Asp Val Leu Val Gly Ser Cys Gly
195 200 205
Gly Gly Gly Ala Gly Gly Thr Ala Ser Ser His Gly Gln Gly Leu Ala
210 215 220
Leu Ser Met Ser Thr Gly Ser Val Ala Ala Ala Gly Gly Gly Gly Ala
225 230 235 240
Val Val Ala Ala Glu Ser Ser Ser Ser Glu Asn Lys Arg Val Asp Ser
245 250 255
Pro Gly Gly Ala Val Asp Gly Ala Val Pro Arg Lys Ser Ile Asp Thr
260 265 270
Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp
275 280 285
Thr Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu
290 295 300
Gly Gln Ser Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys

```
305                 310                 315                 320
Glu Asp Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp
                325                 330                 335
Gly Thr Thr Thr Thr Thr Asn Phe Pro Met Ser Asn Tyr Glu Lys Glu
            340                 345                 350
Leu Glu Glu Met Lys His Met Thr Arg Gln Glu Tyr Ile Ala His Leu
        355                 360                 365
Arg Arg Asn Ser Ser Gly Phe Ser Arg Gly Ala Ser Lys Tyr Arg Gly
    370                 375                 380
Val Thr Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg
385                 390                 395                 400
Val Ala Gly Asn Lys Asp Ile Tyr Leu Gly Thr Phe Ser Thr Glu Glu
                405                 410                 415
Glu Ala Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu
            420                 425                 430
Asn Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Ser Ile
        435                 440                 445
Leu Asp Ser Ser Thr Leu Pro Val Gly Gly Ala Ala Arg Arg Leu Lys
    450                 455                 460
Glu Ala Glu Val Ala Ala Ala Ala Ala Gly Gly Gly Val Ile Val Ser
465                 470                 475                 480
His Leu Ala Asp Gly Gly Val Gly Gly Tyr Tyr Tyr Gly Cys Gly Pro
                485                 490                 495
Thr Ile Ala Phe Gly Gly Gly Gly Gln Gln Pro Ala Pro Leu Ala Val
            500                 505                 510
His Tyr Pro Ser Tyr Gly Gln Ala Ser Gly Trp Cys Lys Pro Glu Gln
        515                 520                 525
Asp Ala Val Ile Ala Ala Gly His Cys Ala Thr Asp Leu Gln His Leu
    530                 535                 540
His Leu Gly Ser Gly Gly Ala Ala Ala Thr His Asn Phe Phe Gln Gln
545                 550                 555                 560
Pro Ala Ser Ser Ser Ala Val Tyr Gly Asn Gly Gly Gly Gly Gly Gly
                565                 570                 575
Asn Ala Phe Met Met Pro Met Gly Ala Val Val Ala Ala Ala Asp His
            580                 585                 590
Gly Gly Gln Ser Ser Ala Tyr Gly Gly Gly Asp Glu Ser Gly Arg Leu
        595                 600                 605
Val Val Gly Tyr Asp Gly Val Val Asp Pro Tyr Ala Ala Met Arg Ser
    610                 615                 620
Ala Tyr Glu Leu Ser Gln Gly Ser Ser Ser Ser Ser Val Ser Val Ala
625                 630                 635                 640
Lys Ala Ala Asn Gly Tyr Pro Asp Asn Trp Ser Ser Pro Phe Asn Gly
                645                 650                 655
Met Gly
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<210> 96
<211> 2112
<212> DNA
<213> Sorghum bicolor

<220>
<221> CDS
<222> (1)...(2112)

<400> 96

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atg gct act gtg aac aac tgg ctc gct ttc tcc ctc tcc ccg cag gag   48
Met Ala Thr Val Asn Asn Trp Leu Ala Phe Ser Leu Ser Pro Gln Glu
 1               5                  10                  15

ctg ccg ccc acc cag acg gac tcc acc ctc atc tct gcc gcc acc acc   96
Leu Pro Pro Thr Gln Thr Asp Ser Thr Leu Ile Ser Ala Ala Thr Thr
            20                  25                  30

gac gat gtc tcc ggc gat gtc tgc ttc aac atc ccc caa gat tgg agc   144
Asp Asp Val Ser Gly Asp Val Cys Phe Asn Ile Pro Gln Asp Trp Ser
        35                  40                  45
```

```
atg agg gga tcc gag ctt tcg gcg ctc gtc gcc gag ccg aag ctg gag   192
Met Arg Gly Ser Glu Leu Ser Ala Leu Val Ala Glu Pro Lys Leu Glu
     50                  55                  60

gac ttc ctc ggc gga atc tcc ttc tcc gag cag cac cac aag gcc aac   240
Asp Phe Leu Gly Gly Ile Ser Phe Ser Glu Gln His His Lys Ala Asn
 65                  70                  75                  80

tgc aac atg atc ccc agc act agc agc aca gct tgc tac gcg agc tcg   288
Cys Asn Met Ile Pro Ser Thr Ser Ser Thr Ala Cys Tyr Ala Ser Ser
                 85                  90                  95

ggt gct acc gcc ggc tac cat cac cag ctg tac cac cag ccc acc agc   336
Gly Ala Thr Ala Gly Tyr His His Gln Leu Tyr His Gln Pro Thr Ser
            100                 105                 110

tcc gcg ctc cac ttc gct gac tcc gtc atg gtg gcc tcc tcg gcc ggc   384
Ser Ala Leu His Phe Ala Asp Ser Val Met Val Ala Ser Ser Ala Gly
        115                 120                 125

ggc gtc cac gac gga ggt gcc atg ctc agc gcg gcc agc gct aat ggt   432
Gly Val His Asp Gly Gly Ala Met Leu Ser Ala Ala Ser Ala Asn Gly
    130                 135                 140

agc gct ggc gct ggc gct gcc agt gcc aat ggc agc ggc agc atc ggg   480
Ser Ala Gly Ala Gly Ala Ala Ser Ala Asn Gly Ser Gly Ser Ile Gly
145                 150                 155                 160

ctg tcc atg atc aag aac tgg ctg cgg agc caa cca gct ccc atg cag   528
Leu Ser Met Ile Lys Asn Trp Leu Arg Ser Gln Pro Ala Pro Met Gln
                165                 170                 175

ccg agg gtg gcg gcg gct gag agc gtg cag ggg ctc tct ttg tcc atg   576
Pro Arg Val Ala Ala Ala Glu Ser Val Gln Gly Leu Ser Leu Ser Met
            180                 185                 190

aac atg gcg ggg gcg acg caa ggc gcc gct ggc atg cca ctt ctt gct   624
Asn Met Ala Gly Ala Thr Gln Gly Ala Ala Gly Met Pro Leu Leu Ala
        195                 200                 205

gga gag cgc ggc cgg gcg ccc gag agt gtc tcg acg tcg gca cag ggt   672
Gly Glu Arg Gly Arg Ala Pro Glu Ser Val Ser Thr Ser Ala Gln Gly
    210                 215                 220

gga gcc gtc gtc acg gct cca aag gag gat agc ggt ggc agc ggt gtt   720
Gly Ala Val Val Thr Ala Pro Lys Glu Asp Ser Gly Gly Ser Gly Val
225                 230                 235                 240

gcc gcc acc ggc gcc cta gta gcc gtg agc acg gac acg ggt ggc agc   768
Ala Ala Thr Gly Ala Leu Val Ala Val Ser Thr Asp Thr Gly Gly Ser
                245                 250                 255

ggc gcg tcg gct gac aac acg gca agg aag acg gtg gac acg ttc ggg   816
Gly Ala Ser Ala Asp Asn Thr Ala Arg Lys Thr Val Asp Thr Phe Gly
            260                 265                 270

cag cgc acg tcg att tac cgt ggc gtg aca agg cat aga tgg act ggg   864
Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly
        275                 280                 285

aga tat gaa gca cat ctg tgg gac aac agt tgc aga agg gaa gga caa   912
Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln
    290                 295                 300

act cgc aag ggt cgt caa gtc tat tta ggt ggc tat gat aaa gag gag   960
Thr Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu
305                 310                 315                 320
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aaa gct gct agg gct tat gat ctg gct gct ctt aag tac tgg ggt ccc    1008
Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Pro
                325                 330                 335

acg aca aca aca aat ttt cca gtg aat aac tac gaa aag gag ctg gag    1056
Thr Thr Thr Thr Asn Phe Pro Val Asn Asn Tyr Glu Lys Glu Leu Glu
            340                 345                 350

gat atg aag cac atg aca agg cag gag ttt gta gcg tct ctg aga agg    1104
Asp Met Lys His Met Thr Arg Gln Glu Phe Val Ala Ser Leu Arg Arg
        355                 360                 365

aag agc agt ggt ttc tcc aga ggt gca tcc att tac agg gga gtg act    1152
Lys Ser Ser Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr
    370                 375                 380

agg cat cac cag cat gga aga tgg caa gca cgg att gga cga gtt gca    1200
Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala
385                 390                 395                 400

ggg aac aag gat ctc tac ttg ggc acc ttc agc acg cag gag gag gca    1248
Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe Ser Thr Gln Glu Glu Ala
                405                 410                 415

gcg gag gca tac gac att gcg gcg atc aag ttc cgc ggc ctc aac gcc    1296
Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Asn Ala
            420                 425                 430

gtc aca aac ttc gac atg agc cgc tac gac gtc aag agc atc ctg gac    1344
Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Ser Ile Leu Asp
        435                 440                 445

agc agt gcg ctc ccc atc ggc agc gcc gcc aag cgt ctc aag gag gcc    1392
Ser Ser Ala Leu Pro Ile Gly Ser Ala Ala Lys Arg Leu Lys Glu Ala
    450                 455                 460

gag gcc gcc gcg tcc gca cag cac cat gcc ggc gtg gtg agc tac gac    1440
Glu Ala Ala Ala Ser Ala Gln His His Ala Gly Val Val Ser Tyr Asp
465                 470                 475                 480

gtc ggc cgc ata gcc tca cag ctc ggc gac ggc ggc gcc ctg gcg gcg    1488
Val Gly Arg Ile Ala Ser Gln Leu Gly Asp Gly Gly Ala Leu Ala Ala
                485                 490                 495

gcg tac ggc gcg cac tac cat ggc gcc tgg ccg acc atc gcg ttc cag    1536
Ala Tyr Gly Ala His Tyr His Gly Ala Trp Pro Thr Ile Ala Phe Gln
            500                 505                 510

ccg agc gcg gcc acg ggc ctg tac cac ccg tac gcg cag ccg atg cgc    1584
Pro Ser Ala Ala Thr Gly Leu Tyr His Pro Tyr Ala Gln Pro Met Arg
        515                 520                 525

ggg tgg tgc aag cag gag cag gac cac gcg gtg atc gcg gcc gcg cac    1632
Gly Trp Cys Lys Gln Glu Gln Asp His Ala Val Ile Ala Ala Ala His
    530                 535                 540

agc ctg cag gag ctc cac cac ctg aac ctg ggt gct gcc gcc ggc gcg    1680
Ser Leu Gln Glu Leu His His Leu Asn Leu Gly Ala Ala Ala Gly Ala
545                 550                 555                 560

cac gac ttc ttc tcg gcg ggg cag cag gcg gcg atg cac ggc ctg ggt    1728
His Asp Phe Phe Ser Ala Gly Gln Gln Ala Ala Met His Gly Leu Gly
                565                 570                 575

agc atg gac aat gca tca ctc gag cac agc acc ggc tcc aac tcc gtc    1776
Ser Met Asp Asn Ala Ser Leu Glu His Ser Thr Gly Ser Asn Ser Val
            580                 585                 590
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gtg tac aac ggt gtt ggt gat agc aac ggc agc acc gtc gtc ggc agt    1824
Val Tyr Asn Gly Val Gly Asp Ser Asn Gly Ser Thr Val Val Gly Ser
        595                 600                 605

ggt ggc tac atg atg cct atg agc gct gcc acg gcg acg gct acc acg    1872
Gly Gly Tyr Met Met Pro Met Ser Ala Ala Thr Ala Thr Ala Thr Thr
    610                 615                 620

gca atg gtg agc cac gag cag gtg cat gca cgg gca cag ggt gat cac    1920
Ala Met Val Ser His Glu Gln Val His Ala Arg Ala Gln Gly Asp His
625                 630                 635                 640

cac gac gaa gcc aag cag gct gct cag atg ggg tac gag agc tac ctg    1968
His Asp Glu Ala Lys Gln Ala Ala Gln Met Gly Tyr Glu Ser Tyr Leu
                645                 650                 655

gtg aac gca gag aac tat ggc ggc ggg agg atg tct gcg gcc tgg gcg    2016
Val Asn Ala Glu Asn Tyr Gly Gly Gly Arg Met Ser Ala Ala Trp Ala
            660                 665                 670

act gtc tca gcg cca ccg gcg gca agc agc aac gat aac atg gcg gac    2064
Thr Val Ser Ala Pro Pro Ala Ala Ser Ser Asn Asp Asn Met Ala Asp
        675                 680                 685

gtc ggc cat ggc ggc gca cag ctc ttc agt gtc tgg aac gat act taa    2112
Val Gly His Gly Gly Ala Gln Leu Phe Ser Val Trp Asn Asp Thr
    690                 695                 700
```

