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(72) Inventeurs/Inventors:
DHUGGA, KANWARPAL S., US;
HELENTJARIS, TIMOTHY G., US;
BOWEN, BENJAMIN A., US;
WANG, XUN, US

(73) Propriétaire/Owner:
PIONEER HI-BRED INTERNATIONAL, INC., US

(74) Agent: TORYS LLP

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(57) **Abrégé/Abstract:**

The invention provides isolated cellulose synthase nucleic acids and their encoded proteins. The present invention provides methods and compositions relating to altering cellulose synthase concentration and/or composition of plants. The invention further provides recombinant expression cassettes, host cells, and transgenic plants.



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Maize Cellulose Synthases and Uses Thereof

TECHNICAL FIELD

The present invention relates generally to plant molecular biology. More specifically, it relates to nucleic acids and methods for modulating their expression in plants.

BACKGROUND OF THE INVENTION

Polysaccharides constitute the bulk of the plant cell walls and have been traditionally classified into three categories: cellulose, hemicellulose, and pectin. Fry, S. C. (1988), *The growing plant cell wall: Chemical and metabolic analysis*. New York: Longman Scientific & Technical. Whereas cellulose is made at the plasma membrane and directly laid down into the cell wall, hemicellulosic and pectic polymers are first made in the Golgi apparatus and then exported to the cell wall by exocytosis. Ray, P. M., *et al.*, (1976), *Ber. Deutsch. Bot. Ges. Bd.* 89, 121-146. The variety of chemical linkages in the pectic and hemicellulosic polysaccharides indicates that there must be tens of polysaccharide synthases in the Golgi apparatus. Darvill *et al.*, (1980). *The primary cell walls of flowering plants*. In *The Plant Cell* (N. E. Tolbert, ed.), *Vol. 1 in Series: The biochemistry of plants: A comprehensive treatise*, eds. P.K. Stumpf and E.E. Conn (New York: Academic Press), pp. 91-162.

Cellulose, by virtue of its ability to form semicrystalline microfibrils, has a very high tensile strength which approaches that of some metals. Niklas, K. J. (1992). *Plant Biomechanics: An engineering approach to plant form and function*, The University of Chicago Press, pp. 607. Bending strength of the culm of normal and brittle-culm mutants of barley has been found to be directly correlated with the concentration of cellulose in the cell wall. Kokubo, *et al.*, (1989), *Plant Physiology* 91, 876-882; Kokubo, *et al.*, (1991) *Plant Physiology* 97, 509-514.

Even though sugar and polysaccharide compositions of the plant cell walls have been well characterized, very limited progress has been made toward identification of the enzymes involved in polysaccharides formation, the reason being their labile nature and recalcitrance to solubilization by available detergents. Sporadic claims for the identification of cellulose synthase from plant sources have been made over the years. Callaghan, T., and Benziman, M. (1984), *Nature* 311, 165-167; Okuda, *et al.*, (1993),

Plant Physiol. 101, 1131-1142. However, these claims have been met with skepticism. Callaghan, T., and Benziman, M. (1985), *Nature* 314, 383-384; Delmer, *et al.*, (1993), Plant Physiol. 103, 307-308. It was only recently that a putative gene for plant cellulose synthase (Cela) was cloned from the developing cotton fibers based on homology to the bacterial gene and a substrate binding and/or catalysis motif was proposed, comprising conserved Asp residues and a conserved amino acid sequence (QXXRW), corresponding, for example to amino acids 300, 367, 462 and 498-502 of the *A. tumefaciens* Cela polypeptide, respectively. Pear, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 93, 12637-12642; Saxena, *et al.*, (1990), *Plant Molecular Biology* 15, 673-684; see also, WO 9818949.

As brittle snap is a major problem in corn breeding, what is needed in the art are compositions and methods for manipulating cellulose concentration in the cell wall and thereby altering plant stalk quality for improved standability or silage. The present invention provides these and other advantages.

SUMMARY OF THE INVENTION

Generally, it is the object of the present invention to provide nucleic acids and proteins relating to cellulose synthases. It is an object of the present invention to provide: 1) nucleic acids and proteins relating to maize cellulose synthases; 2) transgenic plants comprising the nucleic acids of the present invention; 3) methods for modulating, in a transgenic plant, the expression of the nucleic acids of the present invention.

Therefore, in one aspect, the present invention relates to an isolated nucleic acid comprising a member selected from the group consisting of (a) a polynucleotide having a specified sequence identity to a polynucleotide encoding a polypeptide of the present invention;; (b) a polynucleotide which is complementary to the polynucleotide of (a); and (c) a polynucleotide comprising a specified number of contiguous nucleotides from a polynucleotide of (a) or (b). The isolated nucleic acid can be DNA or RNA.

In another aspect, the present invention relates to recombinant expression cassettes, comprising a nucleic acid of the present invention operably linked to a promoter. In some embodiments, the nucleic acid is operably linked in antisense orientation to the promoter.

In another aspect, the present invention is directed to a host cell transfected with the recombinant expression cassette.

In a further aspect, the present invention relates to an isolated protein comprising a polypeptide having a specified number of contiguous amino acids encoded by an isolated nucleic acid of the present invention.

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In another aspect, the present invention relates to an isolated nucleic acid comprising a polynucleotide of specified length which selectively hybridizes under stringent conditions to a polynucleotide of the present invention, or a complement thereof. In some embodiments, the isolated nucleic acid is operably linked to a promoter.

In yet another aspect, the present invention relates to an isolated nucleic acid comprising a polynucleotide, the polynucleotide having a specified sequence identity to an identical length of a nucleic acid of the present invention or a complement thereof.

In another aspect, the present invention relates to an isolated nucleic acid comprising a polynucleotide having a sequence of a nucleic acid amplified from a *Zea mays* nucleic acid library using at least two primers or their complements, one of which selectively hybridizes under stringent conditions to a locus of the nucleic acid comprising the 5' terminal coding region and the other primer selectively hybridizing, under stringent conditions, to a locus of the nucleic acid comprising the 3' terminal coding region, and wherein both primers selectively hybridize within the coding region. In some embodiments, the nucleic acid library is a cDNA library.

In another aspect, the present invention relates to a recombinant expression cassette comprising a nucleic acid, wherein the nucleic acid is operably linked to a promoter. In some embodiments, the present invention relates to a host cell transfected with this recombinant expression cassette. In some embodiments, the present invention relates to a protein of the present invention which is produced from this host cell.

In a further aspect, the present invention relates to a heterologous promoter operably linked to a non-isolated polynucleotide of the present invention, wherein the polypeptide is encoded by a nucleic acid amplified from a nucleic acid library.

In yet another aspect, the present invention relates to a transgenic plant comprising a recombinant expression cassette comprising a plant promoter operably linked to any of the isolated nucleic acids of the present invention. In some embodiments, the transgenic plant is *Zea mays*. The present invention also provides transgenic seed from the transgenic plant.

In a further aspect, the present invention relates to a method of modulating expression of the genes encoding the proteins of the present invention in a plant cell capable of plant regeneration, comprising the steps of (a) transforming a plant cell with a recombinant expression cassette comprising a polynucleotide of the present invention

operably linked to a promoter; (b)growing the plant cell under plant growing conditions; and (c) inducing expression of the polynucleotide for a time sufficient to modulate expression of the genes in the plant. In some embodiments, the plant is maize. Expression of the genes encoding the proteins of the present invention can be increased or decreased relative to a non-transformed control plant.

An aspect of the invention is to provide an isolated nucleic acid molecule comprising a polynucleotide encoding a functional cellulose synthase polypeptide, wherein the polypeptide comprises a cellulose synthase substrate binding and/or catalysis motif, and having at least 90% sequence identity, as determined by the BLAST 2.0 algorithm under default parameters wherein wordlength is 11, expectation value is 10, cutoff is 100, reward score for a pair of matching residues is 5 and penalty score for mismatching residues is -4, to the full length nucleotide sequence selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 25, and 57.

Another aspect of the invention is to provide an isolated nucleic acid molecule encoding a functional cellulose synthase comprising a polynucleotide encoding a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 26, or 58.

Another aspect of the invention is to provide an isolated nucleic acid molecule comprising a polynucleotide encoding a functional cellulose synthase polypeptide, wherein the polypeptide comprises a cellulose synthase substrate binding and/or catalysis motif, and having at least 60 contiguous nucleotides of any of SEQ ID NOS: 1, 5, 9, 13, 17, 25, and 57, and which polynucleotide selectively hybridizes, under stringent hybridization conditions and a wash in 2X SSC at 50°C, along the full length to the complement of a polynucleotide comprising SEQ ID NOS: 1, 5, 9, 13, 17, 25, or 57.

Another aspect of the invention is to provide an isolated nucleic acid molecule encoding a functional cellulose synthase comprising a polynucleotide of SEQ ID NOS: 1, 5, 9, 13, 17, 25, or 57 .

Another aspect of the invention is to provide an isolated nucleic acid molecule comprising a polynucleotide which is complementary to any of the nucleic acids described above.

Another aspect of the invention is to provide an isolated nucleic acid probe for detecting a nucleic acid molecule encoding a cellulose synthase, comprising at least 60 contiguous nucleotides of a nucleic acid molecule described above.

Another aspect of the invention is to provide an isolated nucleic acid primer for amplifying a nucleic acid molecule encoding a cellulose synthase, comprising at least 60 contiguous nucleotides of a nucleic acid molecule described above.

Another aspect of the invention is to provide a recombinant expression cassette, comprising any of the nucleic acid molecules described above operably linked, in sense or anti-sense orientation, to a promoter.

Another aspect of the invention is to provide a host cell transformed with the recombinant expression cassette described above. The host cell may be from a monocot. The host cell may be selected from the group consisting of: maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, and millet.

Another aspect of the invention is to provide a method of modulating the level of cellulose synthase in a plant cell capable of plant regeneration, comprising: (a) transforming the plant cell with a recombinant expression cassette comprising a cellulose synthase polynucleotide of claim 1, 2, 3, or 4, operably linked to a promoter, (b) culturing the transformed plant cell, and (c) inducing expression of said polynucleotide for a time sufficient to modulate the level of cellulose synthase in said transformed plant cell. A plant may be regenerated from the transformed plant cell. The plant may be selected from the group consisting of: maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley and millet. The promoter may be a tissue-preferred promoter. The level of cellulose synthase may be increased. The cellulose synthase polynucleotide may be amplified from a *Zea mays* nucleic acid library using primers which selectively hybridize, under stringent hybridization conditions, to loci within a polynucleotide

selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 25, and 57. The cellulose synthase polynucleotide may be selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 25, and 57.

Another aspect of the invention is to provide an isolated protein for use as an immunogen for eliciting the production of antibodies to a cellulose synthase protein, wherein said isolated protein comprises a polypeptide of at least 60 contiguous amino acids from a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 26, or 58.

Another aspect of the invention is to provide an isolated cellulose synthase protein comprising a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 26, or 58.

Another aspect of the invention is to provide an isolated protein comprising a functional cellulose synthase polypeptide comprising a cellulose synthase substrate binding and/or catalysis motif, and having at least one linear epitope in common with, and having at least 90% sequence identity to, the full length amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 26, or 58, wherein said sequence identity is determined using BLAST 2.0 under default parameters wherein wordlength is 3, expectation value is 10, and matrix is BLOSUM62.

Another aspect of the invention is to provide an isolated cellulose synthase protein comprising a polypeptide encoded by any of the nucleic acids described above.

Another aspect of the invention is to provide a use of the polynucleotide described above for modulating the expression of cellulose synthase in a plant. The level of cellulose synthase may be increased in the plant.

Definitions

Units, prefixes, and symbols may be denoted in their SI accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range and include each integer within the defined range. Amino acids may be referred to herein by either their

commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. Unless otherwise provided for, software, electrical, and electronics terms as used herein are as defined in The New IEEE Standard Dictionary of Electrical and electronics Terms (5th edition, 1993). The terms defined below are more fully defined by reference to the specification as a whole.

By "amplified" is meant the construction of multiple copies of a nucleic acid sequence or multiple copies complementary to the nucleic acid sequence using at least one of the nucleic acid sequences as a template. Amplification systems include the polymerase chain reaction (PCR) system, ligase chain reaction (LCR) system, nucleic acid sequence based amplification (NASBA, Cangene, Mississauga, Ontario), Q-Beta Replicase systems, transcription-based amplification system (TAS), and strand displacement amplification (SDA). See, e.g., *Diagnostic Molecular Microbiology: Principles and Applications*, D. H. Persing *et al.*, Ed., American Society for Microbiology, Washington, D.C. (1993). The product of amplification is termed an amplicon.

The term "antibody" includes reference to antigen binding forms of antibodies (e.g., Fab, F(ab)₂). The term "antibody" frequently refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen). However, while various antibody fragments can be defined in terms of the digestion of an intact antibody, one of

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skill will appreciate that such fragments may be synthesized *de novo* either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments such as single chain Fv, chimeric antibodies (i.e., comprising constant and variable regions from different species), humanized antibodies
5 (i.e., comprising a complementarity determining region (CDR) from a non-human source) and heteroconjugate antibodies (e.g., bispecific antibodies).

The term "antigen" includes reference to a substance to which an antibody can be generated and/or to which the antibody is specifically immunoreactive. The specific immunoreactive sites within the antigen are known as epitopes or antigenic determinants.
10 These epitopes can be a linear array of monomers in a polymeric composition - such as amino acids in a protein - or consist of or comprise a more complex secondary or tertiary structure. Those of skill will recognize that all immunogens (i.e., substances capable of eliciting an immune response) are antigens; however some antigens, such as haptens, are not immunogens but may be made immunogenic by coupling to a carrier molecule. An
15 antibody immunologically reactive with a particular antigen can be generated *in vivo* or by recombinant methods such as selection of libraries of recombinant antibodies in phage or similar vectors. See, e.g., Huse *et al.*, *Science* 246: 1275-1281 (1989); and Ward, *et al.*, *Nature* 341: 544-546 (1989); and Vaughan *et al.*, *Nature Biotech.* 14: 309-314 (1996).

20 As used herein, "antisense orientation" includes reference to a duplex polynucleotide sequence which is operably linked to a promoter in an orientation where the antisense strand is transcribed. The antisense strand is sufficiently complementary to an endogenous transcription product such that translation of the endogenous transcription product is often inhibited.

25 As used herein, "chromosomal region" includes reference to a length of a chromosome which may be measured by reference to the linear segment of DNA which it comprises. The chromosomal region can be defined by reference to two unique DNA sequences, i.e., markers.

The term "conservatively modified variants" applies to both amino acid and
30 nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or conservatively modified variants of the amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein.

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For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one
5 species of conservatively modified variation. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine; and UGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule.
10 Accordingly, each silent variation of a nucleic acid which encodes a polypeptide of the present invention is implicit in each described polypeptide sequence and incorporated herein by reference.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein
15 sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Thus, any number of amino acid residues selected from the group of integers consisting of from 1 to 15 can be so altered. Thus, for example, 1, 2, 3, 4, 5, 7, or 10
20 alterations can be made. Conservatively modified variants typically provide similar biological activity as the unmodified polypeptide sequence from which they are derived. For example, substrate specificity, enzyme activity, or ligand/receptor binding is generally at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the native protein for its native substrate. Conservative substitution tables providing functionally similar
25 amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 30 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

See also, Creighton (1984) Proteins W.H. Freeman and Company.

By "encoding" or "encoded", with respect to a specified nucleic acid, is meant comprising the information for translation into the specified protein. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within
5 translated regions of the nucleic acid, or may lack such intervening non-translated sequences (e.g., as in cDNA). The information by which a protein is encoded is specified by the use of codons. Typically, the amino acid sequence is encoded by the nucleic acid using the "universal" genetic code. However, variants of the universal code, such as are present in some plant, animal, and fungal mitochondria, the bacterium
10 *Mycoplasma capricolum* (*Proc. Natl. Acad. Sci. (USA)*, 82: 2306-2309 (1985)), or the ciliate *Macronucleus*, may be used when the nucleic acid is expressed using these organisms.

When the nucleic acid is prepared or altered synthetically, advantage can be taken of known codon preferences of the intended host where the nucleic acid is to be
15 expressed. For example, although nucleic acid sequences of the present invention may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ (Murray *et al.* *Nucl. Acids Res.* 17: 477-498 (1989)). Thus, the maize preferred codon for a
20 particular amino acid may be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants are listed in Table 4 of Murray *et al.*, *above*.

As used herein "full-length sequence" in reference to a specified polynucleotide or its encoded protein means having the entire amino acid sequence of, a native (non-
25 synthetic), endogenous, catalytically active form of the specified protein. Methods to determine whether a sequence is full-length are well known in the art including such exemplary techniques as northern or western blots, primer extension, S1 protection, and ribonuclease protection. See, e.g., *Plant Molecular Biology: A Laboratory Manual*, Clark, Ed., Springer-Verlag, Berlin (1997). Comparison to known full-length
30 homologous (orthologous and/or paralogous) sequences can also be used to identify full-length sequences of the present invention. Additionally, consensus sequences typically present at the 5' and 3' untranslated regions of mRNA aid in the identification of a polynucleotide as full-length. For example, the consensus sequence ANNNNAUGG,

where the underlined codon represents the N-terminal methionine, aids in determining whether the polynucleotide has a complete 5' end. Consensus sequences at the 3' end, such as polyadenylation sequences, aid in determining whether the polynucleotide has a complete 3' end.

- 5 The term "gene activity" refers to one or more steps involved in gene expression, including transcription, translation, and the functioning of the protein encoded by the gene.

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified
10 from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived, or, if from the same species, one or both are substantially modified from their original form. A heterologous protein may originate from a foreign species or, if from the same
15 species, is substantially modified from its original form by deliberate human intervention.

By "host cell" is meant a cell which contains a vector and supports the replication and/or expression of the expression vector. Host cells may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells.
20 Preferably, host cells are monocotyledonous or dicotyledonous plant cells. A particularly preferred monocotyledonous host cell is a maize host cell.

The term "hybridization complex" includes reference to a duplex nucleic acid structure formed by two single-stranded nucleic acid sequences selectively hybridized with each other.

25 By "immunologically reactive conditions" or "immunoreactive conditions" is meant conditions which allow an antibody, generated to a particular epitope, to bind to that epitope to a detectably greater degree (e.g., at least 2-fold over background) than the antibody binds to substantially all other epitopes in a reaction mixture comprising the particular epitope. Immunologically reactive conditions are dependent upon the format
30 of the antibody binding reaction and typically are those utilized in immunoassay protocols. See Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York (1988), for a description of immunoassay formats and conditions.

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The term "introduced" in the context of inserting a nucleic acid into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

The terms "isolated" refers to material, such as a nucleic acid or a protein, which is: (1) substantially or essentially free from components which normally accompany or interact with it as found in its naturally occurring environment. The isolated material optionally comprises material not found with the material in its natural environment; or (2) if the material is in its natural environment, the material has been synthetically (non-naturally) altered by deliberate human intervention to a composition and/or placed at a locus in the cell (e.g., genome or subcellular organelle) not native to a material found in that environment. The alteration to yield the synthetic material can be performed on the material within or removed from its natural state. For example, a naturally occurring nucleic acid becomes an isolated nucleic acid if it is altered, or if it is transcribed from DNA which has been altered, by non-natural, synthetic (i.e., "man-made") methods performed within the cell from which it originates. See, e.g., Compounds and Methods for Site Directed Mutagenesis in Eukaryotic Cells, Kmiec, U.S. Patent No. 5,565,350; *In Vivo* Homologous Sequence Targeting in Eukaryotic Cells; Zarling *et al.*, PCT/US93/03868. Likewise, a naturally occurring nucleic acid (e.g., a promoter) becomes isolated if it is introduced by non-naturally occurring means to a locus of the genome not native to that nucleic acid. Nucleic acids which are "isolated" as defined herein, are also referred to as "heterologous" nucleic acids.

Unless otherwise stated, the term "cellulose synthase nucleic acid" is a nucleic acid of the present invention and means a nucleic acid comprising a polynucleotide of the present invention (a "cellulose synthase polynucleotide") encoding a cellulose synthase polypeptide. A "cellulose synthase gene" is a gene of the present invention and refers to a non-heterologous genomic form of a full-length cellulose synthase polynucleotide.

As used herein, "localized within the chromosomal region defined by and including" with respect to particular markers includes reference to a contiguous length of a chromosome delimited by and including the stated markers.

As used herein, "marker" includes reference to a locus on a chromosome that serves to identify a unique position on the chromosome. A "polymorphic marker" includes reference to a marker which appears in multiple forms (alleles) such that different forms of the marker, when they are present in a homologous pair, allow transmission of each of the chromosomes in that pair to be followed. A genotype may be defined by use of one or a plurality of markers.

As used herein, "nucleic acid" includes reference to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues having the essential nature of natural nucleotides in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotides (e.g., peptide nucleic acids).

By "nucleic acid library" is meant a collection of isolated DNA or RNA molecules which comprise and substantially represent the entire transcribed fraction of a genome of a specified organism. Construction of exemplary nucleic acid libraries, such as genomic and cDNA libraries, is taught in standard molecular biology references such as Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology*, Vol. 152, Academic Press, Inc., San Diego, CA (Berger); Sambrook *et al.*, *Molecular Cloning - A Laboratory Manual*, 2nd ed., Vol. 1-3 (1989); and *Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (1994 Supplement).

As used herein "operably linked" includes reference to a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

As used herein, the term "plant" includes reference to whole plants, plant parts or organs (e.g., leaves, stems, roots, etc.), plant cells, seeds and progeny of same. Plant cell, as used herein includes, without limitation, cells obtained from or found in: seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. Plant cells can also be understood to include modified cells, such as protoplasts, obtained from the aforementioned tissues.

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The class of plants which can be used in the methods of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants. Particularly preferred plants include maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley and
5 millet.

As used herein, "polynucleotide" includes reference to a deoxyribopolynucleotide, ribopolynucleotide, or analogs thereof that have the essential nature of a natural ribonucleotide in that they hybridize, under stringent hybridization conditions, to substantially the same nucleotide sequence as naturally occurring
10 nucleotides and/or allow translation into the same amino acid(s) as the naturally occurring nucleotide(s). A polynucleotide can be full-length or a subsequence of a native or heterologous structural or regulatory gene. Unless otherwise indicated, the term includes reference to the specified sequence as well as the complementary sequence thereof. Thus, DNAs or RNAs with backbones modified for stability or for other reasons
15 are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as
20 it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including *among other things*, simple and complex cells.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in
25 which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The essential nature of such analogues of naturally occurring amino acids is that, when incorporated into a protein, that protein is specifically reactive to antibodies elicited to the same protein but consisting entirely of naturally occurring
30 amino acids. The terms "polypeptide", "peptide" and "protein" are also inclusive of modifications including, but not limited to, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation. Exemplary modifications are described in most basic texts, such as, *Proteins - Structure*

and *Molecular Properties*, 2nd ed., T. E. Creighton, W. H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as, for example, those provided by Wold, F., *Post-translational Protein Modifications: Perspectives and Prospects*, pp. 1-12 in *Posttranslational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter *et al.*, *Meth. Enzymol.* 182: 626-646 (1990) and Rattan *et al.*, *Protein Synthesis: Posttranslational Modifications and Aging*, *Ann. N.Y. Acad. Sci.* 663: 48-62 (1992). It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslation events, including natural processing event and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translation natural process and by entirely synthetic methods, as well. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in *E. coli* or other cells, prior to proteolytic processing, almost invariably will be N-formylmethionine. During post-translational modification of the peptide, a methionine residue at the NH₂-terminus may be deleted. Accordingly, this invention contemplates the use of both the methionine-containing and the methionine-less amino terminal variants of the protein of the invention. In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized by expressing a polynucleotide in a host cell.

As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. Exemplary plant promoters include, but are not limited to, those that are obtained from plants, plant viruses, and bacteria which comprise genes expressed in plant cells such *Agrobacterium* or *Rhizobium*. Examples of promoters under developmental control include promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, or seeds. Such promoters are

referred to as "tissue preferred". Promoters which initiate transcription only in certain tissue are referred to as "tissue specific". A "cell type" specific promoter primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves. An "inducible" promoter is a promoter which is under environmental control. Examples of environmental conditions that may effect transcription by inducible promoters include anaerobic conditions or the presence of light. Tissue specific, tissue preferred, cell type specific, and inducible promoters constitute the class of "non-constitutive" promoters. A "constitutive" promoter is a promoter which is active under most environmental conditions.

10 The term "cellulose synthase polypeptide" is a polypeptide of the present invention and refers to one or more amino acid sequences, in glycosylated or non-glycosylated form. The term is also inclusive of fragments, variants, homologs, alleles or precursors (e.g., preproteins or proproteins) thereof. A "cellulose synthase protein" is a protein of the present invention and comprises a cellulose synthase polypeptide.

15 As used herein "recombinant" includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found in identical form within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under-expressed or not expressed at all as a result of deliberate human intervention. The term "recombinant" as used herein does not encompass the alteration of the cell or vector by naturally occurring events (e.g., spontaneous mutation, natural transformation/transduction/transposition) such as those occurring without deliberate human intervention.

25 As used herein, a "recombinant expression cassette" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements which permit transcription of a particular nucleic acid in a host cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid to be transcribed, and a promoter.

30 The term "residue" or "amino acid residue" or "amino acid" are used interchangeably herein to refer to an amino acid that is incorporated into a protein,

polypeptide, or peptide (collectively "protein"). The amino acid may be a naturally occurring amino acid and, unless otherwise limited, may encompass known analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

5 The term "selectively hybridizes" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing sequences typically have about at least
10 80% sequence identity, preferably 90% sequence identity, and most preferably 100% sequence identity (i.e., complementary) with each other.

 The term "specifically reactive", includes reference to a binding reaction between an antibody and a protein having an epitope recognized by the antigen binding site of the antibody. This binding reaction is determinative of the presence of a protein having the
15 recognized epitope amongst the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to an analyte having the recognized epitope to a substantially greater degree (e.g., at least 2-fold over background) than to substantially all other analytes lacking the epitope which are present in the sample.

20 The terms "stringent conditions" or "stringent hybridization conditions" includes reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than 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
25 conditions, target sequences can be identified which are 100% complementary to the probe (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, preferably less than 500 nucleotides in length.

30 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

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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 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.

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, *Anal. Biochem.*, 138:267-284 (1984): $T_m = 81.5\text{ }^{\circ}\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\% \text{ form}) - 500/L$; 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 $\geq 90\%$ 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 preferred 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, *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York (1993); and *Current Protocols in Molecular Biology*, Chapter 2, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995).

As used herein, "transgenic plant" includes reference to a plant which comprises within its genome a heterologous polynucleotide. Generally, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is used herein to include any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been altered by the presence of heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

As used herein, "vector" includes reference to a nucleic acid used in transfection of a host cell and into which can be inserted a polynucleotide. Vectors are often replicons. Expression vectors permit transcription of a nucleic acid inserted therein.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", (d) "percentage of sequence identity", and (e) "substantial 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" means includes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence may be compared to a reference sequence and wherein the

portion of 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 sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally
5 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.
10 Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.* 2: 482 (1981); by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48: 443 (1970); by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci.* 85: 2444 (1988); by computerized implementations of these algorithms, including, but not limited
15 to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California, GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wisconsin, USA; the CLUSTAL program is well described by Higgins and Sharp, *Gene* 73: 237-244 (1988); Higgins and Sharp, *CABIOS* 5: 151-153 (1989); Corpet, *et al.*, *Nucleic
20 Acids Research* 16: 10881-90 (1988); Huang, *et al.*, *Computer Applications in the Biosciences* 8: 155-65 (1992), and Pearson, *et al.*, *Methods in Molecular Biology* 24: 307-331 (1994). The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database
25 sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, *Current Protocols in Molecular Biology*, Chapter 19, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995).

30 Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using the BLAST 2.0 suite of programs using default parameters. Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology

Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the
5 neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid
10 sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of
15 either sequence is reached. The BLAST algorithm parameters W , T , and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, $M=5$, $N=-4$, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62
20 scoring matrix (*see* Henikoff & Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability ($P(N)$), which provides
25 an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance.

BLAST searches assume that proteins can be modeled as random sequences. However, many real proteins comprise regions of nonrandom sequences which may be homopolymeric tracts, short-period repeats, or regions enriched in one or more amino
30 acids. Such low-complexity regions may be aligned between unrelated proteins even though other regions of the protein are entirely dissimilar. A number of low-complexity filter programs can be employed to reduce such low-complexity alignments. For example, the SEG (Wooten and Federhen, *Comput. Chem.*, 17:149-163 (1993)) and

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XNU (Claverie and States, *Comput. Chem.*, 17:191-201 (1993)) low-complexity filters can be employed alone or in combination.

(c) As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences which 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. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences which 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., according to the algorithm of Meyers and Miller, *Computer Applic. Biol. Sci.*, 4: 11-17 (1988) e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California, USA).

(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.

(e) (i) The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 70% sequence identity, preferably at least 80%, more preferably at least 90% and most preferably at least 95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 60%, more preferably at least 70%, 80%, 90%, and most preferably at least 95%.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. However, nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This may occur, *e.g.*, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

(e) (ii) The terms "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with at least 70% sequence identity to a reference sequence, preferably 80%, more preferably 85%, most preferably at least 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48: 443 (1970). An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. Peptides which are "substantially similar" share sequences as noted above except that residue positions which are not identical may differ by conservative amino acid changes.

DETAILED DESCRIPTION OF THE INVENTION

Overview

The present invention provides, *among other things*, compositions and methods for modulating (i.e., increasing or decreasing) the level of polypeptides of the present invention in plants. In particular, the polypeptides of the present invention can
5 be expressed at developmental stages, in tissues, and/or in quantities which are uncharacteristic of non-recombinantly engineered plants. Thus, the present invention provides utility in such exemplary applications as improvement of stalk quality for improved stand or silage. Further, the present invention provides for an increased concentration of cellulose in the pericarp; hardening the kernel and thus improving its
10 handling ability.

The present invention also provides isolated nucleic acid comprising polynucleotides of sufficient length and complementarity to a gene of the present invention to use as probes or amplification primers in the detection, quantitation, or isolation of gene transcripts. For example, isolated nucleic acids of the present invention
15 can be used as probes in detecting deficiencies in the level of mRNA in screenings for desired transgenic plants, for detecting mutations in the gene (e.g., substitutions, deletions, or additions), for monitoring upregulation of expression or changes in enzyme activity in screening assays of compounds, for detection of any number of allelic variants (polymorphisms) of the gene, or for use as molecular markers in plant breeding
20 programs. The isolated nucleic acids of the present invention can also be used for recombinant expression of their encoded polypeptides, or for use as immunogens in the preparation and/or screening of antibodies. The isolated nucleic acids of the present invention can also be employed for use in sense or antisense suppression of one or more genes of the present invention in a host cell, tissue, or plant. Attachment of chemical
25 agents which bind, intercalate, cleave and/or crosslink to the isolated nucleic acids of the present invention can also be used to modulate transcription or translation.

The present invention also provides isolated proteins comprising a polypeptide of the present invention (e.g., preproenzyme, proenzyme, or enzymes). The present invention also provides proteins comprising at least one epitope from a polypeptide of the
30 present invention. The proteins of the present invention can be employed in assays for enzyme agonists or antagonists of enzyme function, or for use as immunogens or antigens to obtain antibodies specifically immunoreactive with a protein of the present invention. Such antibodies can be used in assays for expression levels, for identifying

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and/or isolating nucleic acids of the present invention from expression libraries, or for purification of polypeptides of the present invention.

The isolated nucleic acids and proteins of the present invention can be used over a broad range of plant types, particularly monocots such as the species of the Family

5 *Graminae* including *Sorghum bicolor* and *Zea mays*. The isolated nucleic acid and proteins of the present invention can also be used in species from the genera: *Cucurbita*, *Rosa*, *Vitis*, *Juglans*, *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*,

10 *Petunia*, *Digitalis*, *Majorana*, *Ciahorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Heterocallis*, *Nemesis*, *Pelargonium*, *Panieum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Pisum*, *Phaseolus*, *Lolium*, *Oryza*, *Avena*, *Hordeum*, *Secale*, *Triticum*, *Bambusa*, *Dendrocalamus*, and *Melocanna*.

15 Nucleic Acids

The present invention provides, *among other things*, isolated nucleic acids of RNA, DNA, and analogs and/or chimeras thereof, comprising a polynucleotide of the present invention.

A polynucleotide of the present invention is inclusive of:

20 (a) a polynucleotide encoding a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58, and conservatively modified and polymorphic variants thereof, including exemplary polynucleotides of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57;

(b) a polynucleotide which is the product of amplification from a *Zea mays*

25 nucleic acid library using primer pairs which selectively hybridize under stringent conditions to loci within a polynucleotide selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57, wherein the polynucleotide has substantial sequence identity to a polynucleotide selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53,

30 and 57;

(c) a polynucleotide which selectively hybridizes to a polynucleotide of (a) or (b);

(d) a polynucleotide having a specified sequence identity with polynucleotides of (a), (b), or (c);

(e) a polynucleotide encoding a protein having a specified number of contiguous amino acids from a prototype polypeptide, wherein the protein is specifically recognized by antisera elicited by presentation of the protein and wherein the protein does not detectably immunoreact to antisera which has been fully immunosorbed with the protein;

5 (f) complementary sequences of polynucleotides of (a), (b), (c), (d), or (e); and

(g) a polynucleotide comprising at least a specific number of contiguous nucleotides from a polynucleotide of (a), (b), (c), (d), (e), or (f).

10 *A. Polynucleotides Encoding A Polypeptide of the Present Invention or Conservatively Modified or Polymorphic Variants Thereof*

As indicated in (a), above, the present invention provides isolated nucleic acids comprising a polynucleotide of the present invention, wherein the polynucleotide encodes a polypeptide of the present invention, or conservatively modified or polymorphic variants thereof. Those of skill in the art will recognize that the degeneracy of the genetic code allows for a plurality of polynucleotides to encode for the identical amino acid sequence. Such "silent variations" can be used, for example, to selectively hybridize and detect allelic variants of polynucleotides of the present invention. Accordingly, the present invention includes polynucleotides of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57, and silent variations of polynucleotides encoding a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58. The present invention further provides isolated nucleic acids comprising polynucleotides encoding conservatively modified variants of a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58. Additionally, the present invention further provides isolated nucleic acids comprising polynucleotides encoding one or more polymorphic (allelic) variants of polypeptides/polynucleotides. Polymorphic variants are frequently used to follow segregation of chromosomal regions in, for example, marker assisted selection methods for crop improvement.

30

B. Polynucleotides Amplified from a Zea mays Nucleic Acid Library

As indicated in (b), above, the present invention provides an isolated nucleic acid comprising a polynucleotide of the present invention, wherein the polynucleotides are amplified from a *Zea mays* nucleic acid library. *Zea mays* lines B73, PHRE1, A632, BMS-P2#10, W23, and Mo17 are known and publicly available. Other publicly known and available maize lines can be obtained from the Maize Genetics Cooperation (Urbana, IL). The nucleic acid library may be a cDNA library, a genomic library, or a library generally constructed from nuclear transcripts at any stage of intron processing. cDNA libraries can be normalized to increase the representation of relatively rare cDNAs. In optional embodiments, the cDNA library is constructed using a full-length cDNA synthesis method. Examples of such methods include Oligo-Capping (Maruyama, K. and Sugano, S. *Gene* 138: 171-174, 1994), Biotinylated CAP Trapper (Carninci, P., Kvan, C., *et al.* *Genomics* 37: 327-336, 1996), and CAP Retention Procedure (Edery, E., Chu, L.L., *et al.* *Molecular and Cellular Biology* 15: 3363-3371, 1995). cDNA synthesis is often catalyzed at 50-55°C to prevent formation of RNA secondary structure. Examples of reverse transcriptases that are relatively stable at these temperatures are SuperScript II Reverse Transcriptase (Life Technologies, Inc.), AMV Reverse Transcriptase (Boehringer Mannheim) and RetroAmp Reverse Transcriptase (Epicentre). Rapidly growing tissues, or rapidly dividing cells are preferably used as mRNA sources such as from the elongating internode of corn plants.

The polynucleotides of the present invention include those amplified using the following primer pairs:

SEQ ID NOS: 3 and 4 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 1;

SEQ ID NOS: 7 and 8 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 5; and

SEQ ID NOS: 11 and 12 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 9.

SEQ ID NOS: 15 and 16 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 13.

SEQ ID NOS: 19 and 20 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 17;

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SEQ ID NOS: 23 and 24 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 21; and

SEQ ID NOS: 27 and 28 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 25.

5 SEQ ID NOS: 31 and 32 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 29.

SEQ ID NOS: 35 and 36 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 33;

10 SEQ ID NOS: 39 and 40 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 37; and

SEQ ID NOS: 43 and 44 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 41.

SEQ ID NOS: 47 and 48 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 45.

15 SEQ ID NOS: 51 and 52 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 49;

SEQ ID NOS: 55 and 56 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 53; and

20 SEQ ID NOS: 59 and 60 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 57.

The present invention also provides subsequences of the polynucleotides of the present invention. A variety of subsequences can be obtained using primers which selectively hybridize under stringent conditions to at least two sites within a polynucleotide of the present invention, or to two sites within the nucleic acid which flank and comprise a polynucleotide of the present invention, or to a site within a polynucleotide of the present invention and a site within the nucleic acid which comprises it. Primers are chosen to selectively hybridize, under stringent hybridization conditions, to a polynucleotide of the present invention. Generally, the primers are complementary to a subsequence of the target nucleic acid which they amplify. As those skilled in the art will appreciate, the sites to which the primer pairs will selectively hybridize are chosen such that a single contiguous nucleic acid can be formed under the desired amplification conditions.

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In optional embodiments, the primers will be constructed so that they selectively hybridize under stringent conditions to a sequence (or its complement) within the target nucleic acid which comprises the codon encoding the carboxy or amino terminal amino acid residue (i.e., the 3' terminal coding region and 5' terminal coding region, respectively) of the polynucleotides of the present invention. Optionally within these embodiments, the primers will be constructed to selectively hybridize entirely within the coding region of the target polynucleotide of the present invention such that the product of amplification of a cDNA target will consist of the coding region of that cDNA. The primer length in nucleotides is selected from the group of integers consisting of from at least 15 to 50. Thus, the primers can be at least 15, 18, 20, 25, 30, 40, or 50 nucleotides in length. Those of skill will recognize that a lengthened primer sequence can be employed to increase specificity of binding (i.e., annealing) to a target sequence. A non-annealing sequence at the 5' end of a primer (a "tail") can be added, for example, to introduce a cloning site at the terminal ends of the amplicon.

The amplification products can be translated using expression systems well known to those of skill in the art and as discussed, *infra*. The resulting translation products can be confirmed as polypeptides of the present invention by, for example, assaying for the appropriate catalytic activity (e.g., specific activity and/or substrate specificity), or verifying the presence of one or more linear epitopes which are specific to a polypeptide of the present invention. Methods for protein synthesis from PCR derived templates are known in the art and available commercially. See, e.g., Amersham Life Sciences, Inc, Catalog '97, p.354.

Methods for obtaining 5' and/or 3' ends of a vector insert are well known in the art. See, e.g., RACE (Rapid Amplification of Complementary Ends) as described in Frohman, M. A., in PCR Protocols: A Guide to Methods and Applications, M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White, Eds. (Academic Press, Inc., San Diego, 1990), pp. 28-38.); see also, U.S. Pat. No. 5,470,722, and *Current Protocols in Molecular Biology*, Unit 15.6, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995); Frohman and Martin, *Techniques* 1:165 (1989).

C. Polynucleotides Which Selectively Hybridize to a Polynucleotide of (A) or (B)

As indicated in (c), above, the present invention provides isolated nucleic acids comprising polynucleotides of the present invention, wherein the polynucleotides

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selectively hybridize, under selective hybridization conditions, to a polynucleotide of paragraphs (A) or (B) as discussed, *above*. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising the polynucleotides of (A) or (B). For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated or otherwise complementary to a cDNA from a dicot or monocot nucleic acid library. Exemplary species of monocots and dicots include, but are not limited to: corn, canola, soybean, cotton, wheat, sorghum, sunflower, oats, sugar cane, millet, barley, and rice. Optionally, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

D. Polynucleotides Having a Specific Sequence Identity with the Polynucleotides of (A), (B) or (C)

As indicated in (d), above, the present invention provides isolated nucleic acids comprising polynucleotides of the present invention, wherein the polynucleotides have a specified identity at the nucleotide level to a polynucleotide as disclosed above in paragraphs (A), (B), or (C). The percentage of identity to a reference sequence is at least 60% and, rounded upwards to the nearest integer, can be expressed as an integer selected from the group of integers consisting of from 60 to 99. Thus, for example, the percentage of identity to a reference sequence can be at least 70%, 75%, 80%, 85%, 90%, or 95%.

Optionally, the polynucleotides of this embodiment will share an epitope with a polypeptide encoded by the polynucleotides of (A), (B), or (C). Thus, these polynucleotides encode a first polypeptide which elicits production of antisera comprising antibodies which are specifically reactive to a second polypeptide encoded by

a polynucleotide of (A), (B), or (C). However, the first polypeptide does not bind to antisera raised against itself when the antisera has been fully immunosorbed with the first polypeptide. Hence, the polynucleotides of this embodiment can be used to generate antibodies for use in, for example, the screening of expression libraries for nucleic acids
5 comprising polynucleotides of (A), (B), or (C), or for purification of, or in immunoassays for, polypeptides encoded by the polynucleotides of (A), (B), or (C). The polynucleotides of this embodiment embrace nucleic acid sequences which can be employed for selective hybridization to a polynucleotide encoding a polypeptide of the present invention.

10 Screening polypeptides for specific binding to antisera can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. Antibody screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from
15 5-100 amino acids long, and often from about 8 to 15 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such
20 methods are described in PCT patent publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both *in vitro* chemical synthesis and recombinant methods. See, PCT Patent publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Patent Nos. 5,658,754; and 5,643,768. Peptide display libraries, vectors, and screening kits are commercially
25 available from such suppliers as Invitrogen (Carlsbad, CA).