<210> 97
<211> 703
<212> PRT
<213> Sorghum bicolor

<400> 97

```
Met Ala Thr Val Asn Asn Trp Leu Ala Phe Ser Leu Ser Pro Gln Glu
 1               5                  10                  15
Leu Pro Pro Thr Gln Thr Asp Ser Thr Leu Ile Ser Ala Ala Thr Thr
            20                  25                  30
Asp Asp Val Ser Gly Asp Val Cys Phe Asn Ile Pro Gln Asp Trp Ser
        35                  40                  45
Met Arg Gly Ser Glu Leu Ser Ala Leu Val Ala Glu Pro Lys Leu Glu
    50                  55                  60
Asp Phe Leu Gly Gly Ile Ser Phe Ser Glu Gln His His Lys Ala Asn
65                  70                  75                  80
Cys Asn Met Ile Pro Ser Thr Ser Ser Thr Ala Cys Tyr Ala Ser Ser
                85                  90                  95
Gly Ala Thr Ala Gly Tyr His His Gln Leu Tyr His Gln Pro Thr Ser
            100                 105                 110
Ser Ala Leu His Phe Ala Asp Ser Val Met Val Ala Ser Ser Ala Gly
        115                 120                 125
Gly Val His Asp Gly Gly Ala Met Leu Ser Ala Ala Ser Ala Asn Gly
    130                 135                 140
Ser Ala Gly Ala Gly Ala Ala Ser Ala Asn Gly Ser Gly Ser Ile Gly
145                 150                 155                 160
Leu Ser Met Ile Lys Asn Trp Leu Arg Ser Gln Pro Ala Pro Met Gln
                165                 170                 175
Pro Arg Val Ala Ala Ala Glu Ser Val Gln Gly Leu Ser Leu Ser Met
            180                 185                 190
Asn Met Ala Gly Ala Thr Gln Gly Ala Ala Gly Met Pro Leu Leu Ala
        195                 200                 205
Gly Glu Arg Gly Arg Ala Pro Glu Ser Val Ser Thr Ser Ala Gln Gly
    210                 215                 220
Gly Ala Val Val Thr Ala Pro Lys Glu Asp Ser Gly Gly Ser Gly Val
225                 230                 235                 240
Ala Ala Thr Gly Ala Leu Val Ala Val Ser Thr Asp Thr Gly Gly Ser
```