E. Polynucleotides Encoding a Protein Having a Subsequence from a Prototype Polypeptide and is Cross-Reactive to the Prototype Polypeptide

As indicated in (e), above, the present invention provides isolated nucleic acids
30 comprising polynucleotides of the present invention, wherein the polynucleotides encode a protein having a subsequence of contiguous amino acids from a prototype polypeptide of the present invention such as are provided in (a), above. The length of contiguous amino acids from the prototype polypeptide is selected from the group of integers

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consisting of from at least 10 to the number of amino acids within the prototype sequence. Thus, for example, the polynucleotide can encode a polypeptide having a subsequence having at least 10, 15, 20, 25, 30, 35, 40, 45, or 50, contiguous amino acids from the prototype polypeptide. Further, the number of such subsequences
5 encoded by a polynucleotide of the instant embodiment can be any integer selected from the group consisting of from 1 to 20, such as 2, 3, 4, or 5. The subsequences can be separated by any integer of nucleotides from 1 to the number of nucleotides in the sequence such as at least 5, 10, 15, 25, 50, 100, or 200 nucleotides.

The proteins encoded by polynucleotides of this embodiment, when presented as
10 an immunogen, elicit the production of polyclonal antibodies which specifically bind to a prototype polypeptide such as but not limited to, a polypeptide encoded by the polynucleotide of (a) or (b), above. Generally, however, a protein encoded by a polynucleotide of this embodiment does not bind to antisera raised against the prototype polypeptide when the antisera has been fully immunosorbed with the prototype
15 polypeptide. Methods of making and assaying for antibody binding specificity/affinity are well known in the art. Exemplary immunoassay formats include ELISA, competitive immunoassays, radioimmunoassays, Western blots, indirect immunofluorescent assays and the like.

In a preferred assay method, fully immunosorbed and pooled antisera which is
20 elicited to the prototype polypeptide can be used in a competitive binding assay to test the protein. The concentration of the prototype polypeptide required to inhibit 50% of the binding of the antisera to the prototype polypeptide is determined. If the amount of the protein required to inhibit binding is less than twice the amount of the prototype protein, then the protein is said to specifically bind to the antisera elicited to the
25 immunogen. Accordingly, the proteins of the present invention embrace allelic variants, conservatively modified variants, and minor recombinant modifications to a prototype polypeptide.

A polynucleotide of the present invention optionally encodes a protein having a molecular weight as the non-glycosylated protein within 20% of the molecular weight of
30 the full-length non-glycosylated polypeptides of the present invention. Molecular weight can be readily determined by SDS-PAGE under reducing conditions. Preferably, the molecular weight is within 15% of a full length polypeptide of the present invention, more preferably within 10% or 5%, and most preferably within 3%, 2%, or 1% of a full

length polypeptide of the present invention. Molecular weight determination of a protein can be conveniently performed by SDS-PAGE under denaturing conditions.

Optionally, the polynucleotides of this embodiment will encode a protein having a specific activity at least 50%, 60%, 80%, or 90% of the native, endogenous (i.e., non-isolated), full-length polypeptide of the present invention. Further, the proteins encoded by polynucleotides of this embodiment will optionally have a substantially similar affinity constant (K_m) and/or catalytic activity (i.e., the microscopic rate constant, k_{cat}) as the native endogenous, full-length protein. Those of skill in the art will recognize that k_{cat}/K_m value determines the specificity for competing substrates and is often referred to as the specificity constant. Proteins of this embodiment can have a k_{cat}/K_m value at least 10% of a non-isolated full-length polypeptide of the present invention as determined using the endogenous substrate of that polypeptide. Optionally, the k_{cat}/K_m value will be at least 20%, 30%, 40%, 50%, and most preferably at least 60%, 70%, 80%, 90%, or 95% the k_{cat}/K_m value of the non-isolated, full-length polypeptide of the present invention.

Determination of k_{cat} , K_m , and k_{cat}/K_m can be determined by any number of means well known to those of skill in the art. For example, the initial rates (i.e., the first 5% or less of the reaction) can be determined using rapid mixing and sampling techniques (e.g., continuous-flow, stopped-flow, or rapid quenching techniques), flash photolysis, or relaxation methods (e.g., temperature jumps) in conjunction with such exemplary methods of measuring as spectrophotometry, spectrofluorimetry, nuclear magnetic resonance, or radioactive procedures. Kinetic values are conveniently obtained using a Lineweaver-Burk or Eadie-Hofstee plot.

F. Polynucleotides Complementary to the Polynucleotides of (A)-(E)

As indicated in (f), above, the present invention provides isolated nucleic acids comprising polynucleotides complementary to the polynucleotides of paragraphs A-E, above. As those of skill in the art will recognize, complementary sequences base-pair throughout the entirety of their length with the polynucleotides of (A)-(E) (i.e., have 100% sequence identity over their entire length). Complementary bases associate through hydrogen bonding in double stranded nucleic acids. For example, the following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil.

G. Polynucleotides Which are Subsequences of the Polynucleotides of (A)-(F)

As indicated in (g), above, the present invention provides isolated nucleic acids comprising polynucleotides which comprise at least 15 contiguous bases from the polynucleotides of (A) through (F) as discussed above. The length of the polynucleotide
5 is given as an integer selected from the group consisting of from at least 15 to the length of the nucleic acid sequence from which the polynucleotide is a subsequence of. Thus, for example, polynucleotides of the present invention are inclusive of polynucleotides comprising at least 15, 20, 25, 30, 40, 50, 60, 75, or 100 contiguous nucleotides in length from the polynucleotides of (A)-(F). Optionally, the number of such
10 subsequences encoded by a polynucleotide of the instant embodiment can be any integer selected from the group consisting of from 1 to 20, such as 2, 3, 4, or 5. The subsequences can be separated by any integer of nucleotides from 1 to the number of nucleotides in the sequence such as at least 5, 10, 15, 25, 50, 100, or 200 nucleotides.

The subsequences of the present invention can comprise structural characteristics
15 of the sequence from which it is derived. Alternatively, the subsequences can lack certain structural characteristics of the larger sequence from which it is derived. For example, a subsequence from a polynucleotide encoding a polypeptide having at least one linear epitope in common with a prototype polypeptide sequence as provided in (a), above, may encode an epitope in common with the prototype sequence. Alternatively,
20 the subsequence may not encode an epitope in common with the prototype sequence but can be used to isolate the larger sequence by, for example, nucleic acid hybridization with the sequence from which it's derived. Subsequences can be used to modulate or detect gene expression by introducing into the subsequences compounds which bind, intercalate, cleave and/or crosslink to nucleic acids. Exemplary compounds include
25 acridine, psoralen, phenanthroline, naphthoquinone, daunomycin or chloroethylaminoaryl conjugates.

Construction of Nucleic Acids

The isolated nucleic acids of the present invention can be made using (a) standard
30 recombinant methods, (b) synthetic techniques, or combinations thereof. In some embodiments, the polynucleotides of the present invention will be cloned, amplified, or otherwise constructed from a monocot. In preferred embodiments the monocot is *Zea mays*.

The nucleic acids may conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites may be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences may be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. A polynucleotide of the present invention can be attached to a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention. Additional sequences may be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Typically, the length of a nucleic acid of the present invention less the length of its polynucleotide of the present invention is less than 20 kilobase pairs, often less than 15 kb, and frequently less than 10 kb. Use of cloning vectors, expression vectors, adapters, and linkers is well known and extensively described in the art. For a description of various nucleic acids see, for example, Stratagene Cloning Systems, Catalogs 1995, 1996, 1997 (La Jolla, CA); and, Amersham Life Sciences, Inc, Catalog '97 (Arlington Heights, IL).

20 *A. Recombinant Methods for Constructing Nucleic Acids*

The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or a hybrid thereof, can be obtained from plant biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes which selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. While isolation of RNA, and construction of cDNA and genomic libraries is well known to those of ordinary skill in the art, the following highlights some of the methods employed.

30 *A1. mRNA Isolation and Purification*

Total RNA from plant cells comprises such nucleic acids as mitochondrial RNA, chloroplastic RNA, rRNA, tRNA, hnRNA and mRNA. Total RNA preparation typically involves lysis of cells and removal of proteins, followed by precipitation of nucleic

acids. Extraction of total RNA from plant cells can be accomplished by a variety of means. Frequently, extraction buffers include a strong detergent such as SDS and an organic denaturant such as guanidinium isothiocyanate, guanidine hydrochloride or phenol. Following total RNA isolation, poly(A)⁺ mRNA is typically purified from the remainder RNA using oligo(dT) cellulose. Exemplary total RNA and mRNA isolation protocols are described in *Plant Molecular Biology: A Laboratory Manual*, Clark, Ed., Springer-Verlag, Berlin (1997); and, *Current Protocols in Molecular Biology*, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995). Total RNA and mRNA isolation kits are commercially available from vendors such as Stratagene (La Jolla, CA), Clontech (Palo Alto, CA), Pharmacia (Piscataway, NJ), and 5'-3' (Paoli, PA). See also, U.S. Patent Nos. 5,614,391; and, 5,459,253. The mRNA can be fractionated into populations with size ranges of about 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 kb. The cDNA synthesized for each of these fractions can be size selected to the same size range as its mRNA prior to vector insertion. This method helps eliminate truncated cDNA formed by incompletely reverse transcribed mRNA.

A2. Construction of a cDNA Library

Construction of a cDNA library generally entails five steps. First, first strand cDNA synthesis is initiated from a poly(A)⁺ mRNA template using a poly(dT) primer or random hexanucleotides. Second, the resultant RNA-DNA hybrid is converted into double stranded cDNA, typically by a combination of RNase H and DNA polymerase I (or Klenow fragment). Third, the termini of the double stranded cDNA are ligated to adaptors. Ligation of the adaptors will produce cohesive ends for cloning. Fourth, size selection of the double stranded cDNA eliminates excess adaptors and primer fragments, and eliminates partial cDNA molecules due to degradation of mRNAs or the failure of reverse transcriptase to synthesize complete first strands. Fifth, the cDNAs are ligated into cloning vectors and packaged. cDNA synthesis protocols are well known to the skilled artisan and are described in such standard references as: *Plant Molecular Biology: A Laboratory Manual*, Clark, Ed., Springer-Verlag, Berlin (1997); and, *Current Protocols in Molecular Biology*, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995). cDNA synthesis kits are available from a variety of commercial vendors such as Stratagene or Pharmacia.

A number of cDNA synthesis protocols have been described which provide substantially pure full-length cDNA libraries. Substantially pure full-length cDNA libraries are constructed to comprise at least 90%, and more preferably at least 93% or 95% full-length inserts amongst clones containing inserts. The length of insert in such libraries can be from 0 to 8, 9, 10, 11, 12, 13, or more kilobase pairs. Vectors to accommodate inserts of these sizes are known in the art and available commercially. See, e.g., Stratagene's lambda ZAP Express (cDNA cloning vector with 0 to 12 kb cloning capacity).

An exemplary method of constructing a greater than 95% pure full-length cDNA library is described by Carninci *et al.*, *Genomics*, 37:327-336 (1996). In that protocol, the cap-structure of eukaryotic mRNA is chemically labeled with biotin. By using streptavidin-coated magnetic beads, only the full-length first-strand cDNA/mRNA hybrids are selectively recovered after RNase I treatment. The method provides a high yield library with an unbiased representation of the starting mRNA population. Other methods for producing full-length libraries are known in the art. See, e.g., Edery *et al.*, *Mol. Cell Biol.*, 15(6):3363-3371 (1995); and, PCT Application WO 96/34981.

A3. Normalized or Subtracted cDNA Libraries

A non-normalized cDNA library represents the mRNA population of the tissue it was made from. Since unique clones are out-numbered by clones derived from highly expressed genes their isolation can be laborious. Normalization of a cDNA library is the process of creating a library in which each clone is more equally represented.

A number of approaches to normalize cDNA libraries are known in the art. One approach is based on hybridization to genomic DNA. The frequency of each hybridized cDNA in the resulting normalized library would be proportional to that of each corresponding gene in the genomic DNA. Another approach is based on kinetics. If cDNA reannealing follows second-order kinetics, rarer species anneal less rapidly and the remaining single-stranded fraction of cDNA becomes progressively more normalized during the course of the hybridization. Specific loss of any species of cDNA, regardless of its abundance, does not occur at any Cot value. Construction of normalized libraries is described in Ko, *Nucl. Acids. Res.*, 18(19):5705-5711 (1990); Patanjali *et al.*, *Proc. Natl. Acad. U.S.A.*, 88:1943-1947 (1991); U.S. Patents 5,482,685, and 5,637,685. In an exemplary method described by Soares *et al.*, normalization resulted in reduction of

the abundance of clones from a range of four orders of magnitude to a narrow range of only 1 order of magnitude. *Proc. Natl. Acad. Sci. USA*, 91:9228-9232 (1994).

Subtracted cDNA libraries are another means to increase the proportion of less abundant cDNA species. In this procedure, cDNA prepared from one pool of mRNA is
5 depleted of sequences present in a second pool of mRNA by hybridization. The cDNA:mRNA hybrids are removed and the remaining un-hybridized cDNA pool is enriched for sequences unique to that pool. See, Foote *et al.* in, *Plant Molecular Biology: A Laboratory Manual*, Clark, Ed., Springer-Verlag, Berlin (1997); Kho and Zarbl, *Technique*, 3(2):58-63 (1991); Sive and St. John, *Nucl. Acids Res.*, 16(22):10937
10 (1988); *Current Protocols in Molecular Biology*, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995); and, Swaroop *et al.*, *Nucl. Acids Res.*, 19(8):1954 (1991). cDNA subtraction kits are commercially available. See, e.g., PCR-Select (Clontech).

15 A4. Construction of a Genomic Library

To construct genomic libraries, large segments of genomic DNA are generated by random fragmentation, e.g. using restriction endonucleases, and are ligated with vector DNA to form concatemers that can be packaged into the appropriate vector. Methodologies to accomplish these ends, and sequencing methods to verify the sequence
20 of nucleic acids are well known in the art. Examples of appropriate molecular biological techniques and instructions sufficient to direct persons of skill through many construction, cloning, and screening methodologies are found in Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Vols. 1-3 (1989), *Methods in Enzymology*, Vol. 152: *Guide to Molecular Cloning Techniques*, Berger and Kimmel, Eds., San Diego: Academic Press, Inc. (1987), *Current*
25 *Protocols in Molecular Biology*, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995); *Plant Molecular Biology: A Laboratory Manual*, Clark, Ed., Springer-Verlag, Berlin (1997). Kits for construction of genomic libraries are also commercially available.

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A5. Nucleic Acid Screening and Isolation Methods

The cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the present invention such as those disclosed herein.

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Probes may be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different plant species. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the

5 conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant

10 solution through manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100 percent; however, it should be understood that minor sequence variations in the probes and primers may be

15 compensated for by reducing the stringency of the hybridization and/or wash medium.

The nucleic acids of interest can also be amplified from nucleic acid samples using amplification techniques. For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and

20 other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through *in vitro* amplification methods are found in Berger, Sambrook, and

25 Ausubel, as well as Mullis *et al.*, U.S. Patent No. 4,683,202 (1987); and, *PCR Protocols A Guide to Methods and Applications*, Innis *et al.*, Eds., Academic Press Inc., San Diego, CA (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). The T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

30 PCR-based screening methods have also been described. Wilfinger *et al.* describe a PCR-based method in which the longest cDNA is identified in the first step so that incomplete clones can be eliminated from study. *BioTechniques*, 22(3): 481-486 (1997). In that method, a primer pair is synthesized with one primer annealing to the 5'

end of the sense strand of the desired cDNA and the other primer to the vector. Clones are pooled to allow large-scale screening. By this procedure, the longest possible clone is identified amongst candidate clones. Further, the PCR product is used solely as a diagnostic for the presence of the desired cDNA and does not utilize the PCR product itself. Such methods are particularly effective in combination with a full-length cDNA construction methodology, above.

B. Synthetic Methods for Constructing Nucleic Acids

The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by methods such as the phosphotriester method of Narang *et al.*, *Meth. Enzymol.* 68: 90-99 (1979); the phosphodiester method of Brown *et al.*, *Meth. Enzymol.* 68: 109-151 (1979); the diethylphosphoramidite method of Beaucage *et al.*, *Tetra. Lett.* 22: 1859-1862 (1981); the solid phase phosphoramidite triester method described by Beaucage and Caruthers, *Tetra. Letts.* 22(20): 1859-1862 (1981), *e.g.*, using an automated synthesizer, *e.g.*, as described in Needham-VanDevanter *et al.*, *Nucleic Acids Res.*, 12: 6159-6168 (1984); and, the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis generally produces a single stranded oligonucleotide. This may be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill will recognize that while chemical synthesis of DNA is limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

Recombinant Expression Cassettes

The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence coding for the desired polynucleotide of the present invention, for example a cDNA or a genomic sequence encoding a full length polypeptide of the present invention, can be used to construct a recombinant expression cassette which can be introduced into the desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences which will direct the transcription of the polynucleotide in the intended host cell, such as tissues of a transformed plant.

For example, plant expression vectors may include (1) a cloned plant gene under the transcriptional control of 5' and 3' regulatory sequences and (2) a dominant selectable marker. Such plant expression vectors may also contain, if desired, a promoter regulatory region (e.g., one conferring inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific/selective expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

A plant promoter fragment can be employed which will direct expression of a polynucleotide of the present invention in all tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'- promoter derived from T-DNA of *Agrobacterium tumefaciens*, the ubiquitin 1 promoter, the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Patent No. 5,683,439), the *Nos* promoter, the pEmu promoter, the rubisco promoter, the GRP1-8 promoter, the actin promoter, the F3.7 promoter, and other transcription initiation regions from various plant genes known to those of skill.

Alternatively, the plant promoter can direct expression of a polynucleotide of the present invention in a specific tissue or may be otherwise under more precise environmental or developmental control. Such promoters are referred to here as "inducible" promoters. Environmental conditions that may effect transcription by inducible promoters include pathogen attack, anaerobic conditions, or the presence of light. Examples of inducible promoters are the *Adh1* promoter which is inducible by hypoxia or cold stress, the *Hsp70* promoter which is inducible by heat stress, and the *PPDK* promoter which is inducible by light.

Examples of promoters under developmental control include promoters that initiate transcription only, or preferentially, in certain tissues, such as leaves, roots, fruit, seeds, or flowers. The operation of a promoter may also vary depending on its location in the genome. Thus, an inducible promoter may become fully or partially constitutive in certain locations.

Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention. These

promoters can also be used, for example, in recombinant expression cassettes to drive expression of antisense nucleic acids to reduce, increase, or alter concentration and/or composition of the proteins of the present invention in a desired tissue. Thus, in some embodiments, the nucleic acid construct will comprise a promoter functional in a plant cell, such as in *Zea mays*, operably linked to a polynucleotide of the present invention. Promoters useful in these embodiments include the endogenous promoters driving expression of a polypeptide of the present invention.

In some embodiments, isolated nucleic acids which serve as promoter or enhancer elements can be introduced in the appropriate position (generally upstream) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered *in vivo* by mutation, deletion, and/or substitution (see, Kmiec, U.S. Patent 5,565,350; Zarling *et al.*, PCT/US93/03868), or isolated promoters can be introduced into a plant cell in the proper orientation and distance from a gene of the present invention so as to control the expression of the gene. Gene expression can be modulated under conditions suitable for plant growth so as to alter the total concentration and/or alter the composition of the polypeptides of the present invention in plant cell. Thus, the present invention provides compositions, and methods for making, heterologous promoters and/or enhancers operably linked to a native, endogenous (i.e., non-heterologous) form of a polynucleotide of the present invention.

Methods for identifying promoters with a particular expression pattern, in terms of, e.g., tissue type, cell type, stage of development, and/or environmental conditions, are well known in the art. See, e.g., *The Maize Handbook*, Chapters 114-115, Freeling and Walbot, Eds., Springer, New York (1994); *Corn and Corn Improvement*, 3rd edition, Chapter 6, Sprague and Dudley, Eds., American Society of Agronomy, Madison, Wisconsin (1988). A typical step in promoter isolation methods is identification of gene products that are expressed with some degree of specificity in the target tissue. Amongst the range of methodologies are: differential hybridization to cDNA libraries; subtractive hybridization; differential display; differential 2-D protein gel electrophoresis; DNA probe arrays; and isolation of proteins known to be expressed with some specificity in the target tissue. Such methods are well known to those of skill in the art. Commercially available products for identifying promoters are known in the art such as Clontech's (Palo Alto, CA) Universal GenomeWalker Kit.

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For the protein-based methods, it is helpful to obtain the amino acid sequence for at least a portion of the identified protein, and then to use the protein sequence as the basis for preparing a nucleic acid that can be used as a probe to identify either genomic DNA directly, or preferably, to identify a cDNA clone from a library prepared from the target tissue. Once such a cDNA clone has been identified, that sequence can be used to identify the sequence at the 5' end of the transcript of the indicated gene. For differential hybridization, subtractive hybridization and differential display, the nucleic acid sequence identified as enriched in the target tissue is used to identify the sequence at the 5' end of the transcript of the indicated gene. Once such sequences are identified, starting either from protein sequences or nucleic acid sequences, any of these sequences identified as being from the gene transcript can be used to screen a genomic library prepared from the target organism. Methods for identifying and confirming the transcriptional start site are well known in the art.

In the process of isolating promoters expressed under particular environmental conditions or stresses, or in specific tissues, or at particular developmental stages, a number of genes are identified that are expressed under the desired circumstances, in the desired tissue, or at the desired stage. Further analysis will reveal expression of each particular gene in one or more other tissues of the plant. One can identify a promoter with activity in the desired tissue or condition but that do not have activity in any other common tissue.

To identify the promoter sequence, the 5' portions of the clones described here are analyzed for sequences characteristic of promoter sequences. For instance, promoter sequence elements include the TATA box consensus sequence (TATAAT), which is usually an AT-rich stretch of 5-10 bp located approximately 20 to 40 base pairs upstream of the transcription start site. Identification of the TATA box is well known in the art. For example, one way to predict the location of this element is to identify the transcription start site using standard RNA-mapping techniques such as primer extension, S1 analysis, and/or RNase protection. To confirm the presence of the AT-rich sequence, a structure-function analysis can be performed involving mutagenesis of the putative region and quantification of the mutation's effect on expression of a linked downstream reporter gene. See, e.g., *The Maize Handbook*, Chapter 114, Freeling and Walbot, Eds., Springer, New York, (1994).

In plants, further upstream from the TATA box, at positions -80 to -100, there is typically a promoter element (i.e., the CAAT box) with a series of adenines surrounding the trinucleotide G (or T) N G. J. Messing *et al.*, in *Genetic Engineering in Plants*, Kosage, Meredith and Hollaender, Eds., pp. 221-227 1983. In maize, there is no well
5 conserved CAAT box but there are several short, conserved protein-binding motifs upstream of the TATA box. These include motifs for the trans-acting transcription factors involved in light regulation, anaerobic induction, hormonal regulation, or anthocyanin biosynthesis, as appropriate for each gene.

Once promoter and/or gene sequences are known, a region of suitable size
10 is selected from the genomic DNA that is 5' to the transcriptional start, or the translational start site, and such sequences are then linked to a coding sequence. If the transcriptional start site is used as the point of fusion, any of a number of possible 5' untranslated regions can be used in between the transcriptional start site and the partial coding sequence. If the translational start site at the 3' end of the specific promoter is
15 used, then it is linked directly to the methionine start codon of a coding sequence.

If polypeptide expression is desired, it is generally desirable to include a polyadenylation region at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The 3' end sequence to be added can be derived from, for
20 example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

An intron sequence can be added to the 5' untranslated region or the coding sequence of the partial coding sequence to increase the amount of the mature message that accumulates in the cytosol. Inclusion of a spliceable intron in the
25 transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold. Buchman and Berg, *Mol. Cell Biol.* 8: 4395-4405 (1988); Callis *et al.*, *Genes Dev.* 1: 1183-1200 (1987). Such intron enhancement of gene expression is typically greatest when placed near the 5' end of the transcription unit. Use of maize introns Adh1-S intron 1, 2, and
30 6, the Bronze-1 intron are known in the art. See generally, *The Maize Handbook*, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994).

The vector comprising the sequences from a polynucleotide of the present invention will typically comprise a marker gene which confers a selectable phenotype on

plant cells. Usually, the selectable marker gene will encode antibiotic resistance, with suitable genes including genes coding for resistance to the antibiotic spectinomycin (e.g., the *aadA* gene), the streptomycin phosphotransferase (SPT) gene coding for streptomycin resistance, the neomycin phosphotransferase (NPTII) gene encoding kanamycin or geneticin resistance, the hygromycin phosphotransferase (HPT) gene coding for hygromycin resistance, genes coding for resistance to herbicides which act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides which act to inhibit action of glutamine synthase, such as phosphinothricin or basta (e.g., the *bar* gene), or other such genes known in the art. The *bar* gene encodes resistance to the herbicide basta, the *nptII* gene encodes resistance to the antibiotics kanamycin and geneticin, and the ALS gene encodes resistance to the herbicide chlorsulfuron.

Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers *et al.*, Meth. In Enzymol., 153:253-277 (1987). These vectors are plant integrating vectors in that on transformation, the vectors integrate a portion of vector DNA into the genome of the host plant. Exemplary *A. tumefaciens* vectors useful herein are plasmids pKYLX6 and pKYLX7 of Schardl *et al.*, Gene, 61:1-11 (1987) and Berger *et al.*, Proc. Natl. Acad. Sci. U.S.A., 86:8402-8406 (1989). Another useful vector herein is plasmid pBI101.2 that is available from Clontech Laboratories, Inc. (Palo Alto, CA).

A polynucleotide of the present invention can be expressed in either sense or anti-sense orientation as desired. It will be appreciated that control of gene expression in either sense or anti-sense orientation can have a direct impact on the observable plant characteristics. Antisense technology can be conveniently used to gene expression in plants. To accomplish this, a nucleic acid segment from the desired gene is cloned and operably linked to a promoter such that the anti-sense strand of RNA will be transcribed. The construct is then transformed into plants and the antisense strand of RNA is produced. In plant cells, it has been shown that antisense RNA inhibits gene expression by preventing the accumulation of mRNA which encodes the enzyme of interest, see,

e.g., Sheehy *et al.*, *Proc. Nat'l. Acad. Sci. (USA)* 85: 8805-8809 (1988); and Hiatt *et al.*, U.S. Patent No. 4,801,340.

Another method of suppression is sense suppression. Introduction of nucleic acid configured in the sense orientation has been shown to be an effective means by which to
5 block the transcription of target genes. For an example of the use of this method to modulate expression of endogenous genes see, Napoli *et al.*, *The Plant Cell* 2: 279-289 (1990) and U.S. Patent No. 5,034,323.

Catalytic RNA molecules or ribozymes can also be used to inhibit expression of plant genes. It is possible to design ribozymes that specifically pair with virtually any
10 target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs. The
15 design and use of target RNA-specific ribozymes is described in Haseloff *et al.*, *Nature* 334: 585-591 (1988).

A variety of cross-linking agents, alkylating agents and radical generating species as pendant groups on polynucleotides of the present invention can be used to bind, label, detect, and/or cleave nucleic acids. For example, Vlassov, V. V., *et al.*, *Nucleic Acids*
20 *Res* (1986) 14:4065-4076, describe covalent bonding of a single-stranded DNA fragment with alkylating derivatives of nucleotides complementary to target sequences. A report of similar work by the same group is that by Knorre, D. G., *et al.*, *Biochimie* (1985) 67:785-789. Iverson and Dervan also showed sequence-specific cleavage of single-stranded DNA mediated by incorporation of a modified nucleotide which was capable of
25 activating cleavage (*J Am Chem Soc* (1987) 109:1241-1243). Meyer, R. B., *et al.*, *J Am Chem Soc* (1989) 111:8517-8519, effect covalent crosslinking to a target nucleotide using an alkylating agent complementary to the single-stranded target nucleotide sequence. A photoactivated crosslinking to single-stranded oligonucleotides mediated by psoralen was disclosed by Lee, B. L., *et al.*, *Biochemistry* (1988) 27:3197-3203. Use of crosslinking
30 in triple-helix forming probes was also disclosed by Home, *et al.*, *J Am Chem Soc* (1990) 112:2435-2437. Use of N4, N4-ethanocytosine as an alkylating agent to crosslink to single-stranded oligonucleotides has also been described by Webb and Matteucci, *J Am Chem Soc* (1986) 108:2764-2765; *Nucleic Acids Res* (1986) 14:7661-7674; Feteritz

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et al., *J. Am. Chem. Soc.* 113:4000 (1991). Various compounds to bind, detect, label, and/or cleave nucleic acids are known in the art. See, for example, U.S. Patent Nos. 5,543,507; 5,672,593; 5,484,908; 5,256,648; and, 5,681,941.

5 Proteins

The isolated proteins of the present invention comprise a polypeptide having at least 10 amino acids encoded by any one of the polynucleotides of the present invention as discussed more fully, above, or polypeptides which are conservatively modified variants thereof. The proteins of the present invention or variants thereof can comprise
10 any number of contiguous amino acid residues from a polypeptide of the present invention, wherein that number is selected from the group of integers consisting of from 10 to the number of residues in a full-length polypeptide of the present invention. Optionally, this subsequence of contiguous amino acids is at least 15, 20, 25, 30, 35, or 40 amino acids in length, often at least 50, 60, 70, 80, or 90 amino acids in length.
15 Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as 2, 3, 4, or 5.

As those of skill will appreciate, the present invention includes catalytically active polypeptides of the present invention (i.e., enzymes). Catalytically active polypeptides have a specific activity of at least 20%, 30%, or 40%, and preferably at least 50%, 60%,
20 or 70%, and most preferably at least 80%, 90%, or 95% that of the native (non-synthetic), endogenous polypeptide. Further, the substrate specificity (k_{cat}/K_m) is optionally substantially similar to the native (non-synthetic), endogenous polypeptide. Typically, the K_m will be at least 30%, 40%, or 50%, that of the native (non-synthetic), endogenous polypeptide; and more preferably at least 60%, 70%, 80%, or 90%.
25 Methods of assaying and quantifying measures of enzymatic activity and substrate specificity (k_{cat}/K_m), are well known to those of skill in the art.

Generally, the proteins of the present invention will, when presented as an immunogen, elicit production of an antibody specifically reactive to a polypeptide of the present invention. Further, the proteins of the present invention will not bind to antisera
30 raised against a polypeptide of the present invention which has been fully immunosorbed with the same polypeptide. Immunoassays for determining binding are well known to those of skill in the art. A preferred immunoassay is a competitive immunoassay as discussed, *infra*. Thus, the proteins of the present invention can be employed as

immunogens for constructing antibodies immunoreactive to a protein of the present invention for such exemplary utilities as immunoassays or protein purification techniques.

5 Expression of Proteins in Host Cells

Using the nucleic acids of the present invention, one may express a protein of the present invention in a recombinantly engineered cell such as bacteria, yeast, insect, mammalian, or preferably plant cells. The cells produce the protein in a non-natural condition (e.g., in quantity, composition, location, and/or time), because they have been
10 genetically altered through human intervention to do so.

It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made.

15 In brief summary, the expression of isolated nucleic acids encoding a protein of the present invention will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain
20 transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein of the present invention. To obtain high level expression of a cloned gene, it is desirable to construct expression vectors which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation, and a transcription/translation
25 terminator. One of skill would recognize that modifications can be made to a protein of the present invention without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an
30 initiation site, or additional amino acids (e.g., poly His) placed on either terminus to create conveniently located purification sequences. Restriction sites or termination codons can also be introduced.

A. Expression in Prokaryotes

Prokaryotic cells may be used as hosts for expression. 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
5 herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta lactamase (penicillinase) and lactose (lac) promoter systems (Chang et al., Nature 198:1056 (1977)), the tryptophan (trp) promoter system (Goeddel et al., Nucleic
10 Acids Res. 8:4057 (1980)) and the lambda derived P_L promoter and N-gene ribosome binding site (Shimatake *et al.*, Nature 292:128 (1981)). 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
15 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 of the present invention are available using *Bacillus sp.* and *Salmonella* (Palva, *et al.*, *Gene* 22: 229-235 (1983); Mosbach, *et al.*, *Nature* 302: 543-545 (1983)).

20

B. Expression in Eukaryotes

A variety of eukaryotic expression systems such as yeast, insect cell lines, plant and mammalian cells, are known to those of skill in the art. As explained briefly below, a of the present invention can be expressed in these eukaryotic systems. In some
25 embodiments, transformed/transfected plant cells, as discussed *infra*, are employed as expression systems for production of the proteins of the instant invention.

Synthesis of heterologous proteins in yeast is well known. Sherman, F., *et al.*, *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory (1982) is a well recognized work describing the various methods available to produce the protein in yeast. Two
30 widely utilized yeast for production of eukaryotic proteins are *Saccharomyces cerevisiae* and *Pichia pastoris*. Vectors, strains, and protocols for expression in *Saccharomyces* and *Pichia* are known in the art and available from commercial suppliers (e.g., Invitrogen). Suitable vectors usually have expression control sequences, such as

promoters, including 3-phosphoglycerate kinase or alcohol oxidase, and an origin of replication, termination sequences and the like as desired.

A protein of the present invention, once expressed, can be isolated from yeast by lysing the cells and applying standard protein isolation techniques to the lysates. The
5 monitoring of the purification process can be accomplished by using Western blot techniques or radioimmunoassay or other standard immunoassay techniques.

The sequences encoding proteins of the present invention can also be ligated to various expression vectors for use in transfecting cell cultures of, for instance, mammalian, insect, or plant origin. Illustrative of cell cultures useful for the production
10 of the peptides are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions may also be used. A number of suitable host cell lines capable of expressing intact proteins have been developed in the art, and include the HEK293, BHK21, and CHO cell lines. Expression vectors for these cells can include expression control sequences, such as an origin of
15 replication, a promoter (*e.g.*, the CMV promoter, a HSV *tk* promoter or *pgk* (phosphoglycerate kinase) promoter), an enhancer (Queen *et al.*, *Immunol. Rev.* 89: 49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (*e.g.*, an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. Other animal cells useful for production of
20 proteins of the present invention are available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (7th edition, 1992).

Appropriate vectors for expressing proteins of the present invention in insect cells are usually derived from the SF9 baculovirus. Suitable insect cell lines include mosquito larvae, silkworm, armyworm, moth and *Drosophila* cell lines such as a Schneider cell
25 line (See Schneider, *J. Embryol. Exp. Morphol.* 27: 353-365 (1987)).

As with yeast, when higher animal or plant host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript may also
30 be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, *et al.*, *J. Virol.* 45: 773-781 (1983)). Additionally, gene sequences to control replication in the host cell may be incorporated into the vector such as those found in bovine papilloma virus type-vectors. Saveria-Campo, M., Bovine Papilloma Virus DNA a

Eukaryotic Cloning Vector in *DNA Cloning Vol. II a Practical Approach*, D.M. Glover, Ed., IRL Press, Arlington, Virginia pp. 213-238 (1985).

Transfection/Transformation of Cells

5 The method of transformation/transfection is not critical to the instant invention; various methods of transformation or transfection are currently available. As newer methods are available to transform crops or other host cells they may be directly applied. Accordingly, a wide variety of methods have been developed to insert a DNA sequence into the genome of a host cell to obtain the transcription and/or translation of the
10 sequence to effect phenotypic changes in the organism. Thus, any method which provides for efficient transformation/transfection may be employed.

A. Plant Transformation

 A DNA sequence coding for the desired polynucleotide of the present invention,
15 for example a cDNA or a genomic sequence encoding a full length protein, will be used to construct a recombinant expression cassette which can be introduced into the desired plant.

 Isolated nucleic acid acids of the present invention can be introduced into plants according techniques known in the art. Generally, recombinant expression cassettes as
20 described above and suitable for transformation of plant cells are prepared. Techniques for transforming a wide variety of higher plant species are well known and described in the technical, scientific, and patent literature. See, for example, Weising *et al.*, *Ann. Rev. Genet.* 22: 421-477 (1988). For example, the DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation,
25 PEG poration, particle bombardment, silicon fiber delivery, or microinjection of plant cell protoplasts or embryogenic callus. See e.g., Tomes, *et al.*, *Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment*, pp. 197-213 in *Plant Cell, Tissue and Organ Culture, Fundamental Methods*, (eds. O.L. Gamborg and G.C. Phillips, Springer-Verlag Berlin Heidelberg New York, 1995). Alternatively, the DNA
30 constructs may be combined with suitable T-DNA flanking regions and introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent

marker into the plant cell DNA when the cell is infected by the bacteria. See, U.S. Patent No. 5,591,616.

The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski *et al.*, *Embo J.* 3: 2717-2722 (1984). Electroporation
5 techniques are described in Fromm *et al.*, *Proc. Natl. Acad. Sci.* 82: 5824 (1985). Ballistic transformation techniques are described in Klein *et al.*, *Nature* 327: 70-73 (1987).

Agrobacterium tumefaciens-mediated transformation techniques are well described in the scientific literature. See, for example Horsch *et al.*, *Science* 233: 496-498 (1984), and
10 Fraley *et al.*, *Proc. Natl. Acad. Sci.* 80: 4803 (1983). Although *Agrobacterium* is useful primarily in dicots, certain monocots can be transformed by *Agrobacterium*. For instance, *Agrobacterium* transformation of maize is described in U.S. Patent No. 5,550,318.

Other methods of transfection or transformation include (1) *Agrobacterium*
15 *rhizogenes*-mediated transformation (see, e.g., Lichtenstein and Fuller In: Genetic Engineering, vol. 6, PWJ Rigby, Ed., London, Academic Press, 1987; and Lichtenstein, C. P., and Draper, J., In: DNA Cloning, Vol. II, D. M. Glover, Ed., Oxford, IRI Press, 1985), Application PCT/US87/02512 (WO 88/02405 published Apr. 7, 1988) describes the use of *A. rhizogenes* strain A4 and its Ri plasmid along with *A. tumefaciens*
20 vectors pARC8 or pARC16 (2) liposome-mediated DNA uptake (see, e.g., Freeman *et al.*, *Plant Cell Physiol.* 25: 1353, 1984), (3) the vortexing method (see, e.g., Kindle, *Proc. Natl. Acad. Sci.*, USA 87: 1228, (1990).

DNA can also be introduced into plants by direct DNA transfer into pollen as described by Zhou *et al.*, *Methods in Enzymology*, 101:433 (1983); D. Hess, *Intern*
25 *Rev. Cytol.*, 107:367 (1987); Luo *et al.*, *Plant Mol. Biol. Reporter*, 6:165 (1988). Expression of polypeptide coding genes can be obtained by injection of the DNA into reproductive organs of a plant as described by Pena *et al.*, *Nature*, 325.:274 (1987). DNA can also be injected directly into the cells of immature embryos and the rehydration of desiccated embryos as described by Neuhaus *et al.*, *Theor. Appl. Genet.*, 75:30
30 (1987); and Benbrook *et al.*, in *Proceedings Bio Expo 1986*, Butterworth, Stoneham, Mass., pp. 27-54 (1986). A variety of plant viruses that can be employed as vectors are known in the art and include cauliflower mosaic virus (CaMV), geminivirus, brome mosaic virus, and tobacco mosaic virus.

B. Transfection of Prokaryotes, Lower Eukaryotes, and Animal Cells

Animal and lower eukaryotic (e.g., yeast) host cells are competent or rendered competent for transfection by various means. There are several well-known methods of introducing DNA into animal cells. These include: calcium phosphate precipitation, fusion of the recipient cells with bacterial protoplasts containing the DNA, treatment of the recipient cells with liposomes containing the DNA, DEAE dextran, electroporation, biolistics, and micro-injection of the DNA directly into the cells. The transfected cells are cultured by means well known in the art. Kuchler, R.J., *Biochemical Methods in Cell Culture and Virology*, Dowden, Hutchinson and Ross, Inc. (1977).

Synthesis of Proteins

The proteins of the present invention can be constructed using non-cellular synthetic methods. Solid phase synthesis of proteins of less than about 50 amino acids in length may be accomplished by attaching the C-terminal amino acid of the sequence to an insoluble support followed by sequential addition of the remaining amino acids in the sequence. Techniques for solid phase synthesis are described by Barany and Merrifield, *Solid-Phase Peptide Synthesis*, pp. 3-284 in *The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.*; Merrifield, *et al.*, *J. Am. Chem. Soc.* 85: 2149-2156 (1963), and Stewart *et al.*, *Solid Phase Peptide Synthesis, 2nd ed.*, Pierce Chem. Co., Rockford, Ill. (1984). Proteins of greater length may be synthesized by condensation of the amino and carboxy termini of shorter fragments. Methods of forming peptide bonds by activation of a carboxy terminal end (e.g., by the use of the coupling reagent N,N'-dicyclohexylcarbodiimide)) is known to those of skill.

Purification of Proteins

The proteins of the present invention may be purified by standard techniques well known to those of skill in the art. Recombinantly produced proteins of the present invention can be directly expressed or expressed as a fusion protein. The recombinant protein is purified by a combination of cell lysis (e.g., sonication, French press) and affinity chromatography. For fusion products, subsequent digestion of the fusion protein with an appropriate proteolytic enzyme releases the desired recombinant protein.

The proteins of this invention, recombinant or synthetic, may be purified to substantial purity by standard techniques well known in the art, including detergent

solubilization, selective precipitation with such substances as ammonium sulfate, column chromatography, immunopurification methods, and others. See, for instance, R. Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag: New York (1982); Deutscher, *Guide to Protein Purification*, Academic Press (1990). For example,
5 antibodies may be raised to the proteins as described herein. Purification from *E. coli* can be achieved following procedures described in U.S. Patent No. 4,511,503. The protein may then be isolated from cells expressing the protein and further purified by standard protein chemistry techniques as described herein. Detection of the expressed protein is achieved by methods known in the art and include, for example,
10 radioimmunoassays, Western blotting techniques or immunoprecipitation.