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                    245                 250                 255
Gly Ala Ser Ala Asp Asn Thr Ala Arg Lys Thr Val Asp Thr Phe Gly
                260                 265                 270
Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp Thr Gly
            275                 280                 285
Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu Gly Gln
        290                 295                 300
Thr Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys Glu Glu
305                 310                 315                 320
Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp Gly Pro
                325                 330                 335
Thr Thr Thr Thr Asn Phe Pro Val Asn Asn Tyr Glu Lys Glu Leu Glu
            340                 345                 350
Asp Met Lys His Met Thr Arg Gln Glu Phe Val Ala Ser Leu Arg Arg
        355                 360                 365
Lys Ser Ser Gly Phe Ser Arg Gly Ala Ser Ile Tyr Arg Gly Val Thr
    370                 375                 380
Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg Val Ala
385                 390                 395                 400
Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe Ser Thr Gln Glu Glu Ala
                405                 410                 415
Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu Asn Ala
            420                 425                 430
Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Ser Ile Leu Asp
        435                 440                 445
Ser Ser Ala Leu Pro Ile Gly Ser Ala Ala Lys Arg Leu Lys Glu Ala
    450                 455                 460
Glu Ala Ala Ala Ser Ala Gln His His Ala Gly Val Val Ser Tyr Asp
465                 470                 475                 480
Val Gly Arg Ile Ala Ser Gln Leu Gly Asp Gly Gly Ala Leu Ala Ala
                485                 490                 495
Ala Tyr Gly Ala His Tyr His Gly Ala Trp Pro Thr Ile Ala Phe Gln
            500                 505                 510
Pro Ser Ala Ala Thr Gly Leu Tyr His Pro Tyr Ala Gln Pro Met Arg
        515                 520                 525
Gly Trp Cys Lys Gln Glu Gln Asp His Ala Val Ile Ala Ala Ala His
    530                 535                 540
Ser Leu Gln Glu Leu His His Leu Asn Leu Gly Ala Ala Ala Gly Ala
545                 550                 555                 560
His Asp Phe Phe Ser Ala Gly Gln Gln Ala Ala Met His Gly Leu Gly
                565                 570                 575
Ser Met Asp Asn Ala Ser Leu Glu His Ser Thr Gly Ser Asn Ser Val
            580                 585                 590
Val Tyr Asn Gly Val Gly Asp Ser Asn Gly Ser Thr Val Val Gly Ser
        595                 600                 605
Gly Gly Tyr Met Met Pro Met Ser Ala Ala Thr Ala Thr Ala Thr Thr
    610                 615                 620
Ala Met Val Ser His Glu Gln Val His Ala Arg Ala Gln Gly Asp His
625                 630                 635                 640
His Asp Glu Ala Lys Gln Ala Ala Gln Met Gly Tyr Glu Ser Tyr Leu
                645                 650                 655
Val Asn Ala Glu Asn Tyr Gly Gly Gly Arg Met Ser Ala Ala Trp Ala
            660                 665                 670
Thr Val Ser Ala Pro Pro Ala Ala Ser Ser Asn Asp Asn Met Ala Asp
        675                 680                 685
Val Gly His Gly Gly Ala Gln Leu Phe Ser Val Trp Asn Asp Thr
    690                 695                 700
```

<210> 98
<211> 3766
<212> DNA
<213> Sorghum bicolor

<400> 98
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cagacggact ccaccctcat ctctgccgcc accaccgacg atgtctccgg cgatgtctgc 120
ttcaacatcc cccaaggtat gcatctatcg atcgatatat gtacgtacag tgcgcatata 180

tatatatatc tgcagttt gt ggtacgaata ctgattgaag ctagcatgaa atgtcgttt g 240
ttctttcaga ttggagcatg aggggatccg agctttcggc gctcgtcgcc gagccgaagc 300
tggaggactt cctcggcgga atctccttct ccgagcagca ccacaaggcc aactgcaaca 360
tgatccccag cactagcagc acagcttgct acgcgagctc gggtgctacc gccggctacc 420
atcaccagct gtaccaccag cccaccagct ccgcgctcca cttcgctgac tccgtcatgg 480
tggcctcctc ggccggcggc gtccacgacg gaggtgccat gctcagcgcg gccagcgcta 540
atggtagcgc tggcgctggc gctgccagtg ccaatggcag cggcagcatc gggctgtcca 600
tgatcaagaa ctggctgcgg agccaaccag ctcccatgca gccgagggtg gcggcggctg 660
agagcgtgca ggggctcttc ttgtccatga acatggcggg ggcgacgcaa ggcgccgctg 720
gcatgccact tcttgctgga gagcgcggcc gggcgcccga gagtgtctcg acgtcggcac 780
agggtggagc cgtcgtcacg gctccaaagg aggatagcgg tggcagcggt gttgccgcca 840
ccggcgccct agtagccgtg agcacggaca cgggtggcag cggcgcgtcg gctgacaaca 900
cggcaaggaa gacggtggac acgttcgggc agcgcacgtc gattt accgt ggcgtgacaa 960
ggtaataagg gtccggtatt acaatgaatc gtcacttcgt cagagaacta aactagcaca 1020
aatcagcaat gaatcaagta atatcatgaa attt agaaaa gccgttagca atgcaaggag 1080
ctatcatttat agattt gatt gcatctagac agttctgaat taaatgagta gggcaatgtg 1140
tagccttt ga tgatctcgct gattattagg agtgccattt gtattggcta tgattgtggt 1200
atatacagca gtagacaatt aacaaaaggc taccacttt c gaattattt t aggcatagat 1260
ggactgggag atatgaagca catctgtggg acaacagttg cagaagggaa ggacaaactc 1320
gcaagggtcg tcaaggtacc aatataatgc aataccaccgt attt aaatat atatgctttt 1380
ctgtaattaa gttt tatacttt cacaaaact gacattacttt cgcattatca ttttt ggattt 1440
gtcgtcgtca tgattggcgg gattgaaatg aactattgaa tctacagtct attt aggtaa 1500
gcgatttcac ttggttatta attt gggacc aactacttaa tccagttgt ttttcccctta 1560
taaccattat ttttt catct gtgttctcaa ctcttacttt tccatcttgt tccactgata 1620
ggtggctatg ataaagagga gaaagctgct agggcttatg atctggctgc tcttaagtac 1680
tggggtccca cgacaacaac aaatttt cca gtatgtatat gtagaatgca gttttacttc 1740
actgaagatc atacctttgc tatgtctcaa atgccgttca ttagttagtg gatctgaagt 1800
gaaggttctg taatttttgt taactatgta cattgctgga attgtactta aagtcatttg 1860
ttttt gtata tctaggtgaa taactacgaa aaggagctgg aggatatgaa gcacatgaca 1920
aggcaggagt ttgtagcgtc tctgagaagg tcggtcgaac agcattgatt aatcaatgcc 1980
aactctattg aataaacatc tactctgtta attgttaaag tttgagagaa agatctgcat 2040
gttagatctt aatagaccac tgtatatgaa tgcaggaaga gcagtggttt ctccagaggt 2100
gcatccattt acaggggagt gactaggtat gaattcatat aatggcgtca acaaacacac 2160
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cgtgcatcag gtcaaaatag tactatgcct caataagaaa cacatgagca tgcactggca 2700
gcagcagact aatcaagttc tatcatttac taaataaact attaggctac agcatccaaa 2760
agattctacc catt aagcca caactgttca tgcatgcatt catt aaaccag gataccacca 2820
tgcatgcgtg caccgtgttc gtgcttggaa tattgagctg agccgagtgc acccttgcgt 2880
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cggcctcaac gccgtcacaa acttcgacat gagccgctac gacgtcaaga gcatcctgga 3000
cagcagtgcg ctccccatcg gcagcgccgc caagcgtctc aaggaggccg aggccgccgc 3060
gtccgcacag caccatgccg gcgtggtgag ctacgacgtc ggccgcatag cctcacagct 3120
cggcgacggc ggcgcccttgg cggcggcgta cggcgcgcac taccatggcg cctggccgac 3180
catcgcgttc agccgagcg cggccacggg cctgtaccac ccgtacgcgc agccgatgcg 3240
cgggtggtgc aagcaggagc aggaccacgc ggtgatcgcg gccgcgcaca gcctgcagga 3300
gctccaccac ctgaacctgg gtgctgccgc cggcgcgcac gacttcttct cggcggggca 3360
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tggtggctac atgatgcctta tgagcgctgc cacggcgacg gctaccacgg caatggtgag 3540
ccacgagcag gtgcatgcac gggcacaggg tgatcaccac gacgaagcca agcaggctgc 3600
tcagatgggg tacgagagct acctggtgaa cgcagagaac tatggcggcg ggaggatgtc 3660
tgcggcctgg gcgactgtct cagcgccacc ggcggcaagc agcaacgata acatggcgga 3720
cgtcggccat ggcggcgcac agctcttcag tgtctggaac gatact 3766

<210> 99
<211> 2082
<212> DNA
<213> Sorghum bicolor

<220>
<221> CDS

<222> (1)...(2082)

<400> 99

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atg gct tcg acg aac aac cac tgg ctg ggt ttc tcg ctc tcg ggc cag   48
Met Ala Ser Thr Asn Asn His Trp Leu Gly Phe Ser Leu Ser Gly Gln
 1               5                  10                  15

gat aac ccg cag cct aat cat cag gac agc tcg cct gcc gcc gcc ggc   96
Asp Asn Pro Gln Pro Asn His Gln Asp Ser Ser Pro Ala Ala Ala Gly
            20                  25                  30

atc gac atc tcc ggc gcc agc gac ttc tat ggc ttg ccc acg cag cag  144
Ile Asp Ile Ser Gly Ala Ser Asp Phe Tyr Gly Leu Pro Thr Gln Gln
        35                  40                  45

ggc tcc gac ggg aat ctc ggc gtg ccg ggc ctg cgg gac gat cac gct  192
Gly Ser Asp Gly Asn Leu Gly Val Pro Gly Leu Arg Asp Asp His Ala
    50                  55                  60

tct tat ggc atc atg gag gcc ttc aac agg gtt cct caa gaa acc caa  240
Ser Tyr Gly Ile Met Glu Ala Phe Asn Arg Val Pro Gln Glu Thr Gln
65                  70                  75                  80

gat tgg aac atg agg gga ttg gac tac aac ggc ggt ggc tcg gaa ctc  288
Asp Trp Asn Met Arg Gly Leu Asp Tyr Asn Gly Gly Gly Ser Glu Leu
                85                  90                  95

tcg atg ctt gtg ggg tcc agc ggc ggc ggc ggg ggc ggc ggc aag agg  336
Ser Met Leu Val Gly Ser Ser Gly Gly Gly Gly Gly Gly Gly Lys Arg
            100                 105                 110

gcc gtg gaa gac agc gag ccc aag ctc gaa gat ttc ctc ggc ggc aac  384
Ala Val Glu Asp Ser Glu Pro Lys Leu Glu Asp Phe Leu Gly Gly Asn
        115                 120                 125

tcg ttc gtc tcc gag cat gat cag tcc ggc ggt tac ctg ttc tct gga  432
Ser Phe Val Ser Glu His Asp Gln Ser Gly Gly Tyr Leu Phe Ser Gly
    130                 135                 140

gtc ccg atg gcc agc agc acc aac agc aac agc ggg agc aac acc atg  480
Val Pro Met Ala Ser Ser Thr Asn Ser Asn Ser Gly Ser Asn Thr Met
145                 150                 155                 160

gag ctc tcc atg atc aag acc tgg ctc cgg aac aac cag gtg ccc cag  528
Glu Leu Ser Met Ile Lys Thr Trp Leu Arg Asn Asn Gln Val Pro Gln
                165                 170                 175

ccg cag ccg cca gca gct ccg cat cag gcg ccg cag act gag gag atg  576
Pro Gln Pro Pro Ala Ala Pro His Gln Ala Pro Gln Thr Glu Glu Met
            180                 185                 190

agc acc gac gcc aac gcc agc gcc agc agc ttt ggc tgc tcg gat tcg  624
Ser Thr Asp Ala Asn Ala Ser Ala Ser Ser Phe Gly Cys Ser Asp Ser
        195                 200                 205

atg ggg agg aac ggc acg gtg gcg gct gct ggg agc tcc cag agc ctg  672
Met Gly Arg Asn Gly Thr Val Ala Ala Ala Gly Ser Ser Gln Ser Leu
    210                 215                 220

gcg ctc tcg atg agc acg ggc tcg cac ctg ccg atg gtt gtg gcc ggc  720
Ala Leu Ser Met Ser Thr Gly Ser His Leu Pro Met Val Val Ala Gly
225                 230                 235                 240

ggc ggc gcc agc gga gcg gcc tcg gag agc acg tca tcg gag aac aag  768
Gly Gly Ala Ser Gly Ala Ala Ser Glu Ser Thr Ser Ser Glu Asn Lys
                245                 250                 255

cga gcg agc ggc gcc atg gat tcg ccc ggc agc gcg gta gaa gcc gtc  816
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```
Arg Ala Ser Gly Ala Met Asp Ser Pro Gly Ser Ala Val Glu Ala Val
                260                 265                 270

ccg agg aag tcc atc gac acg ttc ggg caa agg acc tct ata tat cga   864
Pro Arg Lys Ser Ile Asp Thr Phe Gly Gln Arg Thr Ser Ile Tyr Arg
        275                 280                 285

ggt gta aca aga cat aga tgg aca ggg cga tat gag gct cat cta tgg   912
Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His Leu Trp
    290                 295                 300

gat aat agt tgt aga aga gaa ggg cag agt cgc aag ggt agg caa gtt   960
Asp Asn Ser Cys Arg Arg Glu Gly Gln Ser Arg Lys Gly Arg Gln Val
305                 310                 315                 320

tac ctt ggt ggc tat gac aag gaa gac aag gca gca agg gct tat gat  1008
Tyr Leu Gly Gly Tyr Asp Lys Glu Asp Lys Ala Ala Arg Ala Tyr Asp
                325                 330                 335

ttg gca gct ctc aag tat tgg ggc act act aca aca aca aat ttc cct  1056
Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr Thr Asn Phe Pro
            340                 345                 350

ata agc aac tat gaa aag gag cta gag gaa atg aaa cat atg act agg  1104
Ile Ser Asn Tyr Glu Lys Glu Leu Glu Glu Met Lys His Met Thr Arg
        355                 360                 365

cag gag tat att gca tac cta aga aga aat agc agt gga ttt tct cgt  1152
Gln Glu Tyr Ile Ala Tyr Leu Arg Arg Asn Ser Ser Gly Phe Ser Arg
    370                 375                 380

ggc gca tca aaa tat cgt gga gta act aga cat cat cag cat ggg aga  1200
Gly Ala Ser Lys Tyr Arg Gly Val Thr Arg His His Gln His Gly Arg
385                 390                 395                 400

tgg caa gca agg ata ggg aga gtt gca gga aac aag gat ctc tac ttg  1248
Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu
                405                 410                 415

ggc aca ttc agc acc gag gag gag gcg gcg gag gcc tac gac atc gcc  1296
Gly Thr Phe Ser Thr Glu Glu Glu Ala Ala Glu Ala Tyr Asp Ile Ala
            420                 425                 430

gcg atc aag ttc cgc ggt ctg aac gcc gtc acc aac ttc gac atg agc  1344
Ala Ile Lys Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Asp Met Ser
        435                 440                 445

cgc tac gac gtc aag agc atc ctc gag agc agc acg ctg cct gtc ggc  1392
Arg Tyr Asp Val Lys Ser Ile Leu Glu Ser Ser Thr Leu Pro Val Gly
    450                 455                 460

ggc gcg gcc agg cgc ctc aag gat gcc gtg gac cac gtg gag gcc ggc  1440
Gly Ala Ala Arg Arg Leu Lys Asp Ala Val Asp His Val Glu Ala Gly
465                 470                 475                 480

gcc acc atc tgg cgc gcc gac atg gac ggc ggc gtg atc tcc cag ctc  1488
Ala Thr Ile Trp Arg Ala Asp Met Asp Gly Gly Val Ile Ser Gln Leu
                485                 490                 495

gcc gaa gcc ggg atg ggc ggc tac gcc tcg tac ggg cac cac gcc tgg  1536
Ala Glu Ala Gly Met Gly Gly Tyr Ala Ser Tyr Gly His His Ala Trp
            500                 505                 510

ccg acc atc gcg ttc cag cag ccg tcg ccg ctc tcc gtc cac tac ccg  1584
Pro Thr Ile Ala Phe Gln Gln Pro Ser Pro Leu Ser Val His Tyr Pro
        515                 520                 525

tac ggg cag ccg ccg tcc cgc ggg tgg tgc aag ccc gag cag gac gcg  1632
```

```
Tyr Gly Gln Pro Pro Ser Arg Gly Trp Cys Lys Pro Glu Gln Asp Ala
    530                 535                 540

gcc gtc gcc gcc gcc gcg cac agc ctg cag gac ctc cag cag ctg cac   1680
Ala Val Ala Ala Ala Ala His Ser Leu Gln Asp Leu Gln Gln Leu His
545                 550                 555                 560

ctc ggc agc gcg gca cac aac ttc ttc cag gcg tcg tcg agc tcg gca   1728
Leu Gly Ser Ala Ala His Asn Phe Phe Gln Ala Ser Ser Ser Ser Ala
                565                 570                 575

gtc tac aac agc ggc ggc ggc ggc gct agc ggc ggg tac cac cag ggc   1776
Val Tyr Asn Ser Gly Gly Gly Gly Ala Ser Gly Gly Tyr His Gln Gly
            580                 585                 590

ctc ggt ggc ggc agc agc tcc ttc ctc atg ccg tcg agc act gtc gtg   1824
Leu Gly Gly Gly Ser Ser Ser Phe Leu Met Pro Ser Ser Thr Val Val
        595                 600                 605

gcg ggg gcc gac cag ggg cac agc agc agc acg gcc aac cag ggg agc   1872
Ala Gly Ala Asp Gln Gly His Ser Ser Ser Thr Ala Asn Gln Gly Ser
    610                 615                 620

acg tgc agc tac ggg gac gat cac cag gaa ggg aag ctc atc ggg tac   1920
Thr Cys Ser Tyr Gly Asp Asp His Gln Glu Gly Lys Leu Ile Gly Tyr
625                 630                 635                 640

gac gcc atg gtg gcg gcg acc gca gcc ggc ggg gac ccg tac gcc gcg   1968
Asp Ala Met Val Ala Ala Thr Ala Ala Gly Gly Asp Pro Tyr Ala Ala
                645                 650                 655

gcg agg agc ggg tac cag ttc tcg tcg cag ggc tcg gga tcc acg gtg   2016
Ala Arg Ser Gly Tyr Gln Phe Ser Ser Gln Gly Ser Gly Ser Thr Val
            660                 665                 670

agc atc gcg agg gcg aac ggg tac tct aac aac tgg agc tct cct ttc   2064
Ser Ile Ala Arg Ala Asn Gly Tyr Ser Asn Asn Trp Ser Ser Pro Phe
        675                 680                 685

aac ggc ggc atg ggg tga                                          2082
Asn Gly Gly Met Gly
    690
```