Transgenic Plant Regeneration

Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant which possesses the transformed
15 genotype. Such regeneration techniques often rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with a polynucleotide of the present invention. For transformation and regeneration of maize see, Gordon-Kamm *et al.*, *The Plant Cell*, 2:603-618 (1990).

20 Plants cells transformed with a plant expression vector can be regenerated, e.g., from single cells, callus tissue or leaf discs according to standard plant tissue culture techniques. It is well known in the art that various cells, tissues, and organs from almost any plant can be successfully cultured to regenerate an entire plant. Plant regeneration from cultured protoplasts is described in Evans *et al.*, *Protoplasts Isolation and Culture*,
25 *Handbook of Plant Cell Culture*, Macmillan Publishing Company, New York, pp. 124-176 (1983); and Binding, *Regeneration of Plants, Plant Protoplasts*, CRC Press, Boca Raton, pp. 21-73 (1985).

The regeneration of plants containing the foreign gene introduced by *Agrobacterium* from leaf explants can be achieved as described by Horsch *et al.*,
30 *Science*, 227:1229-1231 (1985). In this procedure, transformants are grown in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant species being transformed as described by Fraley *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 80:4803 (1983). This procedure typically produces shoots within two to four

weeks and these transformant shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth.

Transgenic plants of the present invention may be fertile or sterile.

5 Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee *et al.*, *Ann. Rev. of Plant Phys.* 38: 467-486 (1987). The regeneration of plants from either single plant protoplasts or various explants is well known in the art. See, for example, *Methods for Plant Molecular Biology*, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, Calif. (1988). This regeneration and growth process includes the steps of
10 selection of transformant cells and shoots, rooting the transformant shoots and growth of the plantlets in soil. For maize cell culture and regeneration see generally, *The Maize Handbook*, Freeling and Walbot, Eds., Springer, New York (1994); *Corn and Corn Improvement*, 3rd edition, Sprague and Dudley Eds., American Society of Agronomy, Madison, Wisconsin (1988).

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

20 In vegetatively propagated crops, mature transgenic plants can be propagated by the taking of cuttings or by tissue culture techniques to produce multiple identical plants. Selection of desirable transgenics is made and new varieties are obtained and propagated vegetatively for commercial use. In seed propagated crops, mature transgenic plants can be self crossed to produce a homozygous inbred plant. The inbred plant produces seed containing the newly introduced heterologous nucleic acid. These seeds can be
25 grown to produce plants that would produce the selected phenotype.

 Parts obtained from the regenerated plant, such as flowers, seeds, leaves, branches, fruit, and the like are included in the invention, provided that these parts comprise cells comprising the isolated nucleic acid of the present invention. Progeny and variants, and mutants of the regenerated plants are also included within the scope of the
30 invention, provided that these parts comprise the introduced nucleic acid sequences.

 Transgenic plants expressing the selectable marker can be screened for transmission of the nucleic acid of the present invention by, for example, standard immunoblot and DNA detection techniques. Transgenic lines are also typically evaluated

on levels of expression of the heterologous nucleic acid. Expression at the RNA level can be determined initially to identify and quantitate expression-positive plants. Standard techniques for RNA analysis can be employed and include PCR amplification assays using oligonucleotide primers designed to amplify only the heterologous RNA templates and solution hybridization assays using heterologous nucleic acid-specific probes. The RNA-positive plants can then analyzed for protein expression by Western immunoblot analysis using the specifically reactive antibodies of the present invention. In addition, *in situ* hybridization and immunocytochemistry according to standard protocols can be done using heterologous nucleic acid specific polynucleotide probes and antibodies, respectively, to localize sites of expression within transgenic tissue. Generally, a number of transgenic lines are usually screened for the incorporated nucleic acid to identify and select plants with the most appropriate expression profiles.

A preferred embodiment is a transgenic plant that is homozygous for the added heterologous nucleic acid; i.e., a transgenic plant that contains two added nucleic acid sequences, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) a heterozygous transgenic plant that contains a single added heterologous nucleic acid, germinating some of the seed produced and analyzing the resulting plants produced for altered expression of a polynucleotide of the present invention relative to a control plant (i.e., native, non-transgenic). Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated.

Modulating Polypeptide Levels and/or Composition

The present invention further provides a method for modulating (i.e., increasing or decreasing) the concentration or composition of the polypeptides of the present invention in a plant or part thereof. Modulation can be effected by increasing or decreasing the concentration and/or the composition (i.e., the ratio of the polypeptides of the present invention) in a plant. The method comprises transforming a plant cell with a recombinant expression cassette comprising a polynucleotide of the present invention as described above to obtain a transformed plant cell, growing the transformed plant cell under plant forming conditions, and inducing expression of a polynucleotide of the present invention in the plant for a time sufficient to modulate concentration and/or composition in the plant or plant part.

In some embodiments, the content and/or composition of polypeptides of the present invention in a plant may be modulated by altering, *in vivo* or *in vitro*, the promoter of a non-isolated gene of the present invention to up- or down-regulate gene expression. In some embodiments, the coding regions of native genes of the present invention can be altered via substitution, addition, insertion, or deletion to decrease activity of the encoded enzyme. See, e.g., Kmiec, U.S. Patent 5,565,350; Zarling *et al.*, PCT/US93/03868. And in some embodiments, an isolated nucleic acid (e.g., a vector) comprising a promoter sequence is transfected into a plant cell. Subsequently, a plant cell comprising the promoter operably linked to a polynucleotide of the present invention is selected for by means known to those of skill in the art such as, but not limited to, Southern blot, DNA sequencing, or PCR analysis using primers specific to the promoter and to the gene and detecting amplicons produced therefrom. A plant or plant part altered or modified by the foregoing embodiments is grown under plant forming conditions for a time sufficient to modulate the concentration and/or composition of polypeptides of the present invention in the plant. Plant forming conditions are well known in the art and discussed briefly, *above*.

In general, concentration or composition is increased or decreased by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% relative to a native control plant, plant part, or cell lacking the aforementioned recombinant expression cassette. Modulation in the present invention may occur during and/or subsequent to growth of the plant to the desired stage of development. Modulating nucleic acid expression temporally and/or in particular tissues can be controlled by employing the appropriate promoter operably linked to a polynucleotide of the present invention in, for example, sense or antisense orientation as discussed in greater detail, *above*. Induction of expression of a polynucleotide of the present invention can also be controlled by exogenous administration of an effective amount of inducing compound. Inducible promoters and inducing compounds which activate expression from these promoters are well known in the art. In preferred embodiments, the polypeptides of the present invention are modulated in monocots, particularly maize.

Molecular Markers

The present invention provides a method of genotyping a plant comprising a polynucleotide of the present invention. Preferably, the plant is a monocot, such as maize or sorghum. Genotyping provides a means of distinguishing homologs of a chromosome pair and can be used to differentiate segregants in a plant population.

5 Molecular marker methods can be used for phylogenetic studies, characterizing genetic relationships among crop varieties, identifying crosses or somatic hybrids, localizing chromosomal segments affecting monogenic traits, map based cloning, and the study of quantitative inheritance. See, e.g., *Plant Molecular Biology: A Laboratory Manual*, Chapter 7, Clark, Ed., Springer-Verlag, Berlin (1997). For molecular marker methods,
10 see generally, *The DNA Revolution* by Andrew H. Paterson 1996 (Chapter 2) in: *Genome Mapping in Plants* (ed. Andrew H. Paterson) by Academic Press/R. G. Landis Company, Austin, Texas, pp.7-21.

The particular method of genotyping in the present invention may employ any number of molecular marker analytic techniques such as, but not limited to, restriction
15 fragment length polymorphisms (RFLPs). RFLPs are the product of allelic differences between DNA restriction fragments caused by nucleotide sequence variability. As is well known to those of skill in the art, RFLPs are typically detected by extraction of genomic DNA and digestion with a restriction enzyme. Generally, the resulting fragments are separated according to size and hybridized with a probe; single copy
20 probes are preferred. Restriction fragments from homologous chromosomes are revealed. Differences in fragment size among alleles represent an RFLP. Thus, the present invention further provides a means to follow segregation of a gene or nucleic acid of the present invention as well as chromosomal sequences genetically linked to these genes or nucleic acids using such techniques as RFLP analysis. Linked
25 chromosomal sequences are within 50 centiMorgans (cM), often within 40 or 30 cM, preferably within 20 or 10 cM, more preferably within 5, 3, 2, or 1 cM of a gene of the present invention.

In the present invention, the nucleic acid probes employed for molecular marker mapping of plant nuclear genomes selectively hybridize, under selective hybridization
30 conditions, to a gene encoding a polynucleotide of the present invention. In preferred embodiments, the probes are selected from polynucleotides of the present invention. Typically, these probes are cDNA probes or *Pst I* genomic clones. The length of the probes is discussed in greater detail, above, but are typically at least 15 bases in length,

more preferably at least 20, 25, 30, 35, 40, or 50 bases in length. Generally, however, the probes are less than about 1 kilobase in length. Preferably, the probes are single copy probes that hybridize to a unique locus in a haploid chromosome complement. Some exemplary restriction enzymes employed in RFLP mapping are *EcoRI*, *EcoRV*, and
5 *SstI*. As used herein the term "restriction enzyme" includes reference to a composition that recognizes and, alone or in conjunction with another composition, cleaves at a specific nucleotide sequence.

The method of detecting an RFLP comprises the steps of (a) digesting genomic DNA of a plant with a restriction enzyme; (b) hybridizing a nucleic acid probe, under
10 selective hybridization conditions, to a sequence of a polynucleotide of the present of said genomic DNA; (c) detecting therefrom a RFLP. Other methods of differentiating polymorphic (allelic) variants of polynucleotides of the present invention can be had by utilizing molecular marker techniques well known to those of skill in the art including such techniques as: 1) single stranded conformation analysis (SSCP); 2) denaturing
15 gradient gel electrophoresis (DGGE); 3) RNase protection assays; 4) allele-specific oligonucleotides (ASOs); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein; and 6) allele-specific PCR. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE); heteroduplex analysis (HA); and
20 chemical mismatch cleavage (CMC). Exemplary polymorphic variants are provided in Table I, above. Thus, the present invention further provides a method of genotyping comprising the steps of contacting, under stringent hybridization conditions, a sample suspected of comprising a polynucleotide of the present invention with a nucleic acid probe. Generally, the sample is a plant sample; preferably, a sample suspected of
25 comprising a maize polynucleotide of the present invention (e.g., gene, mRNA). The nucleic acid probe selectively hybridizes, under stringent conditions, to a subsequence of a polynucleotide of the present invention comprising a polymorphic marker. Selective hybridization of the nucleic acid probe to the polymorphic marker nucleic acid sequence yields a hybridization complex. Detection of the hybridization complex indicates the
30 presence of that polymorphic marker in the sample. In preferred embodiments, the nucleic acid probe comprises a polynucleotide of the present invention.

UTR's and Codon Preference

In general, translational efficiency has been found to be regulated by specific sequence elements in the 5' non-coding or untranslated region (5' UTR) of the RNA. Positive sequence motifs include translational initiation consensus sequences (Kozak, *Nucleic Acids Res.* 15:8125 (1987)) and the 7-methylguanosine cap structure (Drummond *et al.*, *Nucleic Acids Res.* 13:7375 (1985)). Negative elements include stable intramolecular 5' UTR stem-loop structures (Muesing *et al.*, *Cell* 48:691 (1987)) and AUG sequences or short open reading frames preceded by an appropriate AUG in the 5' UTR (Kozak, above, Rao *et al.*, *Mol. and Cell. Biol.* 8:284 (1988)). Accordingly, the present invention provides 5' and/or 3' UTR regions for modulation of translation of heterologous coding sequences.

Further, the polypeptide-encoding segments of the polynucleotides of the present invention can be modified to alter codon usage. Altered codon usage can be employed to alter translational efficiency and/or to optimize the coding sequence for expression in a desired host or to optimize the codon usage in a heterologous sequence for expression in maize. Codon usage in the coding regions of the polynucleotides of the present invention can be analyzed statistically using commercially available software packages such as "Codon Preference" available from the University of Wisconsin Genetics Computer Group (see Devereaux *et al.*, *Nucleic Acids Res.* 12: 387-395 (1984)) or MacVector 4.1 (Eastman Kodak Co., New Haven, Conn.). Thus, the present invention provides a codon usage frequency characteristic of the coding region of at least one of the polynucleotides of the present invention. The number of polynucleotides that can be used to determine a codon usage frequency can be any integer from 1 to the number of polynucleotides of the present invention as provided herein. Optionally, the polynucleotides will be full-length sequences. An exemplary number of sequences for statistical analysis can be at least 1, 5, 10, 20, 50, or 100.

Sequence Shuffling

The present invention provides methods for sequence shuffling using polynucleotides of the present invention, and compositions resulting therefrom. Sequence shuffling is described in PCT publication No. 96/19256. See also, Zhang, J.-H., *et al. Proc. Natl. Acad. Sci. USA* 94:4504-4509 (1997). Generally, sequence shuffling provides a means for generating libraries of polynucleotides having a desired characteristic which can be selected or screened for. Libraries of recombinant

polynucleotides are generated from a population of related sequence polynucleotides which comprise sequence regions which have substantial sequence identity and can be homologously recombined in vitro or in vivo. The population of sequence-recombined polynucleotides comprises a subpopulation of polynucleotides which possess desired or advantageous characteristics and which can be selected by a suitable selection or screening method. The characteristics can be any property or attribute capable of being selected for or detected in a screening system, and may include properties of: an encoded protein, a transcriptional element, a sequence controlling transcription, RNA processing, RNA stability, chromatin conformation, translation, or other expression property of a gene or transgene, a replicative element, a protein-binding element, or the like, such as any feature which confers a selectable or detectable property. In some embodiments, the selected characteristic will be a decreased K_m and/or increased K_{cat} over the wild-type protein as provided herein. In other embodiments, a protein or polynucleotide generated from sequence shuffling will have a ligand binding affinity greater than the non-shuffled wild-type polynucleotide. The increase in such properties can be at least 110%, 120%, 130%, 140% or at least 150% of the wild-type value.

Generic and Consensus Sequences

Polynucleotides and polypeptides of the present invention further include those having: (a) a generic sequence of at least two homologous polynucleotides or polypeptides, respectively, of the present invention; and, (b) a consensus sequence of at least three homologous polynucleotides or polypeptides, respectively, of the present invention. The generic sequence of the present invention comprises each species of polypeptide or polynucleotide embraced by the generic polypeptide or polynucleotide, sequence, respectively. The individual species encompassed by a polynucleotide having an amino acid or nucleic acid consensus sequence can be used to generate antibodies or produce nucleic acid probes or primers to screen for homologs in other species, genera, families, orders, classes, phyla, or kingdoms. For example, a polynucleotide having a consensus sequences from a gene family of *Zea mays* can be used to generate antibody or nucleic acid probes or primers to other *Gramineae* species such as wheat, rice, or sorghum. Alternatively, a polynucleotide having a consensus sequence generated from orthologous genes can be used to identify or isolate orthologs of other taxa. Typically, a polynucleotide having a consensus sequence will be at least 9, 10, 15, 20, 25, 30, or 40

amino acids in length, or 20, 30, 40, 50, 100, or 150 nucleotides in length. As those of skill in the art are aware, a conservative amino acid substitution can be used for amino acids which differ amongst aligned sequence but are from the same conservative substitution group as discussed above. Optionally, no more than 1 or 2 conservative amino acids are substituted for each 10 amino acid length of consensus sequence.

Similar sequences used for generation of a consensus or generic sequence include any number and combination of allelic variants of the same gene, orthologous, or paralogous sequences as provided herein. Optionally, similar sequences used in generating a consensus or generic sequence are identified using the BLAST algorithm's smallest sum probability (P(N)). Various suppliers of sequence-analysis software are listed in chapter 7 of *Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (Supplement 30). A polynucleotide sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, or 0.001, and most preferably less than about 0.0001, or 0.00001. Similar polynucleotides can be aligned and a consensus or generic sequence generated using multiple sequence alignment software available from a number of commercial suppliers such as the Genetics Computer Group's (Madison, WI) PILEUP software, Vector NTI's (North Bethesda, MD) ALIGNX, or Genecode's (Ann Arbor, MI) SEQUENCHER. Conveniently, default parameters of such software can be used to generate consensus or generic sequences.

Detection of Nucleic Acids

The present invention further provides methods for detecting a polynucleotide of the present invention in a nucleic acid sample suspected of comprising a polynucleotide of the present invention, such as a plant cell lysate, particularly a lysate of corn. In some embodiments, a gene of the present invention or portion thereof can be amplified prior to the step of contacting the nucleic acid sample with a polynucleotide of the present invention. The nucleic acid sample is contacted with the polynucleotide to form a hybridization complex. The polynucleotide hybridizes under stringent conditions to a gene encoding a polypeptide of the present invention. Formation of the hybridization complex is used to detect a gene encoding a polypeptide of the present invention in the

nucleic acid sample. Those of skill will appreciate that an isolated nucleic acid comprising a polynucleotide of the present invention should lack cross-hybridizing sequences in common with non-target genes that would yield a false positive result.

Detection of the hybridization complex can be achieved using any number of well known methods. For example, the nucleic acid sample, or a portion thereof, may be assayed by hybridization formats including but not limited to, solution phase, solid phase, mixed phase, or *in situ* hybridization assays. Briefly, in solution (or liquid) phase hybridizations, both the target nucleic acid and the probe or primer are free to interact in the reaction mixture. In solid phase hybridization assays, probes or primers are typically linked to a solid support where they are available for hybridization with target nucleic in solution. In mixed phase, nucleic acid intermediates in solution hybridize to target nucleic acids in solution as well as to a nucleic acid linked to a solid support. In *in situ* hybridization, the target nucleic acid is liberated from its cellular surroundings in such as to be available for hybridization within the cell while preserving the cellular morphology for subsequent interpretation and analysis. The following articles provide an overview of the various hybridization assay formats: Singer *et al.*, *Biotechniques* 4(3): 230-250 (1986); Haase *et al.*, *Methods in Virology*, Vol. VII, pp. 189-226 (1984); Wilkinson, The theory and practice of *in situ* hybridization in: *In situ Hybridization*, D.G. Wilkinson, Ed., IRL Press, Oxford University Press, Oxford; and *Nucleic Acid Hybridization: A Practical Approach*, Hames, B.D. and Higgins, S.J., Eds., IRL Press (1987).

Nucleic Acid Labels and Detection Methods

The means by which nucleic acids of the present invention are labeled is not a critical aspect of the present invention and can be accomplished by any number of methods currently known or later developed. Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, radioisotopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include biotin for staining with labeled streptavidin conjugate, magnetic beads, fluorescent dyes (*e.g.*, fluorescein, texas red, rhodamine, green fluorescent protein, and the like), radiolabels (*e.g.*, ^3H , ^{125}I , ^{35}S , ^{14}C , or ^{32}P), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used

in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, etc.) beads.

Nucleic acids of the present invention can be labeled by any one of several methods typically used to detect the presence of hybridized nucleic acids. One common method of detection is the use of autoradiography using probes labeled with ^3H , ^{125}I , ^{35}S , ^{14}C , or ^{32}P , or the like. The choice of radio-active isotope depends on research preferences due to ease of synthesis, stability, and half lives of the selected isotopes. Other labels include ligands which bind to antibodies labeled with fluorophores, chemiluminescent agents, and enzymes. Alternatively, probes can be conjugated directly with labels such as fluorophores, chemiluminescent agents or enzymes. The choice of label depends on sensitivity required, ease of conjugation with the probe, stability requirements, and available instrumentation. Labeling the nucleic acids of the present invention is readily achieved such as by the use of labeled PCR primers.

In some embodiments, the label is simultaneously incorporated during the amplification step in the preparation of the nucleic acids. Thus, for example, polymerase chain reaction (PCR) with labeled primers or labeled nucleotides will provide a labeled amplification product. In another embodiment, transcription amplification using a labeled nucleotide (*e.g.*, fluorescein-labeled UTP and/or CTP) incorporates a label into the transcribed nucleic acids.

Non-radioactive probes are often labeled by indirect means. For example, a ligand molecule is covalently bound to the probe. The ligand then binds to an anti-ligand molecule which is either inherently detectable or covalently bound to a detectable signal system, such as an enzyme, a fluorophore, or a chemiluminescent compound. Enzymes of interest as labels will primarily be hydrolases, such as phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescers include luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. Ligands and anti-ligands may be varied widely. Where a ligand has a natural anti-ligand, namely ligands such as biotin, thyroxine, and cortisol, it can be used in conjunction with its labeled, naturally occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody.

Probes can also be labeled by direct conjugation with a label. For example, cloned DNA probes have been coupled directly to horseradish peroxidase or alkaline phosphatase, (Renz, M., and Kurz, K., *A Colorimetric Method for DNA Hybridization*, *Nucl. Acids Res.* 12: 3435-3444 (1984)) and synthetic oligonucleotides have been
5 coupled directly with alkaline phosphatase (Jablonski, E., *et al.*, *Preparation of Oligodeoxynucleotide-Alkaline Phosphatase Conjugates and Their Use as Hybridization Probes*, *Nuc. Acids. Res.* 14: 6115-6128 (1986); and Li P., *et al.*, *Enzyme-linked Synthetic Oligonucleotide probes: Non-Radioactive Detection of Enterotoxigenic Escherichia Coli in Faeca Specimens*, *Nucl. Acids Res.* 15: 5275-5287 (1987)).