<210> 100
<211> 693
<212> PRT
<213> Sorghum bicolor

<400> 100

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Met Ala Ser Thr Asn Asn His Trp Leu Gly Phe Ser Leu Ser Gly Gln
 1               5                  10                  15
Asp Asn Pro Gln Pro Asn His Gln Asp Ser Ser Pro Ala Ala Ala Gly
            20                  25                  30
Ile Asp Ile Ser Gly Ala Ser Asp Phe Tyr Gly Leu Pro Thr Gln Gln
        35                  40                  45
Gly Ser Asp Gly Asn Leu Gly Val Pro Gly Leu Arg Asp Asp His Ala
    50                  55                  60
Ser Tyr Gly Ile Met Glu Ala Phe Asn Arg Val Pro Gln Glu Thr Gln
65                  70                  75                  80
Asp Trp Asn Met Arg Gly Leu Asp Tyr Asn Gly Gly Gly Ser Glu Leu
                85                  90                  95
Ser Met Leu Val Gly Ser Ser Gly Gly Gly Gly Gly Gly Gly Lys Arg
            100                 105                 110
Ala Val Glu Asp Ser Glu Pro Lys Leu Glu Asp Phe Leu Gly Gly Asn
        115                 120                 125
Ser Phe Val Ser Glu His Asp Gln Ser Gly Gly Tyr Leu Phe Ser Gly
    130                 135                 140
```

Val Pro Met Ala Ser Ser Thr Asn Ser Asn Ser Gly Ser Asn Thr Met
145 150 155 160
Glu Leu Ser Met Ile Lys Thr Trp Leu Arg Asn Asn Gln Val Pro Gln
165 170 175
Pro Gln Pro Pro Ala Ala Pro His Gln Ala Pro Gln Thr Glu Glu Met
180 185 190
Ser Thr Asp Ala Asn Ala Ser Ala Ser Ser Phe Gly Cys Ser Asp Ser
195 200 205
Met Gly Arg Asn Gly Thr Val Ala Ala Ala Gly Ser Ser Gln Ser Leu
210 215 220
Ala Leu Ser Met Ser Thr Gly Ser His Leu Pro Met Val Val Ala Gly
225 230 235 240
Gly Gly Ala Ser Gly Ala Ala Ser Glu Ser Thr Ser Ser Glu Asn Lys
245 250 255
Arg Ala Ser Gly Ala Met Asp Ser Pro Gly Ser Ala Val Glu Ala Val
260 265 270
Pro Arg Lys Ser Ile Asp Thr Phe Gly Gln Arg Thr Ser Ile Tyr Arg
275 280 285
Gly Val Thr Arg His Arg Trp Thr Gly Arg Tyr Glu Ala His Leu Trp
290 295 300
Asp Asn Ser Cys Arg Arg Glu Gly Gln Ser Arg Lys Gly Arg Gln Val
305 310 315 320
Tyr Leu Gly Gly Tyr Asp Lys Glu Asp Lys Ala Ala Arg Ala Tyr Asp
325 330 335
Leu Ala Ala Leu Lys Tyr Trp Gly Thr Thr Thr Thr Thr Asn Phe Pro
340 345 350
Ile Ser Asn Tyr Glu Lys Glu Leu Glu Glu Met Lys His Met Thr Arg
355 360 365
Gln Glu Tyr Ile Ala Tyr Leu Arg Arg Asn Ser Ser Gly Phe Ser Arg
370 375 380
Gly Ala Ser Lys Tyr Arg Gly Val Thr Arg His His Gln His Gly Arg
385 390 395 400
Trp Gln Ala Arg Ile Gly Arg Val Ala Gly Asn Lys Asp Leu Tyr Leu
405 410 415
Gly Thr Phe Ser Thr Glu Glu Glu Ala Ala Glu Ala Tyr Asp Ile Ala
420 425 430
Ala Ile Lys Phe Arg Gly Leu Asn Ala Val Thr Asn Phe Asp Met Ser
435 440 445
Arg Tyr Asp Val Lys Ser Ile Leu Glu Ser Ser Thr Leu Pro Val Gly
450 455 460
Gly Ala Ala Arg Arg Leu Lys Asp Ala Val Asp His Val Glu Ala Gly
465 470 475 480
Ala Thr Ile Trp Arg Ala Asp Met Asp Gly Gly Val Ile Ser Gln Leu
485 490 495
Ala Glu Ala Gly Met Gly Gly Tyr Ala Ser Tyr Gly His His Ala Trp
500 505 510
Pro Thr Ile Ala Phe Gln Gln Pro Ser Pro Leu Ser Val His Tyr Pro
515 520 525
Tyr Gly Gln Pro Pro Ser Arg Gly Trp Cys Lys Pro Glu Gln Asp Ala
530 535 540
Ala Val Ala Ala Ala Ala His Ser Leu Gln Asp Leu Gln Gln Leu His
545 550 555 560
Leu Gly Ser Ala Ala His Asn Phe Phe Gln Ala Ser Ser Ser Ser Ala
565 570 575
Val Tyr Asn Ser Gly Gly Gly Gly Ala Ser Gly Gly Tyr His Gln Gly
580 585 590
Leu Gly Gly Gly Ser Ser Ser Phe Leu Met Pro Ser Ser Thr Val Val
595 600 605
Ala Gly Ala Asp Gln Gly His Ser Ser Ser Thr Ala Asn Gln Gly Ser
610 615 620
Thr Cys Ser Tyr Gly Asp Asp His Gln Glu Gly Lys Leu Ile Gly Tyr
625 630 635 640
Asp Ala Met Val Ala Ala Thr Ala Ala Gly Gly Asp Pro Tyr Ala Ala
645 650 655
Ala Arg Ser Gly Tyr Gln Phe Ser Ser Gln Gly Ser Gly Ser Thr Val
660 665 670
Ser Ile Ala Arg Ala Asn Gly Tyr Ser Asn Asn Trp Ser Ser Pro Phe
675 680 685

Asn Gly Gly Met Gly
690

<210> 101
<211> 2040
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)...(2040)

<400> 101

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atg gct tca gcg aac aac tgg ctg ggc ttc tcg ctc tcg ggc cag gat   48
Met Ala Ser Ala Asn Asn Trp Leu Gly Phe Ser Leu Ser Gly Gln Asp
 1               5                  10                  15

aac ccg cag cct aac cag gat agc tcg cct gcc gcc ggt atc gac atc   96
Asn Pro Gln Pro Asn Gln Asp Ser Ser Pro Ala Ala Gly Ile Asp Ile
            20                  25                  30

tcc ggc gcc agc gac ttc tat ggc ctg ccc acg cag cag ggc tcc gac  144
Ser Gly Ala Ser Asp Phe Tyr Gly Leu Pro Thr Gln Gln Gly Ser Asp
        35                  40                  45

ggg cat ctc ggc gtg ccg ggc ctg cgg gac gat cac gct tct tat ggt  192
Gly His Leu Gly Val Pro Gly Leu Arg Asp Asp His Ala Ser Tyr Gly
    50                  55                  60

atc atg gag gcc tac aac agg gtt cct caa gaa acc caa gat tgg aac  240
Ile Met Glu Ala Tyr Asn Arg Val Pro Gln Glu Thr Gln Asp Trp Asn
65                  70                  75                  80

atg agg ggc ttg gac tac aac ggc ggt ggc tcg gag ctc tcg atg ctt  288
Met Arg Gly Leu Asp Tyr Asn Gly Gly Gly Ser Glu Leu Ser Met Leu
                85                  90                  95

gtg ggg tcc agc ggc ggc ggc ggg ggc aac ggc aag agg gcc gtg gaa  336
Val Gly Ser Ser Gly Gly Gly Gly Gly Asn Gly Lys Arg Ala Val Glu
            100                 105                 110

gac agc gag ccc aag ctc gaa gat ttc ctc ggc ggc aac tcg ttc gtc  384
Asp Ser Glu Pro Lys Leu Glu Asp Phe Leu Gly Gly Asn Ser Phe Val
        115                 120                 125

tcc gat caa gat cag tcc ggc ggt tac ctg ttc tct gga gtc ccg ata  432
Ser Asp Gln Asp Gln Ser Gly Gly Tyr Leu Phe Ser Gly Val Pro Ile
    130                 135                 140

gcc agc agc gcc aat agc aac agc ggg agc aac acc atg gag ctc tcc  480
Ala Ser Ser Ala Asn Ser Asn Ser Gly Ser Asn Thr Met Glu Leu Ser
145                 150                 155                 160

atg atc aag acc tgg cta cgg aac aac cag gtg gcc cag ccc cag ccg  528
Met Ile Lys Thr Trp Leu Arg Asn Asn Gln Val Ala Gln Pro Gln Pro
                165                 170                 175

cca gct cca cat cag ccg cag cct gag gaa atg agc acc gac gcc agc  576
Pro Ala Pro His Gln Pro Gln Pro Glu Glu Met Ser Thr Asp Ala Ser
            180                 185                 190

ggc agc agc ttt gga tgc tcg gat tcg atg gga agg aac agc atg gtg  624
Gly Ser Ser Phe Gly Cys Ser Asp Ser Met Gly Arg Asn Ser Met Val
        195                 200                 205

gcg gct ggt ggg agc tcg cag agc ctg gcg ctc tcg atg agc acg ggc  672
Ala Ala Gly Gly Ser Ser Gln Ser Leu Ala Leu Ser Met Ser Thr Gly
```

```
    210                 215                 220

tcg cac ctg ccc atg gtt gtg ccc agc ggc gcc gcc agc gga gcg gcc   720
Ser His Leu Pro Met Val Val Pro Ser Gly Ala Ala Ser Gly Ala Ala
225                 230                 235                 240

tcg gag agc aca tcg tcg gag aac aag cga gcg agc ggt gcc atg gat   768
Ser Glu Ser Thr Ser Ser Glu Asn Lys Arg Ala Ser Gly Ala Met Asp
                245                 250                 255

tcg ccc ggc agc gcg gta gaa gcc gta ccg agg aag tcc atc gac acg   816
Ser Pro Gly Ser Ala Val Glu Ala Val Pro Arg Lys Ser Ile Asp Thr
            260                 265                 270

ttc ggg caa agg acc tct ata tat cga ggt gta aca agg cat aga tgg   864
Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp
        275                 280                 285

aca ggg cgg tat gag gct cat cta tgg gat aat agt tgt aga agg gaa   912
Thr Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu
    290                 295                 300

ggg cag agt cgc aag ggt agg caa gtt tac ctt ggt ggc tat gac aag   960
Gly Gln Ser Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys
305                 310                 315                 320

gag gac aag gca gca agg gct tat gat ttg gca gct ctc aag tat tgg  1008
Glu Asp Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp
                325                 330                 335

ggc act acg aca aca aca aat ttc cct ata agc aac tac gaa aag gag  1056
Gly Thr Thr Thr Thr Thr Asn Phe Pro Ile Ser Asn Tyr Glu Lys Glu
            340                 345                 350

cta gaa gaa atg aaa cat atg act aga cag gag tac att gca tac cta  1104
Leu Glu Glu Met Lys His Met Thr Arg Gln Glu Tyr Ile Ala Tyr Leu
        355                 360                 365

aga aga aat agc agt gga ttt tct cgt ggg gcg tca aag tat cgt gga  1152
Arg Arg Asn Ser Ser Gly Phe Ser Arg Gly Ala Ser Lys Tyr Arg Gly
    370                 375                 380

gta act aga cat cat cag cat ggg aga tgg caa gca agg ata ggg aga  1200
Val Thr Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg
385                 390                 395                 400

gtt gca gga aac aag gat ctc tac ttg ggc aca ttc agc acc gag gag  1248
Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe Ser Thr Glu Glu
                405                 410                 415

gag gcg gcg gag gcc tac gac atc gcc gcg atc aag ttc cgc ggt ctc  1296
Glu Ala Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu
            420                 425                 430

aac gcc gtc acc aac ttc gac atg agc cgc tac gac gtg aag agc atc  1344
Asn Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Ser Ile
        435                 440                 445

ctc gag agc agc aca ctg cct gtc ggc ggt gcg gcc agg cgc ctc aag  1392
Leu Glu Ser Ser Thr Leu Pro Val Gly Gly Ala Ala Arg Arg Leu Lys
    450                 455                 460

gac gcc gtg gac cac gtg gag gcc ggc gcc acc atc tgg cgc gcc gac  1440
Asp Ala Val Asp His Val Glu Ala Gly Ala Thr Ile Trp Arg Ala Asp
465                 470                 475                 480

atg gac ggc gcc gtg atc tcc cag ctg gcc gaa gcc ggg atg ggc ggc  1488
Met Asp Gly Ala Val Ile Ser Gln Leu Ala Glu Ala Gly Met Gly Gly
```

```
                    485                 490                 495

tac gcc tcg tac ggc cac cac ggc tgg ccg acc atc gcg ttc cag cag   1536
Tyr Ala Ser Tyr Gly His His Gly Trp Pro Thr Ile Ala Phe Gln Gln
            500                 505                 510

ccg tcg ccg ctc tcc gtc cac tac ccg tac ggc cag ccg tcc cgc ggg   1584
Pro Ser Pro Leu Ser Val His Tyr Pro Tyr Gly Gln Pro Ser Arg Gly
        515                 520                 525

tgg tgc aaa ccc gag cag gac gcg gcc gcc gcc gcg gcg cac agc ctg   1632
Trp Cys Lys Pro Glu Gln Asp Ala Ala Ala Ala Ala Ala His Ser Leu
    530                 535                 540

cag gac ctc cag cag ctg cac ctc ggc agc gcg gcc cac aac ttc ttc   1680
Gln Asp Leu Gln Gln Leu His Leu Gly Ser Ala Ala His Asn Phe Phe
545                 550                 555                 560

cag gcg tcg tcg agc tcc aca gtc tac aac ggc ggc gcc ggc gcc agt   1728
Gln Ala Ser Ser Ser Ser Thr Val Tyr Asn Gly Gly Ala Gly Ala Ser
                565                 570                 575

ggt ggg tac cag ggc ctc ggt ggt ggc agc tct ttc ctc atg ccg tcg   1776
Gly Gly Tyr Gln Gly Leu Gly Gly Gly Ser Ser Phe Leu Met Pro Ser
            580                 585                 590

agc act gtc gtg gcg gcg gcc gac cag ggg cac agc agc acg gcc aac   1824
Ser Thr Val Val Ala Ala Ala Asp Gln Gly His Ser Ser Thr Ala Asn
        595                 600                 605

cag ggg agc acg tgc agc tac ggg gac gac cac cag gag ggg aag ctc   1872
Gln Gly Ser Thr Cys Ser Tyr Gly Asp Asp His Gln Glu Gly Lys Leu
    610                 615                 620

atc ggt tac gac gcc gcc atg gtg gcg acc gca gct ggt gga gac ccg   1920
Ile Gly Tyr Asp Ala Ala Met Val Ala Thr Ala Ala Gly Gly Asp Pro
625                 630                 635                 640

tac gct gcg gcg agg aac ggg tac cag ttc tcg cag ggc tcg gga tcc   1968
Tyr Ala Ala Ala Arg Asn Gly Tyr Gln Phe Ser Gln Gly Ser Gly Ser
                645                 650                 655

acg gtg agc atc gcg agg gcg aac ggg tac gct aac aac tgg agc tct   2016
Thr Val Ser Ile Ala Arg Ala Asn Gly Tyr Ala Asn Asn Trp Ser Ser
            660                 665                 670

cct ttc aac aac ggc atg ggg tga                                  2040
Pro Phe Asn Asn Gly Met Gly
        675
```

<210> 102
<211> 679
<212> PRT
<213> Zea mays

<400> 102

```
Met Ala Ser Ala Asn Asn Trp Leu Gly Phe Ser Leu Ser Gly Gln Asp
 1               5                  10                  15
Asn Pro Gln Pro Asn Gln Asp Ser Ser Pro Ala Ala Gly Ile Asp Ile
            20                  25                  30
Ser Gly Ala Ser Asp Phe Tyr Gly Leu Pro Thr Gln Gln Gly Ser Asp
        35                  40                  45
Gly His Leu Gly Val Pro Gly Leu Arg Asp Asp His Ala Ser Tyr Gly
    50                  55                  60
Ile Met Glu Ala Tyr Asn Arg Val Pro Gln Glu Thr Gln Asp Trp Asn
65                  70                  75                  80
Met Arg Gly Leu Asp Tyr Asn Gly Gly Gly Ser Glu Leu Ser Met Leu
```

85 90 95
Val Gly Ser Ser Gly Gly Gly Gly Gly Asn Gly Lys Arg Ala Val Glu
100 105 110
Asp Ser Glu Pro Lys Leu Glu Asp Phe Leu Gly Gly Asn Ser Phe Val
115 120 125
Ser Asp Gln Asp Gln Ser Gly Gly Tyr Leu Phe Ser Gly Val Pro Ile
130 135 140
Ala Ser Ser Ala Asn Ser Asn Ser Gly Ser Asn Thr Met Glu Leu Ser
145 150 155 160
Met Ile Lys Thr Trp Leu Arg Asn Asn Gln Val Ala Gln Pro Gln Pro
165 170 175
Pro Ala Pro His Gln Pro Gln Pro Glu Glu Met Ser Thr Asp Ala Ser
180 185 190
Gly Ser Ser Phe Gly Cys Ser Asp Ser Met Gly Arg Asn Ser Met Val
195 200 205
Ala Ala Gly Gly Ser Ser Gln Ser Leu Ala Leu Ser Met Ser Thr Gly
210 215 220
Ser His Leu Pro Met Val Val Pro Ser Gly Ala Ala Ser Gly Ala Ala
225 230 235 240
Ser Glu Ser Thr Ser Ser Glu Asn Lys Arg Ala Ser Gly Ala Met Asp
245 250 255
Ser Pro Gly Ser Ala Val Glu Ala Val Pro Arg Lys Ser Ile Asp Thr
260 265 270
Phe Gly Gln Arg Thr Ser Ile Tyr Arg Gly Val Thr Arg His Arg Trp
275 280 285
Thr Gly Arg Tyr Glu Ala His Leu Trp Asp Asn Ser Cys Arg Arg Glu
290 295 300
Gly Gln Ser Arg Lys Gly Arg Gln Val Tyr Leu Gly Gly Tyr Asp Lys
305 310 315 320
Glu Asp Lys Ala Ala Arg Ala Tyr Asp Leu Ala Ala Leu Lys Tyr Trp
325 330 335
Gly Thr Thr Thr Thr Thr Asn Phe Pro Ile Ser Asn Tyr Glu Lys Glu
340 345 350
Leu Glu Glu Met Lys His Met Thr Arg Gln Glu Tyr Ile Ala Tyr Leu
355 360 365
Arg Arg Asn Ser Ser Gly Phe Ser Arg Gly Ala Ser Lys Tyr Arg Gly
370 375 380
Val Thr Arg His His Gln His Gly Arg Trp Gln Ala Arg Ile Gly Arg
385 390 395 400
Val Ala Gly Asn Lys Asp Leu Tyr Leu Gly Thr Phe Ser Thr Glu Glu
405 410 415
Glu Ala Ala Glu Ala Tyr Asp Ile Ala Ala Ile Lys Phe Arg Gly Leu
420 425 430
Asn Ala Val Thr Asn Phe Asp Met Ser Arg Tyr Asp Val Lys Ser Ile
435 440 445
Leu Glu Ser Ser Thr Leu Pro Val Gly Gly Ala Ala Arg Arg Leu Lys
450 455 460
Asp Ala Val Asp His Val Glu Ala Gly Ala Thr Ile Trp Arg Ala Asp
465 470 475 480
Met Asp Gly Ala Val Ile Ser Gln Leu Ala Glu Ala Gly Met Gly Gly
485 490 495
Tyr Ala Ser Tyr Gly His His Gly Trp Pro Thr Ile Ala Phe Gln Gln
500 505 510
Pro Ser Pro Leu Ser Val His Tyr Pro Tyr Gly Gln Pro Ser Arg Gly
515 520 525
Trp Cys Lys Pro Glu Gln Asp Ala Ala Ala Ala Ala Ala His Ser Leu
530 535 540
Gln Asp Leu Gln Gln Leu His Leu Gly Ser Ala Ala His Asn Phe Phe
545 550 555 560
Gln Ala Ser Ser Ser Ser Thr Val Tyr Asn Gly Gly Ala Gly Ala Ser
565 570 575
Gly Gly Tyr Gln Gly Leu Gly Gly Gly Ser Ser Phe Leu Met Pro Ser
580 585 590
Ser Thr Val Val Ala Ala Ala Asp Gln Gly His Ser Ser Thr Ala Asn
595 600 605
Gln Gly Ser Thr Cys Ser Tyr Gly Asp Asp His Gln Glu Gly Lys Leu
610 615 620
Ile Gly Tyr Asp Ala Ala Met Val Ala Thr Ala Ala Gly Gly Asp Pro

```
625                 630                 635                 640
Tyr Ala Ala Ala Arg Asn Gly Tyr Gln Phe Ser Gln Gly Ser Gly Ser
                645                 650                 655
Thr Val Ser Ile Ala Arg Ala Asn Gly Tyr Ala Asn Asn Trp Ser Ser
            660                 665                 670
Pro Phe Asn Asn Gly Met Gly
        675
```

<210> 103
<211> 975
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)...(975)