10 Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate,
15 and colorimetric labels are detected by simply visualizing the colored label.

Antibodies to Proteins

Antibodies can be raised to a protein of the present invention, including individual, allelic, strain, or species variants, and fragments thereof, both in their
20 naturally occurring (full-length) forms and in recombinant forms. Additionally, antibodies are raised to these proteins in either their native configurations or in non-native configurations. Anti-idiotypic antibodies can also be generated. Many methods of making antibodies are known to persons of skill. The following discussion is presented as a general overview of the techniques available; however, one of skill will
25 recognize that many variations upon the following methods are known.

A number of immunogens are used to produce antibodies specifically reactive with a protein of the present invention. An isolated recombinant, synthetic, or native polynucleotide of the present invention are the preferred immunogens (antigen) for the production of monoclonal or polyclonal antibodies. Those of skill will readily
30 understand that the proteins of the present invention are typically denatured, and optionally reduced, prior to formation of antibodies for screening expression libraries or other assays in which a putative protein of the present invention is expressed or

denatured in a non-native secondary, tertiary, or quaternary structure. Non-isolated polypeptides of the present invention can be used either in pure or impure form.

The protein of the present invention is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies can be generated for subsequent use in immunoassays to measure the presence and quantity of the protein of the present invention. Methods of producing polyclonal antibodies are known to those of skill in the art. In brief, an immunogen (antigen), preferably a purified protein, a protein coupled to an appropriate carrier (*e.g.*, GST, keyhole limpet hemanocyanin, *etc.*), or a protein incorporated into an immunization vector such as a recombinant vaccinia virus (see, U.S. Patent No. 4,722,848) is mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the protein of interest. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein is performed where desired (See, *e.g.*, Coligan, *Current Protocols in Immunology*, Wiley/Greene, NY (1991); and Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, NY (1989)).

Antibodies, including binding fragments and single chain recombinant versions thereof, against predetermined fragments of a protein of the present invention are raised by immunizing animals, *e.g.*, with conjugates of the fragments with carrier proteins as described above. Typically, the immunogen of interest is a protein of at least about 5 amino acids, more typically the protein is 10 amino acids in length, preferably, 15 amino acids in length and more preferably the protein is 20 amino acids in length or greater. The peptides are typically coupled to a carrier protein (*e.g.*, as a fusion protein), or are recombinantly expressed in an immunization vector. Antigenic determinants on peptides to which antibodies bind are typically 3 to 10 amino acids in length.

Monoclonal antibodies are prepared from cells secreting the desired antibody. Monoclonal antibodies are screened for binding to a protein from which the immunogen was derived. Specific monoclonal and polyclonal antibodies will usually have an antibody binding site with an affinity constant for its cognate monovalent antigen at least between 10^6 - 10^7 , usually at least 10^8 , preferably at least 10^9 , more preferably at least 10^{10} , and most preferably at least 10^{11} liters/mole.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, *etc.* Description of techniques for preparing such monoclonal antibodies are found in, *e.g.*, *Basic and Clinical Immunology*, 4th ed., Stites *et al.*, Eds., Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane, *Supra*; Goding, *Monoclonal Antibodies: Principles and Practice*, 2nd ed., Academic Press, New York, NY (1986); and Kohler and Milstein, *Nature* 256: 495-497 (1975). Summarized briefly, this method proceeds by injecting an animal with an immunogen comprising a protein of the present invention. The animal is then sacrificed and cells taken from its spleen, which are fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing *in vitro*. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve selection of libraries of recombinant antibodies in phage or similar vectors (*see, e.g.*, Huse *et al.*, *Science* 246: 1275-1281 (1989); and Ward, *et al.*, *Nature* 341: 544-546 (1989); and Vaughan *et al.*, *Nature Biotechnology*, 14: 309-314 (1996)). Alternatively, high avidity human monoclonal antibodies can be obtained from transgenic mice comprising fragments of the unrearranged human heavy and light chain Ig loci (*i.e.*, minilocus transgenic mice). Fishwild *et al.*, *Nature Biotech.*, 14: 845-851 (1996). Also, recombinant immunoglobulins may be produced. See, Cabilly, U.S. Patent No. 4,816,567; and Queen *et al.*, *Proc. Nat'l Acad. Sci.* 86: 10029-10033 (1989).

The antibodies of this invention are also used for affinity chromatography in isolating proteins of the present invention. Columns are prepared, *e.g.*, with the antibodies linked to a solid support, *e.g.*, particles, such as agarose, Sephadex, or the like, where a cell lysate is passed through the column, washed, and treated with increasing concentrations of a mild denaturant, whereby purified protein are released.

The antibodies can be used to screen expression libraries for particular expression products such as normal or abnormal protein. Usually the antibodies in such a procedure are labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies raised against a protein of the present invention can also be used to raise anti-idiotypic antibodies. These are useful for detecting or diagnosing various pathological conditions related to the presence of the respective antigens.

5 Frequently, the proteins and antibodies of the present invention will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionucleotides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like.

10

Protein Immunoassays

Means of detecting the proteins of the present invention are not critical aspects of the present invention. In a preferred embodiment, the proteins are detected and/or quantified using any of a number of well recognized immunological binding assays (*see*, 15 *e.g.*, U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of the general immunoassays, see also *Methods in Cell Biology*, Vol. 37: *Antibodies in Cell Biology*, Asai, Ed., Academic Press, Inc. New York (1993); *Basic and Clinical Immunology* 7th Edition, Stites & Terr, Eds. (1991). Moreover, the immunoassays of the present invention can be performed in any of several configurations, *e.g.*, those 20 reviewed in *Enzyme Immunoassay*, Maggio, Ed., CRC Press, Boca Raton, Florida (1980); Tijan, *Practice and Theory of Enzyme Immunoassays*, *Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers B.V., Amsterdam (1985); Harlow and Lane, above; *Immunoassay: A Practical Guide*, Chan, Ed., Academic Press, Orlando, FL (1987); *Principles and Practice of Immunoassays*, Price and Newman Eds., Stockton Press, NY (1991); and *Non-isotopic Immunoassays*, Ngo, 25 Ed., Plenum Press, NY (1988). Immunological binding assays (or immunoassays) typically utilize a "capture agent" to specifically bind to and often immobilize the analyte (in this case, a protein of the present invention). The capture agent is a moiety that specifically binds to the analyte. In a preferred embodiment, the capture agent is an 30 antibody that specifically binds a protein(s) of the present invention. The antibody may be produced by any of a number of means known to those of skill in the art as described herein.

Immunoassays also often utilize a labeling agent to specifically bind to and label the binding complex formed by the capture agent and the analyte. The labeling agent may itself be one of the moieties comprising the antibody/analyte complex. Thus, the labeling agent may be a labeled protein of the present invention or a labeled antibody specifically reactive to a protein of the present invention. Alternatively, the labeling agent may be a third moiety, such as another antibody, that specifically binds to the antibody/protein complex.

In a preferred embodiment, the labeling agent is a second antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G may also be used as the label agent. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (See, generally Kronval, *et al.*, *J. Immunol.* 111: 1401-1406 (1973), and Akerstrom, *et al.*, *J. Immunol.* 135: 2589-2542 (1985)).

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

While the details of the immunoassays of the present invention may vary with the particular format employed, the method of detecting a protein of the present invention in a biological sample generally comprises the steps of contacting the biological sample with an antibody which specifically reacts, under immunologically reactive conditions, to a protein of the present invention. The antibody is allowed to bind to the protein under immunologically reactive conditions, and the presence of the bound antibody is detected directly or indirectly.

A. *Non-Competitive Assay Formats*

Immunoassays for detecting proteins of the present invention include competitive and noncompetitive formats. Noncompetitive immunoassays are assays in which the amount of captured analyte (i.e., a protein of the present invention) is directly
5 measured. In one preferred "sandwich" assay, for example, the capture agent (e.g., an antibody specifically reactive, under immunoreactive conditions, to a protein of the present invention) can be bound directly to a solid substrate where they are immobilized. These immobilized antibodies then capture the protein present in the test sample. The protein thus immobilized is then bound by a labeling agent, such as a second antibody
10 bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

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B. *Competitive Assay Formats*

In competitive assays, the amount of analyte present in the sample is measured indirectly by measuring the amount of an added (exogenous) analyte (e.g., a protein of the present invention) displaced (or competed away) from a capture agent (e.g., an
20 antibody specifically reactive, under immunoreactive conditions, to the protein) by the analyte present in the sample. In one competitive assay, a known amount of analyte is added to the sample and the sample is then contacted with a capture agent that specifically binds a protein of the present invention. The amount of protein bound to the capture agent is inversely proportional to the concentration of analyte present in the
25 sample.

In a particularly preferred embodiment, the antibody is immobilized on a solid substrate. The amount of protein bound to the antibody may be determined either by measuring the amount of protein present in a protein/antibody complex, or alternatively by measuring the amount of remaining uncomplexed protein. The amount of protein may
30 be detected by providing a labeled protein.

A hapten inhibition assay is another preferred competitive assay. In this assay a known analyte, (such as a protein of the present invention) is immobilized on a solid substrate. A known amount of antibody specifically reactive, under immunoreactive

conditions, to the protein is added to the sample, and the sample is then contacted with the immobilized protein. In this case, the amount of antibody bound to the immobilized protein is inversely proportional to the amount of protein present in the sample. Again, the amount of immobilized antibody may be detected by detecting either the immobilized
5 fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled or indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

C. Generation of pooled antisera for use in immunoassays

10 A protein that specifically binds to or that is specifically immunoreactive with an antibody generated against a defined immunogen is determined in an immunoassay. The immunoassay uses a polyclonal antiserum which is raised to a polypeptide of the present invention (i.e., the immunogenic polypeptide). This antiserum is selected to have low crossreactivity against other proteins and any such crossreactivity is removed by
15 immunoabsorbtion prior to use in the immunoassay (e.g., by immunosorbtion of the antisera with a protein of different substrate specificity (e.g., a different enzyme) and/or a protein with the same substrate specificity but of a different form).

In order to produce antisera for use in an immunoassay, a polypeptide of the present invention is isolated as described herein. For example, recombinant protein can
20 be produced in a mammalian or other eukaryotic cell line. An inbred strain of mice is immunized with the protein using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, above). Alternatively, a synthetic polypeptide derived from the sequences disclosed herein and conjugated to a carrier protein is used as an immunogen. Polyclonal sera are collected and titered
25 against the immunogenic polypeptide in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against polypeptides of different forms or substrate specificity, using a competitive binding immunoassay such as the one described in Harlow and Lane, above, at pages 570-573.
30 Preferably, two or more distinct forms of polypeptides are used in this determination. These distinct types of polypeptides are used as competitors to identify antibodies which are specifically bound by the polypeptide being assayed for. The competitive

polypeptides can be produced as recombinant proteins and isolated using standard molecular biology and protein chemistry techniques as described herein.

Immunoassays in the competitive binding format are used for crossreactivity determinations. For example, the immunogenic polypeptide is immobilized to a solid support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the immunogenic polypeptide. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with a distinct form of a polypeptide are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorbtion with a distinct form of a polypeptide.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described herein to compare a second "target" polypeptide to the immunogenic polypeptide. In order to make this comparison, the two polypeptides are each assayed at a wide range of concentrations and the amount of each polypeptide required to inhibit 50% of the binding of the antisera to the immobilized protein is determined using standard techniques. If the amount of the target polypeptide required is less than twice the amount of the immunogenic polypeptide that is required, then the target polypeptide is said to specifically bind to an antibody generated to the immunogenic protein. As a final determination of specificity, the pooled antisera is fully immunosorbed with the immunogenic polypeptide until no binding to the polypeptide used in the immunosorbtion is detectable. The fully immunosorbed antisera is then tested for reactivity with the test polypeptide. If no reactivity is observed, then the test polypeptide is specifically bound by the antisera elicited by the immunogenic protein.

D. Other Assay Formats

In a particularly preferred embodiment, Western blot (immunoblot) analysis is used to detect and quantify the presence of protein of the present invention in the sample. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically bind a protein of the present invention. The antibodies specifically bind to the protein on the solid support. These

antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (*e.g.*, labeled sheep anti-mouse antibodies) that specifically bind to the antibodies.

5

E. Quantification of Proteins.

The proteins of the present invention may be detected and quantified by any of a number of means well known to those of skill in the art. These include analytic biochemical methods such as electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like, and various immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, and the like.

15

F. Reduction of Non-Specific Binding

One of skill will appreciate that it is often desirable to reduce non-specific binding in immunoassays and during analyte purification. Where the assay involves an antigen, antibody, or other capture agent immobilized on a solid substrate, it is desirable to minimize the amount of non-specific binding to the substrate. Means of reducing such non-specific binding are well known to those of skill in the art. Typically, this involves coating the substrate with a proteinaceous composition. In particular, protein compositions such as bovine serum albumin (BSA), nonfat powdered milk, and gelatin are widely used.

25

G. Immunoassay Labels

The labeling agent can be, *e.g.*, a monoclonal antibody, a polyclonal antibody, a binding protein or complex, or a polymer such as an affinity matrix, carbohydrate or lipid. Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, radioisotopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Detection may proceed by any known method, such as immunoblotting, western analysis, gel-mobility shift assays, fluorescent *in situ* hybridization analysis (FISH), tracking of radioactive or

30

bioluminescent markers, nuclear magnetic resonance, electron paramagnetic resonance, stopped-flow spectroscopy, column chromatography, capillary electrophoresis, or other methods which track a molecule based upon an alteration in size and/or charge. The particular label or detectable group used in the assay is not a critical aspect of the invention. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads, fluorescent dyes, radiolabels, enzymes, and colorimetric labels or colored glass or plastic beads, as discussed for nucleic acid labels, above.

The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on the sensitivity required, ease of conjugation of the compound, stability requirements, available instrumentation, and disposal provisions.

Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (*e.g.*, biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (*e.g.*, streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, for example, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody.

The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, *e.g.*,

luminol. For a review of various labeling or signal producing systems which may be used, see, U.S. Patent No. 4,391,904, which is incorporated herein by reference.

Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence, *e.g.*, by microscopy, visual inspection, via photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing appropriate substrates for the enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

Assays for Compounds that Modulate Enzymatic Activity or Expression

The present invention also provides means for identifying compounds that bind to (e.g., substrates), and/or increase or decrease (i.e., modulate) the enzymatic activity of, catalytically active polypeptides of the present invention. The method comprises contacting a polypeptide of the present invention with a compound whose ability to bind to or modulate enzyme activity is to be determined. The polypeptide employed will have at least 20%, preferably at least 30% or 40%, more preferably at least 50% or 60%, and most preferably at least 70% or 80% of the specific activity of the native, full-length polypeptide of the present invention (e.g., enzyme). Generally, the polypeptide will be present in a range sufficient to determine the effect of the compound, typically about 1 nM to 10 μ M. Likewise, the compound will be present in a concentration of from about 1 nM to 10 μ M. Those of skill will understand that such factors as enzyme concentration, ligand concentrations (i.e., substrates, products, inhibitors, activators), pH, ionic strength, and temperature will be controlled so as to obtain useful kinetic data

and determine the presence or absence of a compound that binds or modulates polypeptide activity. Methods of measuring enzyme kinetics is well known in the art. See, e.g., Segel, *Biochemical Calculations*, 2nd ed., John Wiley and Sons, New York (1976).

5 Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

10 Example 1

 This example describes the construction cDNA libraries.

Total RNA Isolation

 Total RNA was isolated from corn tissues with TRIzol™ Reagent (Life Technology
15 Inc. Gaithersburg, MD) using a modification of the guanidine isothiocyanate/acid-phenol
procedure described by Chomczynski and Sacchi (Chomczynski, P., and Sacchi, N.
Anal. Biochem. 162, 156 (1987)). In brief, plant tissue samples were pulverized in
liquid nitrogen before the addition of the TRIzol™ Reagent, and then were further
homogenized with a mortar and pestle. Addition of chloroform followed by
20 centrifugation was conducted for separation of an aqueous phase and an organic phase.
The total RNA was recovered by precipitation with isopropyl alcohol from the aqueous
phase.

Poly(A)+ RNA Isolation

25 The selection of poly(A)+ RNA from total RNA was performed using PolyATact™
system (Promega Corporation, Madison, WI). In brief, biotinylated oligo(dT) primers
were used to hybridize to the 3' poly(A) tails on mRNA. The hybrids were captured
using streptavidin coupled to paramagnetic particles and a magnetic separation stand.
The mRNA was washed at high stringent condition and eluted by RNase-free deionized
30 water.

cDNA Library Construction

cDNA synthesis was performed and unidirectional cDNA libraries were constructed using the SuperScript™ Plasmid System (Life Technology Inc. Gaithersburg, MD). The first strand of cDNA was synthesized by priming an oligo(dT) primer containing a Not I site. The reaction was catalyzed by SuperScript™ Reverse
5 Transcriptase II at 45°C. The second strand of cDNA was labeled with alpha-³²P-dCTP and a portion of the reaction was analyzed by agarose gel electrophoresis to determine cDNA sizes. cDNA molecules smaller than 500 base pairs and unligated adapters were removed by Sephacryl-S400 chromatography. The selected cDNA molecules were ligated into pSPORT1 vector in between of Not I and Sal I sites.

10

Example 2

This example describes cDNA sequencing and library subtraction.

Sequencing Template Preparation

Individual colonies were picked and DNA was prepared either by PCR with M13
15 forward primers and M13 reverse primers, or by plasmid isolation. All the cDNA clones were sequenced using M13 reverse primers.

Q-bot Subtraction Procedure

cDNA libraries subjected to the subtraction procedure were plated out on 22 x 22
20 cm² agar plate at density of about 3,000 colonies per plate. The plates were incubated in a 37°C incubator for 12-24 hours. Colonies were picked into 384-well plates by a robot colony picker, Q-bot™ (GENETIX Limited). These plates were incubated overnight at 37°C.

Once sufficient colonies were picked, they were pinned onto 22 x 22 cm² nylon
25 membranes using Q-bot™. Each membrane contained 9,216 colonies or 36,864 colonies. These membranes were placed onto agar plate with appropriate antibiotic. The plates were incubated at 37°C for overnight.

After colonies were recovered on the second day, these filters were placed on filter paper prewetted with denaturing solution for four minutes, then were incubated on
30 top of a boiling water bath for additional four minutes. The filters were then placed on filter paper prewetted with neutralizing solution for four minutes. After excess solution was removed by placing the filters on dry filter papers for one minute, the colony side of the filters were place into Proteinase K solution, incubated at 37°C for 40-50 minutes.

The filters were placed on dry filter papers to dry overnight. DNA was then cross-linked to nylon membrane by UV light treatment.

Colony hybridization was conducted as described by Sambrook, J., Fritsch, E.F. and Maniatis, T., (in *Molecular Cloning: A laboratory Manual*, 2nd Edition). The following probes were used in colony hybridization:

1. First strand cDNA from the same tissue as the library was made from to remove the most redundant clones.
2. 48-192 most redundant cDNA clones from the same library based on previous sequencing data.
3. 192 most redundant cDNA clones in the entire corn partial sequence database.
4. A Sal-A20 oligo nucleotide: TCG ACC CAC GCG TCC GAA AAA AAA AAA AAA AAA AAA, removes clones containing a poly A tail but no cDNA.
5. cDNA clones derived from rRNA.

The image of the autoradiography was scanned into computer and the signal intensity and cold colony addresses of each colony was analyzed. Re-arraying of cold-colonies from 384 well plates to 96 well plates was conducted using Q-bot.

Example 3

This example describes identification of the gene from a computer homology search. Gene identities were determined by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches under default parameters for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm. The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish, W. and States, D. J. (1993) *Nature Genetics* 3:266-272) provided by the NCBI. In some cases, the sequencing data from two or more clones containing overlapping segments of DNA were used to construct contiguous DNA sequences.

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The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims.