<400> 103

```
atg gag acg cca cag cag caa tcc gcc gcc gcc gcc gcc gcc gcc gcc   48
Met Glu Thr Pro Gln Gln Gln Ser Ala Ala Ala Ala Ala Ala Ala Ala
 1               5                  10                  15

cac ggg cag gac gac ggc ggg tcg ccg ccg atg tcg ccg gcc tcc gcc   96
His Gly Gln Asp Asp Gly Gly Ser Pro Pro Met Ser Pro Ala Ser Ala
            20                  25                  30

gcg gcg gcg gcg ctg gcg aac gcg cgg tgg aac ccg acc aag gag cag   144
Ala Ala Ala Ala Leu Ala Asn Ala Arg Trp Asn Pro Thr Lys Glu Gln
        35                  40                  45

gtg gcc gtg ctg gag ggg ctg tac gag cac ggc ctg cgc acc ccc agc   192
Val Ala Val Leu Glu Gly Leu Tyr Glu His Gly Leu Arg Thr Pro Ser
    50                  55                  60

gcg gag cag ata cag cag atc acg ggc agg ctg cgg gag cac ggc gcc   240
Ala Glu Gln Ile Gln Gln Ile Thr Gly Arg Leu Arg Glu His Gly Ala
65                  70                  75                  80

atc gag ggc aag aac gtc ttc tac tgg ttc cag aac cac aag gcc cgc   288
Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg
                85                  90                  95

cag cgc cag agg cag aag cag gac agc ttc gcc tac ttc agc agg ctc   336
Gln Arg Gln Arg Gln Lys Gln Asp Ser Phe Ala Tyr Phe Ser Arg Leu
            100                 105                 110

ctc cgc cgg ccc ccg ccg ctg ccc gtg ctc tcc atg ccc ccc gcg cca   384
Leu Arg Arg Pro Pro Pro Leu Pro Val Leu Ser Met Pro Pro Ala Pro
        115                 120                 125

ccg tac cat cac gcc cgc gtc ccg gcg ccg ccc gcg ata ccg atg ccg   432
Pro Tyr His His Ala Arg Val Pro Ala Pro Pro Ala Ile Pro Met Pro
    130                 135                 140

atg gcg ccg ccg ccg ccc gct gca tgc aac gac aac ggc ggc gcg cgt   480
Met Ala Pro Pro Pro Pro Ala Ala Cys Asn Asp Asn Gly Gly Ala Arg
145                 150                 155                 160

gtg atc tac agg aac cca ttc tac gtg gct gcg ccg cag gcg ccc cct   528
Val Ile Tyr Arg Asn Pro Phe Tyr Val Ala Ala Pro Gln Ala Pro Pro
                165                 170                 175

gca aat gcc gcc tac tac tac cca cag cca cag cag cag cag cag cag   576
Ala Asn Ala Ala Tyr Tyr Tyr Pro Gln Pro Gln Gln Gln Gln Gln Gln
            180                 185                 190

cag gtg aca gtc atg tac cag tac ccg aga atg gag gta gcc ggc cag   624
```

```
Gln Val Thr Val Met Tyr Gln Tyr Pro Arg Met Glu Val Ala Gly Gln
        195                 200                 205

gac aag atg atg acc agg gcc gcg gcg cac cag cag cag cag cac aac   672
Asp Lys Met Met Thr Arg Ala Ala Ala His Gln Gln Gln Gln His Asn
    210                 215                 220

ggc gcc ggg caa caa ccg gga cgc gcc ggc cac ccc agc cgc gag acg   720
Gly Ala Gly Gln Gln Pro Gly Arg Ala Gly His Pro Ser Arg Glu Thr
225                 230                 235                 240

ctc cag ctg ttc ccg ctc cag ccc acc ttc gtg ctg cgg cac gac aag   768
Leu Gln Leu Phe Pro Leu Gln Pro Thr Phe Val Leu Arg His Asp Lys
                245                 250                 255

ggg cgc gcc gcc aac ggc agt aat aac gac tcc ctg acg tcg acg tcg   816
Gly Arg Ala Ala Asn Gly Ser Asn Asn Asp Ser Leu Thr Ser Thr Ser
            260                 265                 270

acg gcg act gcg aca gcg aca gcg aca gcg aca gcg tcc gct tcc atc   864
Thr Ala Thr Ala Thr Ala Thr Ala Thr Ala Thr Ala Ser Ala Ser Ile
        275                 280                 285

tcc gag gac tcg gat ggc ctg gag agc ggc agc tcc ggc aag ggc gtc   912
Ser Glu Asp Ser Asp Gly Leu Glu Ser Gly Ser Ser Gly Lys Gly Val
    290                 295                 300

gag gag gcg ccc gcg ctg ccg ttc tat gac ttc ttc ggg ctc cag tcc   960
Glu Glu Ala Pro Ala Leu Pro Phe Tyr Asp Phe Phe Gly Leu Gln Ser
305                 310                 315                 320

tcc gga ggc cgc tga                                               975
Ser Gly Gly Arg
```

<210> 104
<211> 324
<212> PRT
<213> Zea mays

<400> 104

```
Met Glu Thr Pro Gln Gln Gln Ser Ala Ala Ala Ala Ala Ala Ala Ala
 1               5                  10                  15
His Gly Gln Asp Asp Gly Gly Ser Pro Pro Met Ser Pro Ala Ser Ala
            20                  25                  30
Ala Ala Ala Ala Leu Ala Asn Ala Arg Trp Asn Pro Thr Lys Glu Gln
        35                  40                  45
Val Ala Val Leu Glu Gly Leu Tyr Glu His Gly Leu Arg Thr Pro Ser
    50                  55                  60
Ala Glu Gln Ile Gln Gln Ile Thr Gly Arg Leu Arg Glu His Gly Ala
65                  70                  75                  80
Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg
                85                  90                  95
Gln Arg Gln Arg Gln Lys Gln Asp Ser Phe Ala Tyr Phe Ser Arg Leu
            100                 105                 110
Leu Arg Arg Pro Pro Pro Leu Pro Val Leu Ser Met Pro Pro Ala Pro
        115                 120                 125
Pro Tyr His His Ala Arg Val Pro Ala Pro Pro Ala Ile Pro Met Pro
    130                 135                 140
Met Ala Pro Pro Pro Pro Ala Ala Cys Asn Asp Asn Gly Gly Ala Arg
145                 150                 155                 160
Val Ile Tyr Arg Asn Pro Phe Tyr Val Ala Ala Pro Gln Ala Pro Pro
                165                 170                 175
Ala Asn Ala Ala Tyr Tyr Tyr Pro Gln Pro Gln Gln Gln Gln Gln Gln
            180                 185                 190
Gln Val Thr Val Met Tyr Gln Tyr Pro Arg Met Glu Val Ala Gly Gln
        195                 200                 205
```

```
Asp Lys Met Met Thr Arg Ala Ala Ala His Gln Gln Gln His Asn
    210                 215                 220
Gly Ala Gly Gln Gln Pro Gly Arg Ala Gly His Pro Ser Arg Glu Thr
225                 230                 235                 240
Leu Gln Leu Phe Pro Leu Gln Pro Thr Phe Val Leu Arg His Asp Lys
                245                 250                 255
Gly Arg Ala Ala Asn Gly Ser Asn Asn Asp Ser Leu Thr Ser Thr Ser
            260                 265                 270
Thr Ala Thr Ala Thr Ala Thr Ala Thr Ala Thr Ala Ser Ala Ser Ile
        275                 280                 285
Ser Glu Asp Ser Asp Gly Leu Glu Ser Gly Ser Ser Gly Lys Gly Val
    290                 295                 300
Glu Glu Ala Pro Ala Leu Pro Phe Tyr Asp Phe Phe Gly Leu Gln Ser
305                 310                 315                 320
Ser Gly Gly Arg
```

<210> 105
<211> 909
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)...(909)

<400> 105

```
atg gcg gcc aat gcg ggc ggc ggt gga gcg gga gga ggc agc ggc agc    48
Met Ala Ala Asn Ala Gly Gly Gly Gly Ala Gly Gly Gly Ser Gly Ser
 1               5                  10                  15

ggc agc gtg gct gcg ccg gcg gtg tgc cgc ccc agc ggc tcg cgg tgg    96
Gly Ser Val Ala Ala Pro Ala Val Cys Arg Pro Ser Gly Ser Arg Trp
            20                  25                  30

acg ccg acg ccg gag cag atc agg atg ctg aag gag ctc tac tac ggc   144
Thr Pro Thr Pro Glu Gln Ile Arg Met Leu Lys Glu Leu Tyr Tyr Gly
        35                  40                  45

tgc ggc atc cgg tcg ccc agc tcg gag cag atc cag cgc atc acc gcc   192
Cys Gly Ile Arg Ser Pro Ser Ser Glu Gln Ile Gln Arg Ile Thr Ala
    50                  55                  60

atg ctg cgg cag cac ggc aag atc gag ggc aag aac gtc ttc tac tgg   240
Met Leu Arg Gln His Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp
65                  70                  75                  80

ttc cag aac cac aag gcc cgc gag cgc cag aag cgc cgc ctc acc agc   288
Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Arg Arg Leu Thr Ser
                85                  90                  95

ctc gac gtc aac gtg ccc gcc gcc ggc gcg gcc gac gcc acc acc agc   336
Leu Asp Val Asn Val Pro Ala Ala Gly Ala Ala Asp Ala Thr Thr Ser
            100                 105                 110

caa ctc ggc gtc ctc tcg ctg tcg tcg ccg ccg cct tca ggc gcg gcg   384
Gln Leu Gly Val Leu Ser Leu Ser Ser Pro Pro Pro Ser Gly Ala Ala
        115                 120                 125

cct ccc tcg ccc acc ctc ggc ttc tac gcc gcc ggc aat ggc ggc gga   432
Pro Pro Ser Pro Thr Leu Gly Phe Tyr Ala Ala Gly Asn Gly Gly Gly
    130                 135                 140

tcg gct gtg ctg ctg gac acg agt tcc gac tgg ggc agc agc ggc gct   480
Ser Ala Val Leu Leu Asp Thr Ser Ser Asp Trp Gly Ser Ser Gly Ala
145                 150                 155                 160
```

```
gcc atg gcc acc gag aca tgc ttc ctg cag gac tac atg ggc gtg acg   528
Ala Met Ala Thr Glu Thr Cys Phe Leu Gln Asp Tyr Met Gly Val Thr
                165                 170                 175

gac acg ggc agc tcg tcg cag tgg cca cgc ttc tcg tcg tcg gac acg   576
Asp Thr Gly Ser Ser Ser Gln Trp Pro Arg Phe Ser Ser Ser Asp Thr
            180                 185                 190

ata atg gcg gcg gcc gcg gcg cgg gcg gcg acg acg cgg gcg ccc gag   624
Ile Met Ala Ala Ala Ala Ala Arg Ala Ala Thr Thr Arg Ala Pro Glu
        195                 200                 205

acg ctc cct ctc ttc ccg acc tgc ggc gac gac ggc ggc agc ggt agc   672
Thr Leu Pro Leu Phe Pro Thr Cys Gly Asp Asp Gly Gly Ser Gly Ser
    210                 215                 220

agc agc tac ttg ccg ttc tgg ggt gcc gcg tcc aca act gcc ggc gcc   720
Ser Ser Tyr Leu Pro Phe Trp Gly Ala Ala Ser Thr Thr Ala Gly Ala
225                 230                 235                 240

act tct tcc gtt gcg atc cag cag caa cac cag ctg cag gag cag tac   768
Thr Ser Ser Val Ala Ile Gln Gln Gln His Gln Leu Gln Glu Gln Tyr
                245                 250                 255

agc ttt tac agc aac agc aac agc acc cag ctg gcc ggc acc ggc aac   816
Ser Phe Tyr Ser Asn Ser Asn Ser Thr Gln Leu Ala Gly Thr Gly Asn
            260                 265                 270

caa gac gta tcg gca aca gca gca gca gcc gcc gcc ctg gag ctg agc   864
Gln Asp Val Ser Ala Thr Ala Ala Ala Ala Ala Ala Leu Glu Leu Ser
        275                 280                 285

ctc agc tca tgg tgc tcc cct tac cct gct gca ggg agt atg tga       909
Leu Ser Ser Trp Cys Ser Pro Tyr Pro Ala Ala Gly Ser Met
    290                 295                 300
```