SEQUENCE LISTING

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<120> Maize Cellulose Synthases and Uses
Thereof

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<150> 60/096,822

<151> August 17, 1998

<160> 60

<170> FastSEQ for Windows Version 3.0

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<211> 3568

<212> DNA

<213> Zea mays

<220>

<221> CDS

<222> (63)...(3237)

<400> 1

```

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cc atg gac cag cgg aac ggc cag gtg tgc cag att tgc ggc gac gac      107
  Met Asp Gln Arg Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Asp
    1             5             10             15

gtg ggg cgc aac ccc gac ggg gag cct ttc gtg gcc tgc aac gag tgc      155
Val Gly Arg Asn Pro Asp Gly Glu Pro Phe Val Ala Cys Asn Glu Cys
          20             25             30

gcc ttc ccc atc tgc cgg gac tgc tac gag tac gag cgc cgc gag ggc      203
Ala Phe Pro Ile Cys Arg Asp Cys Tyr Glu Tyr Glu Arg Arg Glu Gly
          35             40             45

acg cag aac tgc ccc cag tgc aag acc cgc ttc aag cgc ttc aag ggg      251
Thr Gln Asn Cys Pro Gln Cys Lys Thr Arg Phe Lys Arg Phe Lys Gly
          50             55             60

tgc gcg cgc gtg ccc ggg gac gag gag gag gac ggc gtc gac gac ctg      299
Cys Ala Arg Val Pro Gly Asp Glu Glu Glu Asp Gly Val Asp Asp Leu
          65             70             75

gag aac gag ttc aac tgg agc gac aag cac gac tcc cag tac ctc gcc      347
Glu Asn Glu Phe Asn Trp Ser Asp Lys His Asp Ser Gln Tyr Leu Ala
          80             85             90             95

gag tcc atg ctc cac gcc cac atg agc tac ggc cgc ggc gcc gac ctc      395
Glu Ser Met Leu His Ala His Met Ser Tyr Gly Arg Gly Ala Asp Leu
          100            105            110

gac ggc gtg ccg cag cca ttc cac ccc atc ccc aat gtt ccc ctc ctc      443
Asp Gly Val Pro Gln Pro Phe His Pro Ile Pro Asn Val Pro Leu Leu
          115            120            125

acc aac gga cag atg gtc gat gac atc ccg ccg gac cag cac gcc ctt      491

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Val	Pro	Ser	Phe	Val	Gly	Gly	Gly	Gly	Lys	Arg	Ile	His	Pro	Leu	Pro	
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Tyr	Ala	Asp	Pro	Asn	Leu	Pro	Val	Gln	Pro	Arg	Ser	Met	Asp	Pro	Ser	
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Ile	Asn	Pro	Tyr	Arg	Met	Ile	Ile	Ile	Ile	Arg	Leu	Val	Val	Leu	Cys	
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Phe	Phe	Phe	His	Tyr	Arg	Val	Met	His	Pro	Val	Pro	Asp	Ala	Phe	Ala	
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Ile	Leu	Asp	Gln	Phe	Pro	Lys	Trp	Phe	Pro	Ile	Glu	Arg	Glu	Thr	Tyr	
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Leu	Ala	Pro	Val	Asp	Phe	Phe	Val	Ser	Thr	Val	Asp	Pro	Leu	Lys	Glu	
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Tyr	Pro	Val	Asp	Lys	Val	Ser	Cys	Tyr	Val	Ser	Asp	Asp	Gly	Ala	Ala	
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Gln	Ala	Leu	Tyr	Gly	Tyr	Asp	Ala	Pro	Lys	Thr	Lys	Lys	Pro	Pro	Ser			
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aga act tgc aac tgc tgg cca aag tgg tgc att tgc tgt tgc tgt ttt	1979
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Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His	
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Cys His Gly Trp Arg Ser Ile Tyr Cys Ile Pro Lys Arg Ala Ala Phe	
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Lys Gly Ser Ala Pro Leu Asn Leu Ser Asp Arg Phe His Gln Val Leu	
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Tyr Cys Thr Leu Pro Ala Ile Cys Leu Leu Thr Gly Lys Phe Ile Thr	
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Ala	Asp	Pro	Asn	Leu	Pro	Val	Gln	Pro	Arg	Ser	Met	Asp	Pro	Ser	Lys
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Tyr	Phe	Gln	Gln	Lys	Ile	Asp	Tyr	Leu	Lys	Asp	Lys	Val	Ala	Pro	Asn
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Thr 775	Glu	Asp	Ile	Leu	Thr 780	Gly	Phe	Lys	Met	His 785	Ala	Arg	Gly	Trp	Arg 790	
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Ser	Val	Glu	Ile	Leu	Phe	Ser	Arg	His 830	Cys	Pro	Leu	Trp 835	Tyr	Gly	Tyr	
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Gly	Ala	Ser	Pro	Asp	His	His	Met	Met	Ser	Pro	Thr	Gly	Asn	Ile	Gly
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Lys	Arg	Ala	Pro	Phe	Pro	Tyr	Val	Asn	His	Ser	Pro	Asn	Pro	Ser	Arg
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Glu	Phe	Ser	Gly	Ser	Ile	Gly	Asn	Val	Ala	Trp	Lys	Glu	Arg	Val	Asp
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Gln	Pro	Leu	Ser	Arg	Lys	Val	Pro	Leu	Pro	Ser	Ser	Arg	Ile	Asn	Pro
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Gln	Phe	Pro	Lys	Trp	Phe	Pro	Ile	Asn	Arg	Glu	Thr	Tyr	Leu	Asp	Arg
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Lys	Asp	Arg	Arg	Ala	Met	Lys	Arg	Glu	Tyr	Glu	Glu	Phe	Lys	Val	Arg
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Cys	Asp	His	Tyr	Ile	Asn	Asn	Ser	Lys	Ala	Leu	Arg	Glu	Ala	Met	Cys
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Cys Gly Asp Ser Val Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala	
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Leu Asp Gln Phe Pro Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu	
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Asp Arg Leu Ala Leu Arg Tyr Asp Arg Glu Gly Glu Pro Ser Gln Leu	
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Ala Pro Ile Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro	
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Pro Leu Ile Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr	
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Pro Val Asp Lys Val Ser Cys Tyr Val Ser Asp Asp Gly Ser Ala Met	
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Leu Thr Phe Glu Ser Leu Ser Glu Thr Ala Glu Phe Ala Arg Lys Trp	
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Val Pro Phe Cys Lys Lys His Asn Ile Glu Pro Arg Ala Pro Glu Phe	
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Tyr Phe Ala Gln Lys Ile Asp Tyr Leu Lys Asp Lys Ile Gln Pro Ser	
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Phe Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys	

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Ala Pro Ile Phe Asn Met Glu Asp Ile Glu Glu Gly Ile Glu Gly Tyr					
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Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser
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Val Ala Gly Ile Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly
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ccg ctc ttt gga aag ctg ttc ttc tcg atc tgg gtg atc ctc cat ctc      3257
Pro Leu Phe Gly Lys Leu Phe Phe Ser Ile Trp Val Ile Leu His Leu
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Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr
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Ile Val Ile Val Trp Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu
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Trp Val Lys Ile Asp Pro Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala
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Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala
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Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn
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Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly Ser
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Leu	Gln	Gly	Asp	Asp	Ala	Asp	Leu	Ser	Ser	Ser	Ala	Arg	His	Glu	Pro	130	135	140
His	His	Arg	Ile	Pro	Arg	Leu	Thr	Ser	Gly	Gln	Gln	Ile	Ser	Gly	Glu	145	150	155
Ile	Pro	Asp	Ala	Ser	Pro	Asp	Arg	His	Ser	Ile	Arg	Ser	Pro	Thr	Ser	165	170	175
Ser	Tyr	Val	Asp	Pro	Ser	Val	Pro	Val	Pro	Val	Arg	Ile	Val	Asp	Pro	180	185	190
Ser	Lys	Asp	Leu	Asn	Ser	Tyr	Gly	Leu	Asn	Ser	Val	Asp	Trp	Lys	Glu	195	200	205
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Arg	Ala	Met	Lys	Arg	Glu	Tyr	Glu	Glu	Phe	Lys	Val	Arg	Ile	Asn	Ala	450	455	460
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Asp	Gly	Thr	Ala	Trp	Pro	Gly	Asn	Asn	Pro	Arg	Asp	His	Pro	Gly	Met	485	490	495
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Glu	Leu	Pro	Arg	Leu	Val	Tyr	Val	Ser	Arg	Glu	Lys	Arg	Pro	Gly	Phe	515	520	525
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Ala	Val	Leu	Thr	Asn	Gly	Ala	Tyr	Leu	Leu	Asn	Val	Asp	Cys	Asp	His	545	550	555
Tyr	Phe	Asn	Ser	Ser	Lys	Ala	Leu	Arg	Glu	Ala	Met	Cys	Phe	Met	Met	565	570	575
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 Met Glu Ala Ser Ala Gly Leu Val Ala Gly
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 Ser His Asn Arg Asn Glu Leu Val Val Ile Arg Arg Asp Gly Asp Pro
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aac gag tgc gcc ttc ccc gtc tgc cgg gac tgc tac gaa tac gag cgc Asn Glu Cys Ala Phe Pro Val Cys Arg Asp Cys Tyr Glu Tyr Glu Arg 60 65 70	365
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Ser	Arg	Glu	Lys	Arg	Pro	Gly	Tyr	Asn	His	His	Lys	Lys	Ala	Gly	Ala		
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Met	Asn	Ala	Leu	Val	Arg	Val	Ser	Ala	Val	Leu	Thr	Asn	Ala	Pro	Tyr		
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Cys	Tyr	Val	Gln	Phe	Pro	Gln	Arg	Phe	Asp	Gly	Ile	Asp	Arg	His	Asp		
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Arg	Tyr	Ala	Asn	Arg	Asn	Val	Val	Phe	Phe	Asp	Ile	Asn	Met	Lys	Gly		
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Arg	Arg	Gln	Ala	Leu	Tyr	Gly	Tyr	Asp	Ala	Pro	Lys	Thr	Lys	Lys	Pro		
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cca	tca	agg	act	tgc	aac	tgc	tgg	ccc	aag	tgg	tgc	ttt	tgc	tgt	tgc		2141
Pro	Ser	Arg	Thr	Cys	Asn	Cys	Trp	Pro	Lys	Trp	Cys	Phe	Cys	Cys	Cys		
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tgc	ttt	ggc	aat	agg	aag	caa	aag	aag	act	acc	aaa	ccc	aaa	aca	gag		2189
Cys	Phe	Gly	Asn	Arg	Lys	Gln	Lys	Lys	Thr	Thr	Lys	Pro	Lys	Thr	Glu		
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Lys	Lys	Lys	Leu	Leu	Phe	Phe	Lys	Lys	Glu	Glu	Asn	Gln	Ser	Pro	Ala		
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tat	gct	ctt	ggt	gaa	att	gac	gaa	gct	gct	cca	gga	gct	gag	aat	gaa		2285
Tyr	Ala	Leu	Gly	Glu	Ile	Asp	Glu	Ala	Ala	Pro	Gly	Ala	Glu	Asn	Glu		
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aag	gcc	ggt	att	gta	aat	caa	caa	aaa	tta	gaa	aag	aaa	ttt	ggc	caa		2333
Lys	Ala	Gly	Ile	Val	Asn	Gln	Gln	Lys	Leu	Glu	Lys	Lys	Phe	Gly	Gln		
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Ser	Ser	Val	Phe	Val	Thr	Ser	Thr	Leu	Leu	Glu	Asn	Gly	Gly	Thr	Leu		
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aag	agt	gca	agt	cct	gct	tct	ctt	ttg	aaa	gaa	gct	ata	cat	gtc	att		2429
Lys	Ser	Ala	Ser	Pro	Ala	Ser	Leu	Leu	Lys	Glu	Ala	Ile	His	Val	Ile		

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Ile	Tyr	Gly	Ser	Val	Thr	Glu	Asp	Ile	Leu	Thr	Gly	Phe	Lys	Met	His						
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Cys	His	Gly	Trp	Arg	Ser	Ile	Tyr	Cys	Ile	Pro	Lys	Arg	Val	Ala	Phe						
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Lys	Gly	Ser	Ala	Pro	Leu	Asn	Leu	Ser	Asp	Arg	Leu	His	Gln	Val	Leu						
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Arg	Trp	Ala	Leu	Gly	Ser	Ile	Glu	Ile	Phe	Phe	Ser	Asn	His	Cys	Pro						
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Leu	Trp	Tyr	Gly	Tyr	Gly	Gly	Gly	Leu	Lys	Phe	Leu	Glu	Arg	Phe	Ser						
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tac	atc	aac	tcc	atc	gtg	tat	cct	tgg	aca	tct	att	ccc	ctc	ttg	gct	2765					
Tyr	Ile	Asn	Ser	Ile	Val	Tyr	Pro	Trp	Thr	Ser	Ile	Pro	Leu	Leu	Ala						
	860					865					870										
tac	tgt	aca	ttg	cct	gcc	atc	tgt	tta	ttg	aca	ggg	aaa	ttt	atc	act	2813					
Tyr	Cys	Thr	Leu	Pro	Ala	Ile	Cys	Leu	Leu	Thr	Gly	Lys	Phe	Ile	Thr						
875					880					885					890						
cca	gag	ctg	aat	aat	gtt	gcc	agc	ctg	tgg	ttc	atg	tca	ctt	ttt	atc	2861					
Pro	Glu	Leu	Asn	Asn	Val	Ala	Ser	Leu	Trp	Phe	Met	Ser	Leu	Phe	Ile						
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Ser	Ser	His	Leu	Phe	Ala	Val	Phe	Gln	Gly	Leu	Leu	Lys	Val	Ile	Ala						
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Glu	Phe	Ser	Glu	Leu	Tyr	Thr	Phe	Lys	Trp	Thr	Thr	Leu	Leu	Ile	Pro						
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cct	acc	acc	ttg	ctt	cta	ttg	aac	ttc	att	ggt	gtg	gtc	gct	ggc	gtt	3149					
Pro	Thr	Thr	Leu	Leu	Leu	Leu	Asn	Phe	Ile	Gly	Val	Val	Ala	Gly	Val						
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 Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu
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 Lys Gly Leu Val Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val
 1035 1040 1045 1050

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 35 40 45
 Ala Pro Gly Gly Asp Pro Phe Val Ala Cys Asn Glu Cys Ala Phe Pro
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 Val Cys Arg Asp Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Thr Gln Asn
 65 70 75 80
 Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Leu Lys Gly Cys Gln Arg
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 Phe Asn Trp Asp Gly His Asp Ser Gln Ser Val Ala Glu Ser Met Leu
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Cys	Lys	Arg	Tyr	Asn	Ile	Glu	Pro	Arg	Ala	Pro	Glu	Trp	Tyr	Phe	Gln	
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Gln	Lys	Ile	Asp	Tyr	Leu	Lys	Asp	Lys	Val	Ala	Ala	Asn	Phe	Val	Arg	
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Gln	Arg	Phe	Asp	Gly	Ile	Asp	Arg	His	Asp	Arg	Tyr	Ala	Asn	Arg	Asn	
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Gln	Asn	Arg	Thr	Pro	Thr	Ile	Val	Ile	Val	Trp	Ser	Ile	Leu	Leu	Ala		
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cgccggcctc gtcgggtgtcg gtggagtgtg aatcgggtgtg tgtaggagga gcgcggag 178
atg gcg gcc aac aag ggg atg gtg gca ggc tct cac aac cgc aac gag 226
Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu
1 5 10 15

ttc gtc atg atc cgc cac gac ggc gac gcg cct gtc ccg gct aag ccc 274
Phe Val Met Ile Arg His Asp Gly Asp Ala Pro Val Pro Ala Lys Pro
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acg aag agt gcg aat ggg cag gtc tgc cag att tgt ggc gac act gtt 322
Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Thr Val
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Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala
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Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn
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Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly Ser
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Pro Arg Val His Gly Asp Asp Glu Glu Glu Asp Val Asp Asp Leu Asp
100 105 110

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Asn Glu Phe Asn Tyr Lys Gln Gly Asn Gly Lys Gly Pro Glu Trp Gln
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Leu Gln Gly Asp Asp Ala Asp Leu Ser Ser Ser Ala Arg His Asp Pro
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cgc Arg	att Ile	gtg Val	cca Pro 260	att Ile	tcc Ser	tca Ser	aac Asn 265	cag Gln	ctc Leu	aac Asn	ctt Leu	tac Tyr 270	cgg Arg	ata Ile	gta Val	994
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act Thr 370	gtt Val	ttg Leu	tcc Ser	att Ile	ctt Leu	gct Ala 375	gtg Val	gat Asp	tac Tyr	cct Pro	gtt Val 380	gac Asp	aaa Lys	gtg Val	tca Ser	1330
tgc Cys	tat Tyr	gtt Val	tct Ser	gat Asp	gat Asp	ggc Gly	tca Ser	gct Ala	atg Met	ctg Leu	act Thr	ttt Phe	gag Glu	tct Ser	ctc Leu	1378

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Ser Glu Thr Ala Glu Phe Ala Arg Lys Trp Val Pro Phe Cys Lys Lys							
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His Asn Ile Glu Pro Arg Ala Pro Glu Phe Tyr Phe Ala Gln Lys Ile							
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Val Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr Met Ala Asp							
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Gly Thr Ala Trp Pro Gly Asn Asn Pro Arg Asp His Pro Gly Met Ile							
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cag gtg ttc ttg ggg cac agt ggt ggg ctt gac act gat gga aat gaa							1714
Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp Thr Asp Gly Asn Glu							
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Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val Asp Cys Asp His Tyr							
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Pro Ala Leu Gly Arg Lys Thr Cys Tyr Val Gln Phe Pro Gln Arg Phe							
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Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn Arg Asn Ile Val Phe							
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Glu Asp Ile Glu Glu Gly Ile Glu Gly Tyr Glu Asp Glu Arg Ser Val	
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Asn Pro Ala Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly	
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acc att gtt tat cca atc aca tct gtt ccg ctt atc gcc tat tgt gtg	2770
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Ile	Pro	Asp	Ala	Ser	Pro	Asp	Arg	His	Ser	Ile	Arg	Ser	Pro	Thr	Ser
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Val	Leu	Thr	Asn	Gly	Ala	Tyr	Leu	Leu	Asn	Val	Asp	Cys	Asp	His	Tyr
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Phe	Asn	Ser	Ser	Lys	Ala	Leu	Arg	Glu	Ala	Met	Cys	Phe	Met	Met	Asp
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Met Glu Ala Ser Ala Gly Leu	
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gtg gcc ggc tcg cat aac cgg aac gag ctg gtg gtg atc cgc cgc gac	283
Val Ala Gly Ser His Asn Arg Asn Glu Leu Val Val Ile Arg Arg Asp	
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cgc gag tcg gga gcc gcg ggc ggc ggc gcg gcg cgc cgg gcg gag gcg	331
Arg Glu Ser Gly Ala Ala Gly Gly Gly Ala Ala Arg Arg Ala Glu Ala	
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ccg tgc cag ata tgc ggc gac gag gtc ggg gtg ggc ttc gac ggg gag	379
Pro Cys Gln Ile Cys Gly Asp Glu Val Gly Val Gly Phe Asp Gly Glu	

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ccc ttc gtg gcg tgc aac gag tgc gcc ttc ccc gtc tgc cgc gcc tgc				427
Pro Phe Val Ala Cys Asn Glu Cys Ala Phe Pro Val Cys Arg Ala Cys	60	65	70	
tac gag tac gag cgc cgc gag ggc tcg caa gcg tgc ccg cag tgc agg				475
Tyr Glu Tyr Glu Arg Arg Glu Gly Ser Gln Ala Cys Pro Gln Cys Arg	75	80	85	
acc cgc tac aag cgc ctc aag ggc tgc ccg cgg gtg gcc ggc gac gag				523
Thr Arg Tyr Lys Arg Leu Lys Gly Cys Pro Arg Val Ala Gly Asp Glu	90	95	100	
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Glu Glu Asp Gly Val Asp Asp Leu Glu Gly Glu Phe Gly Leu Gln Asp	105	110	115	
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Gly Ala Ala His Glu Asp Asp Pro Gln Tyr Val Ala Glu Ser Met Leu	120	125	130	135
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Arg Ala Gln Met Ser Tyr Gly Arg Gly Gly Asp Ala His Pro Gly Phe	140	145	150	
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Ser Pro Val Pro Asn Val Pro Leu Leu Thr Asn Gly Gln Met Val Asp	155	160	165	
gac atc ccg ccg gag cag cac gcg ctc gtg ccg tcc tac atg agc ggc				763
Asp Ile Pro Pro Glu Gln His Ala Leu Val Pro Ser Tyr Met Ser Gly	170	175	180	
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Gly Gly Gly Gly Gly Lys Arg Ile His Pro Leu Pro Phe Ala Asp Pro	185	190	195	
aac ctt cca gtg caa ccg aga tcc atg gac ccg tcc aag gat ctg gcc				859
Asn Leu Pro Val Gln Pro Arg Ser Met Asp Pro Ser Lys Asp Leu Ala	200	205	210	215
gcc tac gga tat ggc agc gtg gcc tgg aag gag aga atg gag ggc tgg				907
Ala Tyr Gly Tyr Gly Ser Val Ala Trp Lys Glu Arg Met Glu Gly Trp	220	225	230	
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Lys Gln Lys Gln Glu Arg Leu Gln His Val Arg Ser Glu Gly Gly Gly	235	240	245	
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Asp Trp Asp Gly Asp Asp Ala Asp Leu Pro Leu Met Asp Glu Ala Arg	250	255	260	
cag cca ttg tcc aga aaa gtc cct ata tca tca agc cga att aat ccc				1051
Gln Pro Leu Ser Arg Lys Val Pro Ile Ser Ser Ser Arg Ile Asn Pro	265	270	275	
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Tyr Arg Met Ile Ile Val Ile Arg Leu Val Val Leu Gly Phe Phe Phe	280	285	290	295

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His Tyr Arg Val Met His Pro Ala Lys Asp Ala Phe Ala Leu Trp Leu	
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Ile Ser Val Ile Cys Glu Ile Trp Phe Ala Met Ser Trp Ile Leu Asp	
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cag ttc cca aag tgg ctt cca atc gag aga gag act tac ctg gac cgt	1243
Gln Phe Pro Lys Trp Leu Pro Ile Glu Arg Glu Thr Tyr Leu Asp Arg	
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Leu Ser Leu Arg Phe Asp Lys Glu Gly Gln Pro Ser Gln Leu Ala Pro	