<210> 106
<211> 302
<212> PRT
<213> Zea mays

<400> 106

```
Met Ala Ala Asn Ala Gly Gly Gly Gly Ala Gly Gly Gly Ser Gly Ser
 1               5                  10                  15
Gly Ser Val Ala Ala Pro Ala Val Cys Arg Pro Ser Gly Ser Arg Trp
            20                  25                  30
Thr Pro Thr Pro Glu Gln Ile Arg Met Leu Lys Glu Leu Tyr Tyr Gly
        35                  40                  45
Cys Gly Ile Arg Ser Pro Ser Ser Glu Gln Ile Gln Arg Ile Thr Ala
    50                  55                  60
Met Leu Arg Gln His Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp
65                  70                  75                  80
Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Arg Arg Leu Thr Ser
                85                  90                  95
Leu Asp Val Asn Val Pro Ala Ala Gly Ala Ala Asp Ala Thr Thr Ser
            100                 105                 110
Gln Leu Gly Val Leu Ser Leu Ser Ser Pro Pro Pro Ser Gly Ala Ala
        115                 120                 125
Pro Pro Ser Pro Thr Leu Gly Phe Tyr Ala Ala Gly Asn Gly Gly Gly
    130                 135                 140
Ser Ala Val Leu Leu Asp Thr Ser Ser Asp Trp Gly Ser Ser Gly Ala
145                 150                 155                 160
Ala Met Ala Thr Glu Thr Cys Phe Leu Gln Asp Tyr Met Gly Val Thr
                165                 170                 175
Asp Thr Gly Ser Ser Ser Gln Trp Pro Arg Phe Ser Ser Ser Asp Thr
            180                 185                 190
```

```
Ile Met Ala Ala Ala Ala Ala Arg Ala Ala Thr Thr Arg Ala Pro Glu
        195                 200                 205
Thr Leu Pro Leu Phe Pro Thr Cys Gly Asp Asp Gly Gly Ser Gly Ser
    210                 215                 220
Ser Ser Tyr Leu Pro Phe Trp Gly Ala Ala Ser Thr Thr Ala Gly Ala
225                 230                 235                 240
Thr Ser Ser Val Ala Ile Gln Gln Gln His Gln Leu Gln Glu Gln Tyr
                245                 250                 255
Ser Phe Tyr Ser Asn Ser Asn Ser Thr Gln Leu Ala Gly Thr Gly Asn
            260                 265                 270
Gln Asp Val Ser Ala Thr Ala Ala Ala Ala Ala Ala Leu Glu Leu Ser
        275                 280                 285
Leu Ser Ser Trp Cys Ser Pro Tyr Pro Ala Ala Gly Ser Met
    290                 295                 300


<210> 107
<211> 978
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)...(978)

<400> 107
atg gcg gcc aat gcg ggc ggc ggt gga gcg gga gga ggc agc ggc agc    48
Met Ala Ala Asn Ala Gly Gly Gly Gly Ala Gly Gly Gly Ser Gly Ser
 1               5                  10                  15

ggc agc gtg gct gcg ccg gcg gtg tgc cgc ccc agc ggc tcg cgg tgg    96
Gly Ser Val Ala Ala Pro Ala Val Cys Arg Pro Ser Gly Ser Arg Trp
            20                  25                  30

acg ccg acg ccg gag cag atc agg atg ctg aag gag ctc tac tac ggc   144
Thr Pro Thr Pro Glu Gln Ile Arg Met Leu Lys Glu Leu Tyr Tyr Gly
        35                  40                  45

tgc ggc atc cgg tcg ccc agc tcg gag cag atc cag cgc atc acc gcc   192
Cys Gly Ile Arg Ser Pro Ser Ser Glu Gln Ile Gln Arg Ile Thr Ala
    50                  55                  60

atg ctg cgg cag cac ggc aag atc gag ggc aag aac gtc ttc tac tgg   240
Met Leu Arg Gln His Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp
65                  70                  75                  80

ttc cag aac cac aag gcc cgc gag cgc cag aag cgc cgc ctc acc agc   288
Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Arg Arg Leu Thr Ser
                85                  90                  95

ctc gac gtc aac gtg ccc gcc gcc ggc gcg gcc gac gcc acc acc agc   336
Leu Asp Val Asn Val Pro Ala Ala Gly Ala Ala Asp Ala Thr Thr Ser
            100                 105                 110

caa ctc ggc gtc ctc tcg ctg tcg tcg ccg cct tca ggc gcg gcg cct   384
Gln Leu Gly Val Leu Ser Leu Ser Ser Pro Pro Ser Gly Ala Ala Pro
        115                 120                 125

ccc tcg ccc acc ctc ggc ttc tac gcc gcc ggc aat ggc ggc gga tcg   432
Pro Ser Pro Thr Leu Gly Phe Tyr Ala Ala Gly Asn Gly Gly Gly Ser
    130                 135                 140

gct ggg ctg ctg gac acg agt tcc gac tgg ggc agc agc ggc gct gct   480
Ala Gly Leu Leu Asp Thr Ser Ser Asp Trp Gly Ser Ser Gly Ala Ala
145                 150                 155                 160

atg gcc acc gag aca tgc ttc ctg cag gac tac atg ggc gtg acg gac   528
Met Ala Thr Glu Thr Cys Phe Leu Gln Asp Tyr Met Gly Val Thr Asp
```

```
                    165                 170                 175

acg ggc agc tcg tcg cag tgg cca tgc ttc tcg tcg tcg gac acg ata   576
Thr Gly Ser Ser Ser Gln Trp Pro Cys Phe Ser Ser Ser Asp Thr Ile
                180                 185                 190

atg gcg gcg gcg gcg gcc gcg gcg cgg gtg gcg acg acg cgg gcg ccc   624
Met Ala Ala Ala Ala Ala Ala Ala Arg Val Ala Thr Thr Arg Ala Pro
        195                 200                 205

gag aca ctc cct ctc ttc ccg acc tgc ggc gac gac gac gac gac gac   672
Glu Thr Leu Pro Leu Phe Pro Thr Cys Gly Asp Asp Asp Asp Asp Asp
    210                 215                 220

agc cag ccc ccg ccg cgg ccg cgg cac gca gtc cca gtc ccg gca ggc   720
Ser Gln Pro Pro Pro Arg Pro Arg His Ala Val Pro Val Pro Ala Gly
225                 230                 235                 240

gag acc atc cgc ggc ggc ggc ggc agc agc agc agc tac ttg ccg ttc   768
Glu Thr Ile Arg Gly Gly Gly Gly Ser Ser Ser Ser Tyr Leu Pro Phe
                245                 250                 255

tgg ggt gcc ggt gcc gcg tcc aca act gcc ggc gcc act tct tcc gtt   816
Trp Gly Ala Gly Ala Ala Ser Thr Thr Ala Gly Ala Thr Ser Ser Val
            260                 265                 270

gcg atc cag cag caa cac cag ctg cag gag cag tac agc ttt tac agc   864
Ala Ile Gln Gln Gln His Gln Leu Gln Glu Gln Tyr Ser Phe Tyr Ser
        275                 280                 285

aac agc acc cag ctg gcc ggc acc ggc agc caa gac gta tcg gct tca   912
Asn Ser Thr Gln Leu Ala Gly Thr Gly Ser Gln Asp Val Ser Ala Ser
    290                 295                 300

gcg gcc gcc ctg gag ctg agc ctc agc tca tgg tgc tcc cct tac cct   960
Ala Ala Ala Leu Glu Leu Ser Leu Ser Ser Trp Cys Ser Pro Tyr Pro
305                 310                 315                 320

gct gca ggg agc atg tga                                           978
Ala Ala Gly Ser Met
                325


<210> 108
<211> 325
<212> PRT
<213> Zea mays

<400> 108
Met Ala Ala Asn Ala Gly Gly Gly Gly Ala Gly Gly Gly Ser Gly Ser
 1               5                  10                  15
Gly Ser Val Ala Ala Pro Ala Val Cys Arg Pro Ser Gly Ser Arg Trp
            20                  25                  30
Thr Pro Thr Pro Glu Gln Ile Arg Met Leu Lys Glu Leu Tyr Tyr Gly
        35                  40                  45
Cys Gly Ile Arg Ser Pro Ser Ser Glu Gln Ile Gln Arg Ile Thr Ala
    50                  55                  60
Met Leu Arg Gln His Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp
65                  70                  75                  80
Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Arg Arg Leu Thr Ser
                85                  90                  95
Leu Asp Val Asn Val Pro Ala Ala Gly Ala Ala Asp Ala Thr Thr Ser
            100                 105                 110
Gln Leu Gly Val Leu Ser Leu Ser Ser Pro Pro Ser Gly Ala Ala Pro
        115                 120                 125
Pro Ser Pro Thr Leu Gly Phe Tyr Ala Ala Gly Asn Gly Gly Gly Ser
    130                 135                 140
Ala Gly Leu Leu Asp Thr Ser Ser Asp Trp Gly Ser Ser Gly Ala Ala
```

```
145                 150                 155                 160
Met Ala Thr Glu Thr Cys Phe Leu Gln Asp Tyr Met Gly Val Thr Asp
                165                 170                 175
Thr Gly Ser Ser Ser Gln Trp Pro Cys Phe Ser Ser Ser Asp Thr Ile
            180                 185                 190
Met Ala Ala Ala Ala Ala Ala Ala Arg Val Ala Thr Thr Arg Ala Pro
        195                 200                 205
Glu Thr Leu Pro Leu Phe Pro Thr Cys Gly Asp Asp Asp Asp Asp Asp
    210                 215                 220
Ser Gln Pro Pro Pro Arg Pro Arg His Ala Val Pro Val Pro Ala Gly
225                 230                 235                 240
Glu Thr Ile Arg Gly Gly Gly Gly Ser Ser Ser Ser Tyr Leu Pro Phe
                245                 250                 255
Trp Gly Ala Gly Ala Ala Ser Thr Thr Ala Gly Ala Thr Ser Ser Val
            260                 265                 270
Ala Ile Gln Gln Gln His Gln Leu Gln Glu Gln Tyr Ser Phe Tyr Ser
        275                 280                 285
Asn Ser Thr Gln Leu Ala Gly Thr Gly Ser Gln Asp Val Ser Ala Ser
    290                 295                 300
Ala Ala Ala Leu Glu Leu Ser Leu Ser Ser Trp Cys Ser Pro Tyr Pro
305                 310                 315                 320
Ala Ala Gly Ser Met
                325


<210> 109
<211> 663
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)...(663)

<400> 109
atg gag gcg ctg agc ggg cgg gta ggc gtc aag tgc ggg cgg tgg aac   48
Met Glu Ala Leu Ser Gly Arg Val Gly Val Lys Cys Gly Arg Trp Asn
 1               5                  10                  15

cct acg gcg gag cag gtg aag gtc ctg acg gag ctc ttc cgc gcg ggg   96
Pro Thr Ala Glu Gln Val Lys Val Leu Thr Glu Leu Phe Arg Ala Gly
            20                  25                  30

ctg cgg acg ccc agc acg gag cag atc cag cgc atc tcc acc cac ctc   144
Leu Arg Thr Pro Ser Thr Glu Gln Ile Gln Arg Ile Ser Thr His Leu
        35                  40                  45

agc gcc ttc ggc aag gtg gag agc aag aac gtc ttc tac tgg ttc cag   192
Ser Ala Phe Gly Lys Val Glu Ser Lys Asn Val Phe Tyr Trp Phe Gln
    50                  55                  60

aac cac aag gcc cgc gag cgc cac cac cac aag aag cgc cgc cgc ggc   240
Asn His Lys Ala Arg Glu Arg His His His Lys Lys Arg Arg Arg Gly
65                  70                  75                  80

gcg tcg tcg tcc tcc ccc gac agc ggc agc ggc agg gga agc aac aac   288
Ala Ser Ser Ser Ser Pro Asp Ser Gly Ser Gly Arg Gly Ser Asn Asn
                85                  90                  95

gag gaa gac ggc cgt ggt gcc gcc tcg cag tcg cac gac gcc gac gcc   336
Glu Glu Asp Gly Arg Gly Ala Ala Ser Gln Ser His Asp Ala Asp Ala
            100                 105                 110

gac gcc gac ctc gtg ctg caa ccg cca gag agc aag cgg gag gcc aga   384
Asp Ala Asp Leu Val Leu Gln Pro Pro Glu Ser Lys Arg Glu Ala Arg
        115                 120                 125

agc tat ggc cac cat cac cgg ctc gtg aca tgc tac gtc agg gac gtg   432
```

```
Ser Tyr Gly His His His Arg Leu Val Thr Cys Tyr Val Arg Asp Val
    130                 135                 140

gtg gag cag cag gag gcg tcg ccg tcg tgg gag cgg ccg acg agg gag   480
Val Glu Gln Gln Glu Ala Ser Pro Ser Trp Glu Arg Pro Thr Arg Glu
145                 150                 155                 160

gtg gag acg cta gag ctc ttc ccc ctc aag tcg tac ggc gac ctc gag   528
Val Glu Thr Leu Glu Leu Phe Pro Leu Lys Ser Tyr Gly Asp Leu Glu
                165                 170                 175

gcg gcg gag aag gtc cgg tcg tac gtc aga ggc atc gcc gcc acc agc   576
Ala Ala Glu Lys Val Arg Ser Tyr Val Arg Gly Ile Ala Ala Thr Ser
            180                 185                 190

gag cag tgc agg gag ttg tcc ttc ttc gac gtc tcc gcc ggc cgg gat   624
Glu Gln Cys Arg Glu Leu Ser Phe Phe Asp Val Ser Ala Gly Arg Asp
        195                 200                 205

ccg ccg ctc gag ctc agg ctc tgc agc ttc ggt ccc tag               663
Pro Pro Leu Glu Leu Arg Leu Cys Ser Phe Gly Pro
    210                 215                 220


<210> 110
<211> 220
<212> PRT
<213> Zea mays

<400> 110
Met Glu Ala Leu Ser Gly Arg Val Gly Val Lys Cys Gly Arg Trp Asn
 1               5                  10                  15
Pro Thr Ala Glu Gln Val Lys Val Leu Thr Glu Leu Phe Arg Ala Gly
            20                  25                  30
Leu Arg Thr Pro Ser Thr Glu Gln Ile Gln Arg Ile Ser Thr His Leu
        35                  40                  45
Ser Ala Phe Gly Lys Val Glu Ser Lys Asn Val Phe Tyr Trp Phe Gln
    50                  55                  60
Asn His Lys Ala Arg Glu Arg His His His Lys Lys Arg Arg Arg Gly
65                  70                  75                  80
Ala Ser Ser Ser Ser Pro Asp Ser Gly Ser Gly Arg Gly Ser Asn Asn
                85                  90                  95
Glu Glu Asp Gly Arg Gly Ala Ala Ser Gln Ser His Asp Ala Asp Ala
            100                 105                 110
Asp Ala Asp Leu Val Leu Gln Pro Pro Glu Ser Lys Arg Glu Ala Arg
        115                 120                 125
Ser Tyr Gly His His His Arg Leu Val Thr Cys Tyr Val Arg Asp Val
    130                 135                 140
Val Glu Gln Gln Glu Ala Ser Pro Ser Trp Glu Arg Pro Thr Arg Glu
145                 150                 155                 160
Val Glu Thr Leu Glu Leu Phe Pro Leu Lys Ser Tyr Gly Asp Leu Glu
                165                 170                 175
Ala Ala Glu Lys Val Arg Ser Tyr Val Arg Gly Ile Ala Ala Thr Ser
            180                 185                 190
Glu Gln Cys Arg Glu Leu Ser Phe Phe Asp Val Ser Ala Gly Arg Asp
        195                 200                 205
Pro Pro Leu Glu Leu Arg Leu Cys Ser Phe Gly Pro
    210                 215                 220


<210> 111
<211> 896
<212> DNA
<213> Zea mays

<400> 111
gtgcagcgtg acccggtcgt gcccctctct agagataatg agcattgcat gtctaagtta 60
taaaaaatta ccacatattt ttttttgtcac acttgtttga agtgcagttt atctatcttt 120
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atacatatat ttaaacttta ctctacgaat aatataatct atagtactac aataatatca 180
gtgttttaga gaatcatata aatgaacagt tagacatggt ctaaaggaca attgagtatt 240
ttgacaacag gactctacag ttttatcttt ttagtgtgca tgtgttctcc tttttttttg 300
caaatagctt cacctatata atacttcatc catttttatta gtacatccat ttagggttta 360
gggttaatgg ttttttataga ctaatttttt tagtacatct attttattct attttagcct 420
ctaaattaag aaaactaaaa ctctatttta gttttttttat ttaataattt agatataaaa 480
tagaataaaa taaagtgact aaaaattaaa caaatacccct ttaagaaatt aaaaaaacta 540
aggaaacatt tttcttgttt cgagtagata atgccagcct gttaaacgcc gtcgacgagt 600
ctaacggaca ccaaccagcg aaccagcagc gtcgcgtcgg gccaagcgaa gcagacggca 660
cggcatctct gtcgctgcct ctggacccct ctcgagagtt ccgctccacc gttggacttg 720
ctccgctgtc ggcatccaga aattgcgtgg cggagcggca gacgtgagcc ggcacggcag 780
gcggcctcct cctcctctca cggcaccggc agctacgggg gattcctttc ccaccgctcc 840
ttcgctttcc cttcctcgcc cgccgtaata aatagacacc ccctccacac cctctt 896

<210> 112
<211> 82
<212> DNA
<213> Zea mays

<400> 112
tccccaacct cgtgttgttc ggagcgcaca cacacacaac cagatctccc ccaaatccac 60
ccgtcggcac ctccgcttca ag 82

<210> 113
<211> 1013
<212> DNA
<213> Zea mays

<400> 113
gtacgccgct cgtcctcccc ccccccccctc tctaccttct ctagatcggc gttccggtcc 60
atgcatggtt agggcccggt agttctactt ctgttcatgt ttgtgttaga tccgtgtttg 120
tgttagatcc gtgctgctag cgttcgtaca cggatgcgac ctgtacgtca gacacgttct 180
gattgctaac ttgccagtgt ttctctttgg ggaatcctgg gatggctcta gccgttccgc 240
agacgggatc gatttcatga tttttttttgt ttcgttgcat agggttttggt ttgccctttt 300
cctttatttc aatatatgcc gtgcacttgt ttgtcgggtc atctttttcat gctttttttt 360
gtcttggttg tgatgatgtg gtctggttgg gcggtcgttc tagatcggag tagaattctg 420
tttcaaactа cctggtggat ttattaattt tggatctgta tgtgtgtgcc atacatattc 480
atagttacga attgaagatg atggatggaa atatcgatct aggataggta tacatgttga 540
tgcgggtttt actgatgcat atacagagat gctttttgtt cgcttggttg tgatgatgtg 600
gtgtggttgg gcggtcgttc attcgttcta gatcggagta gaatactgtt tcaaactacc 660
tggtgtattt attaatttttg gaactgtatg tgtgtgtcat acatcttcat agttacgagt 720
ttaagatgga tggaaatatc gatctaggat aggtatacat gttgatgtgg gttttactga 780
tgcatatаca tgatggcata tgcagcatct attcatatgc tctaaccttg agtacctatc 840
tattataata aacaagtatg ttttataatt attttgatct tgatatactt ggatgatggc 900
atatgcagca gctatatgtg gatttttttа gccctgcctt catacgctat ttatttgctt 960
ggtactgttt cttttgtcga tgctcaccct gttgttttggt gttacttctg cag 1013

<210> 114
<211> 11
<212> DNA
<213> Triticum monococcum

<400> 114
cctcgttttg g 11

<210> 115
<211> 1036
<212> DNA
<213> Triticum monococcum

<400> 115
atttgcctga tgagacgctt gacaacagtg tattgatgga tgtctggtcg gtatacacgc 60
acagcacagt acccctactc ctaggactgg cgagtatctt tcattcattc cagaaatacg 120
cgggtcggcc aaaagtagaa aaatacactg cgcccactca atccacgtag cgcactgcac 180
tgcacagcaa cgcttcatgt caaaagtcga gctcaagcat gcacgcgatg gacgcggcgc 240
gaatgacccg ggcggcacga cgcgagtgcc cgccgcgccc gcccgcctgc cccgcagccg 300
acctctccca aacgggacaa gcgagacggc ccaaaacgag caaggaaagc agcctcctac 360
tgtggcagcc cgccccacg accgtcatct cacctttccat tccatttttcc ctggacggac 420

cagacccgtc cgagccgccc tgacctagcc agccagcatt tcctcttt cg tcccccgccg 480
ccgtgaccaa aaaagcaaaa aaggaaaaag ggaaaatgct aaaggaaaaa actccgctct 540
ttccctt ctt ctaggcctag ggtacagtag aatattatt aa aaggaaaaat tctgctcgtt 600
ttttgctctg tggtgtgtgt ttgtggcgag agaaaatgat ttggggaaag caaaatcggg 660
agatt cgcac gtacgatcgt tcgacacgtc gacgcccggc gggcccgtgg tggggcatcg 720
tgtggctgca ggaccgcggg gccccgcggg gcgggccggg ccaatgggtg ctcgacagcg 780
gctatgctcc agaccagccc ggtattgcat accgcgctcg gggccagatc cctttaaaaa 840
ccccctcccc cctgccggaa ccctcgtttt ggcctggcca tcctccctct cctccccctct 900
cttccacctc acccaaccac ctgatagcca tggctccgcc gcctcgcctc cgcctgcgcc 960
agtcggagta gccgtcgcgg tctgcgggtg ttggagggta ggggcgtagg gttggcccgg 1020
ttctcgagcg gagatg 1036