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Ile Asp Phe Phe Val Ser Thr Val Asp Pro Thr Lys Glu Pro Pro Leu	
360 365 370 375	
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Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro Val	
380 385 390	
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Glu Lys Val Ser Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr	
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Phe Glu Ala Leu Ser Glu Thr Ser Glu Phe Ala Lys Lys Trp Val Pro	
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Phe Ser Lys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe	
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Gln Gln Lys Ile Asp Tyr Leu Lys Asp Lys Val Ala Ala Ser Phe Val	
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Arg Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg	
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Ile Asn Ala Leu Val Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp	
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Thr Met Gln Asp Gly Ser Pro Trp Pro Gly Asn Asn Val Arg Asp His	
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Pro Gly Met Ile Gln Val Phe Leu Gly Gln Ser Gly Gly Arg Asp Val	
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Arg Val Ser Ala Val Leu Ser Asn Ala Ala Tyr Leu Leu Asn Leu Asp	
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Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Ile Lys Glu Ala Met Cys	
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Phe Met Met Asp Pro Leu Val Gly Lys Lys Val Cys Tyr Val Gln Phe	
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Tyr Gly Tyr Asp Ala Pro Lys Thr Lys Lys Pro Pro Ser Arg Thr Cys	
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Asn Gln Gln Lys Leu Glu Lys Lys Phe Gly Gln Ser Ser Val Phe Val	
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Ala Ser Thr Leu Leu Glu Asn Gly Gly Thr Leu Lys Ser Ala Ser Pro	
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Ser	Ile	Tyr	Cys	Ile	Pro	Lys	Arg	Pro	Ala	Phe	Lys	Gly	Ser	Ala	Pro	
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Phe	Thr	Val	Thr	Ser	Lys	Ala	Gly	Asp	Asp	Glu	Glu	Phe	Ser	Glu	Leu	
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Tyr	Thr	Phe	Lys	Trp	Thr	Thr	Leu	Leu	Ile	Pro	Pro	Thr	Thr	Leu	Leu	
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Pro	Arg	Val	Ala	Gly	Asp	Glu	Glu	Glu	Asp	Gly	Val	Asp	Asp	Leu	Glu	
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ctg ctc aag gtg ctg gcc ggc atc gac acc aac ttc acc gtc acc tcc			3185
Leu Leu Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser			
940	945	950	955
aag gcc tcg gac gag gac ggc gac ttc gcg gag ctg tac atg ttc aag			3233
Lys Ala Ser Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Met Phe Lys			
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tgg acg acg ctc ctg atc ccg ccc acc acc atc ctg atc atc aac ctg			3281
Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Ile Leu Ile Ile Asn Leu			
975	980	985	
gtc ggc gtc gtc gcc ggc atc tcc tac gcc atc aac agc gga tac cag			3329
Val Gly Val Val Ala Gly Ile Ser Tyr Ala Ile Asn Ser Gly Tyr Gln			
990	995	1000	
tcg tgg ggc ccg ctc ttc ggc aag ctc ttc ttc gcc ttc tgg gtc atc			3377
Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile			
1005	1010	1015	
gtc cac ctg tac ccg ttc ctc aag ggc ctc atg ggc agg cag aac cgc			3425
Val His Leu Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg			
1020	1025	1030	1035
acc ccg acc atc gtc gtc gtc tgg gcc atc ctg ctg gcg tcc atc ttc			3473
Thr Pro Thr Ile Val Val Val Trp Ala Ile Leu Leu Ala Ser Ile Phe			
1040	1045	1050	
tcc ttg ctg tgg gtt cgc atc gac ccc ttc acc acc cgc gtc act ggc			3521
Ser Leu Leu Trp Val Arg Ile Asp Pro Phe Thr Thr Arg Val Thr Gly			
1055	1060	1065	
ccg gat acc cag acg tgt ggc atc aac t gctaggggaag tggaagggtt			3569
Pro Asp Thr Gln Thr Cys Gly Ile Asn			
1070	1075		
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ataagcagca agtggcggtta tttacagcta cgtacagacc agtggatatt gtttaccaca			3689
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<212> PRT

<213> Zea mays

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Asp	Val	Phe	Ala	Ala	Cys	Asp	Val	Cys	Gly	Phe	Pro	Val	Cys	Arg	Pro

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Cys	Tyr	Glu	Tyr	Glu	Arg	Lys	Asp	Gly	Thr	Gln	Ala	Cys	Pro	Gln	Cys	
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Lys	Thr	Lys	Tyr	Lys	Arg	His	Lys	Gly	Ser	Pro	Ala	Ile	Arg	Gly	Glu	
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Glu	Gly	Asp	Asp	Thr	Asp	Ala	Asp	Ser	Asp	Phe	Asn	Tyr	Leu	Ala	Ser	
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Gly	Asn	Glu	Asp	Gln	Lys	Gln	Lys	Ile	Ala	Asp	Arg	Met	Arg	Ser	Trp	
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Arg	Met	Asn	Val	Gly	Gly	Ser	Gly	Asp	Val	Gly	Arg	Pro	Lys	Tyr	Asp	
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Ser	Gly	Glu	Ile	Gly	Leu	Thr	Lys	Tyr	Asp	Ser	Gly	Glu	Ile	Pro	Arg	
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Gly	Tyr	Ile	Pro	Ser	Val	Thr	Asn	Ser	Gln	Ile	Ser	Gly	Glu	Ile	Pro	
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Gly	Ala	Ser	Pro	Asp	His	His	Met	Met	Ser	Pro	Thr	Gly	Asn	Ile	Gly	
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Lys	Arg	Ala	Pro	Phe	Pro	Tyr	Val	Asn	His	Ser	Pro	Asn	Pro	Ser	Arg	
		180						185					190			
Glu	Phe	Ser	Gly	Ser	Ile	Gly	Asn	Val	Ala	Trp	Lys	Glu	Arg	Val	Asp	
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Thr	Ser	Ile	Ala	Pro	Ser	Glu	Gly	Arg	Gly	Val	Gly	Asp	Ile	Asp	Ala	
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Ser	Thr	Asp	Tyr	Asn	Met	Glu	Asp	Ala	Leu	Leu	Asn	Asp	Glu	Thr	Arg	
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Gln	Pro	Leu	Ser	Arg	Lys	Val	Pro	Leu	Pro	Ser	Ser	Arg	Ile	Asn	Pro	
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Tyr	Arg	Met	Val	Ile	Val	Leu	Arg	Leu	Ile	Val	Leu	Ser	Ile	Phe	Leu	
	275						280					285				
His	Tyr	Arg	Ile	Thr	Asn	Pro	Val	Arg	Asn	Ala	Tyr	Pro	Leu	Trp	Leu	
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Leu	Ser	Val	Ile	Cys	Glu	Ile	Trp	Phe	Ala	Leu	Ser	Trp	Ile	Leu	Asp	
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Gln	Phe	Pro	Lys	Trp	Phe	Pro	Ile	Asn	Arg	Glu	Thr	Tyr	Leu	Asp	Arg	
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Phe	Asp	Ala	Leu	Ala	Glu	Thr	Ser	Glu	Phe	Ala	Arg	Lys	Trp	Val	Pro	
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Lys	Asp	Arg	Arg	Ala	Met	Lys	Arg	Glu	Tyr	Glu	Glu	Phe	Lys	Val	Arg	
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Val	Asn	Gly	Leu	Val	Ala	Lys	Ala	Gln	Lys	Val	Pro	Glu	Glu	Gly	Trp	
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Ile	Met	Gln	Asp	Gly	Thr	Pro	Trp	Pro	Gly	Asn	Asn	Thr	Xaa	Asp	His	
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Pro	Gly	Met	Ile	Gln	Val	Phe	Leu	Gly	His	Ser	Gly	Gly	Leu	Asp	Thr	
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Glu	Gly	Asn	Glu	Leu	Pro	Arg	Leu	Val	Tyr	Val	Ser	Arg	Glu	Lys	Arg	
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Pro	Gly	Phe	Gln	His	His	Lys	Lys	Ala	Gly	Ala	Met	Asn	Ala	Leu	Val
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Arg	Val	Ser	Ala	Val	Leu	Thr	Asn	Gly	Gln	Tyr	Met	Leu	Asn	Leu	Asp
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Cys	Asp	His	Tyr	Ile	Asn	Asn	Ser	Lys	Ala	Leu	Arg	Glu	Ala	Met	Cys
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Phe	Leu	Met	Asp	Pro	Asn	Leu	Gly	Arg	Ser	Val	Cys	Tyr	Val	Gln	Phe
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Pro	Gln	Arg	Phe	Asp	Gly	Ile	Asp	Arg	Asn	Asp	Arg	Tyr	Ala	Asn	Arg
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Tyr	Gly	Tyr	Glu	Pro	Pro	Ile	Lys	Gln	Lys	Lys	Gly	Gly	Phe	Leu	Ser
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Gln	Ser	Ala	Ala	Phe	Val	Ala	Ser	Thr	Leu	Met	Glu	Tyr	Gly	Gly	Val
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Pro	Gln	Ser	Ala	Thr	Pro	Glu	Ser	Leu	Leu	Lys	Glu	Ala	Ile	His	Val
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Phe	Lys	Gly	Ser	Ala	Pro	Ile	Asn	Leu	Ser	Asp	Arg	Leu	Asn	Gln	Val
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gag atg gcg gcc aac aag ggg atg gtg gcg ggc tcg cac aac cgc aac	228
Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn	
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Glu Phe Val Met Ile Arg His Asp Gly Asp Val Pro Gly Ser Ala Lys	
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Pro Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Ser	
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gtg ggt gtt tca gcc act ggt gat gtc ttt gtt gcc tgc aat gag tgt	372
Val Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys	
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gcc ttc cct gtc tgc cgc cca tgc tat gag tat gag cgc aag gag ggg	420
Ala Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly	
65 70 75	

aac caa tgc tgc ccc cag tgc aag act aga tac aag aga cag aaa ggt	468
Asn Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly	

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Ser Pro Arg Val His Gly Asp Glu Asp Glu Glu Asp Val Asp Asp Leu							
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gac aat gaa ttc aac tac aag caa ggc agt ggg aaa ggc cca gag tgg							564
Asp Asn Glu Phe Asn Tyr Lys Gln Gly Ser Gly Lys Gly Pro Glu Trp							
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caa ctg caa gga gat gat gct gat ctg tct tca tct gct cgc cat gag							612
Gln Leu Gln Gly Asp Asp Ala Asp Leu Ser Ser Ser Ala Arg His Glu							
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cca cat cat cgg att cca cgc ctg aca agc ggt caa cag ata tct gga							660
Pro His His Arg Ile Pro Arg Leu Thr Ser Gly Gln Gln Ile Ser Gly							
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gag att cct gat gct tcc cct gac cgt cat tct atc cgc agt cca aca							708
Glu Ile Pro Asp Ala Ser Pro Asp Arg His Ser Ile Arg Ser Pro Thr							
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tcg agc tat gtt gat cca agc gtc cca gtt cct gtg agg att gtg gac							756
Ser Ser Tyr Val Asp Pro Ser Val Pro Val Pro Val Arg Ile Val Asp							
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ccc tcg aag gac ttg aat tcc tat ggg ctt aat agt gtt gac tgg aag							804
Pro Ser Lys Asp Leu Asn Ser Tyr Gly Leu Asn Ser Val Asp Trp Lys							
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gaa aga gtt gag agc tgg agg gtt aaa cag gac aaa aat atg atg caa							852
Glu Arg Val Glu Ser Trp Arg Val Lys Gln Asp Lys Asn Met Met Gln							
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gtg act aat aaa tat cca gag gct aga gga gga gac atg gag ggg act							900
Val Thr Asn Lys Tyr Pro Glu Ala Arg Gly Gly Asp Met Glu Gly Thr							
		225		230		235	
ggc tca aat gga gaa nat atg caa atg gtt gat gat gca cgg cta cct							948
Gly Ser Asn Gly Glu Xaa Met Gln Met Val Asp Asp Ala Arg Leu Pro							
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Leu Ser Arg Ile Val Pro Ile Ser Ser Asn Gln Leu Asn Leu Tyr Arg							
		260		265		270	
gta gtg atc att ctc cgt ctt atc atc ctg tgc ttc ttc ttc cag tat							1044
Val Val Ile Ile Leu Arg Leu Ile Ile Leu Cys Phe Phe Phe Gln Tyr							
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cgt gtc agt cat cca gtg cgt gat gct tat gga tta tgg cta gta tct							1092
Arg Val Ser His Pro Val Arg Asp Ala Tyr Gly Leu Trp Leu Val Ser							
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Val Ile Cys Glu Val Trp Phe Ala Leu Ser Trp Leu Leu Asp Gln Phe							
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cca aaa tgg tat cca atc aac cgt gag aca tat ctt gac agg ctt gca							1188
Pro Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu Asp Arg Leu Ala							
		320		325		330	335

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Leu Arg Tyr Asp Arg Glu Gly Glu Pro Ser Gln Leu Ala Pro Ile Asp	
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Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro Leu Ile Thr	
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Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro Val Asp Lys	
370 375 380	
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Val Ser Cys Tyr Val Ser Asp Asp Gly Ser Ala Met Leu Thr Phe Glu	
385 390 395	
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Ser Leu Ser Glu Thr Ala Glu Phe Ala Arg Lys Trp Val Pro Phe Cys	
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Lys Lys His Asn Ile Glu Pro Arg Ala Pro Glu Phe Tyr Phe Ala Gln	
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Lys Ile Asp Tyr Leu Lys Asp Lys Ile Gln Pro Ser Phe Val Lys Glu	
435 440 445	
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Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn	
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Ala Leu Val Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr Met	
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Ala Asp Gly Thr Ala Trp Pro Gly Asn Asn Pro Arg Asp His Pro Gly	
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Met Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp Thr Asp Gly	
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Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly	
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Phe Gln His His Lys Lys Ala Gly Ala Met Asn Ala Leu Ile Arg Val	
530 535 540	
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Ser Ala Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val Asp Cys Asp	
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His Tyr Phe Asn Ser Ser Lys Ala Leu Arg Glu Ala Met Cys Phe Met	
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Met Asp Pro Ala Leu Gly Arg Lys Thr Cys Tyr Val Gln Phe Pro Gln	
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Arg Phe Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn Arg Asn Ile	
595 600 605	
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Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro	
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Val Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala Leu Tyr Gly	
625 630 635	
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Tyr Asp Pro Val Leu Thr Glu Ala Asp Leu Glu Pro Asn Ile Val Ile	
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Lys Ser Cys Cys Gly Arg Arg Lys Lys Lys Asn Lys Ser Tyr Met Asp	
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Ser Gln Ser Arg Ile Met Lys Arg Thr Glu Ser Ser Ala Pro Ile Phe	
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Asn Met Glu Asp Ile Glu Glu Gly Ile Glu Gly Tyr Glu Asp Glu Arg	
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Ser Val Leu Met Ser Gln Arg Lys Leu Glu Lys Arg Phe Gly Gln Ser	
705 710 715	
cct att ttc att gca tcc acc ttt atg aca caa ggt ggc ata cca cct	2388
Pro Ile Phe Ile Ala Ser Thr Phe Met Thr Gln Gly Gly Ile Pro Pro	
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Ser Thr Asn Pro Ala Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser	
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Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp Ile	
755 760 765	
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Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Ala	
770 775 780	
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Arg Gly Trp Gln Ser Ile Tyr Cys Met Pro Pro Arg Pro Cys Phe Lys	
785 790 795	
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Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg	
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Trp	Tyr	Gly	Tyr	Asn	Gly	Arg	Leu	Lys	Leu	Leu	Glu	Arg	Leu	Ala	Tyr	
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 Gly Val Asn

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 Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala
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 Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn
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 Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly Ser
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 Pro Arg Val His Gly Asp Glu Asp Glu Glu Asp Val Asp Asp Leu Asp
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 Asn Glu Phe Asn Tyr Lys Gln Gly Ser Gly Lys Gly Pro Glu Trp Gln
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 Leu Gln Gly Asp Asp Ala Asp Leu Ser Ser Ser Ala Arg His Glu Pro
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 Ile Pro Asp Ala Ser Pro Asp Arg His Ser Ile Arg Ser Pro Thr Ser
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 Ser Tyr Val Asp Pro Ser Val Pro Val Pro Val Arg Ile Val Asp Pro
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 Ser Lys Asp Leu Asn Ser Tyr Gly Leu Asn Ser Val Asp Trp Lys Glu
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 Arg Val Glu Ser Trp Arg Val Lys Gln Asp Lys Asn Met Met Gln Val
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 Val Ser His Pro Val Arg Asp Ala Tyr Gly Leu Trp Leu Val Ser Val
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 Ile Cys Glu Val Trp Phe Ala Leu Ser Trp Leu Leu Asp Gln Phe Pro
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 Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu Asp Arg Leu Ala Leu
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Lys	His	Asn	Ile	Glu	Pro	Arg	Ala	Pro	Glu	Phe	Tyr	Phe	Ala	Gln	Lys	420	425	430
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Ile	Gln	Val	Phe	Leu	Gly	His	Ser	Gly	Gly	Leu	Asp	Thr	Asp	Gly	Asn	500	505	510
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Asp	Pro	Ala	Leu	Gly	Arg	Lys	Thr	Cys	Tyr	Val	Gln	Phe	Pro	Gln	Arg	580	585	590
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Phe	Phe	Asp	Ile	Asn	Met	Lys	Gly	Leu	Asp	Gly	Ile	Gln	Gly	Pro	Val	610	615	620
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Ser	Ala	Pro	Ile	Asn	Leu	Ser	Asp	Arg	Leu	Asn	Gln	Val	Leu	Arg	Trp	805	810	815
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Asp	Thr	Asn	Phe	Thr	Val	Thr	Ser	Lys	Ala	Ser	Asp	Glu	Asp	Gly	Asp		
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Lys Ser Ala Ser Pro Ala Ser Leu Leu Lys Glu Ala Ile His Val Ile							
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agt tgt ggt tat gaa gac aag aca gac tgg gga aaa gag att ggc tgg							2477
Ser Cys Gly Tyr Glu Asp Lys Thr Asp Trp Gly Lys Glu Ile Gly Trp							
		765		770		775	
atc tat gga tca gtt aca gaa gat att cta act ggt ttc aag atg cat							2525
Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His							
		780		785		790	
tgt cat ggt tgg cgg tca att tac tgc ata cct aaa cgg gtt gca ttc							2573
Cys His Gly Trp Arg Ser Ile Tyr Cys Ile Pro Lys Arg Val Ala Phe							
		795		800		805	
aaa ggt tct gca cct ctg aat ctt tca gat cgt ctt cac cag gtg ctt							2621
Lys Gly Ser Ala Pro Leu Asn Leu Ser Asp Arg Leu His Gln Val Leu							
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cgg tgg gct ctt ggg tct att gag atc ttc ttc agc aat cat tgc cct							2669
Arg Trp Ala Leu Gly Ser Ile Glu Ile Phe Phe Ser Asn His Cys Pro							
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ctt tgg tat ggg tat ggt ggc ggt ctg aaa ttt ttg gaa aga ttt tcc							2717
Leu Trp Tyr Gly Tyr Gly Gly Gly Leu Lys Phe Leu Glu Arg Phe Ser							
		845		850		855	
tac atc aac tcc atc gtg tat cct tgg aca tct att ccc ctc ttg gct							2765
Tyr Ile Asn Ser Ile Val Tyr Pro Trp Thr Ser Ile Pro Leu Leu Ala							
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tac tgt aca ttg cct gcc atc tgt tta ttg aca ggg aaa ttt atc act							2813
Tyr Cys Thr Leu Pro Ala Ile Cys Leu Leu Thr Gly Lys Phe Ile Thr							
		875		880		885	
cca gag ctg aat aat gtt gcc agc ctg tgg ttc atg tca ctt ttt atc							2861
Pro Glu Leu Asn Asn Val Ala Ser Leu Trp Phe Met Ser Leu Phe Ile							
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tgc att ttt gct acg agc atc cta gaa atg aga tgg agt ggt gtt gga							2909
Cys Ile Phe Ala Thr Ser Ile Leu Glu Met Arg Trp Ser Gly Val Gly							
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att gat gac tgg tgg agg aat gag cag ttc tgg gtc att gga ggt gtg							2957
Ile Asp Asp Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Val							
		925		930		935	
tcc tca cac ctc ttt gct gtg ttc cag gga ctt ctc aag gtc ata gct							3005
Ser Ser His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Ile Ala							
		940		945		950	
ggt gtt gat aca agc ttc acc gtg aca tca aag ggt gga gat gat gag							3053
Gly Val Asp Thr Ser Phe Thr Val Thr Ser Lys Gly Gly Asp Asp Glu							
		955		960		965	
						970	

gag ttc tca gag cta tat aca ttc aaa tgg act acc tta ttg ata cct 3101
 Glu Phe Ser Glu Leu Tyr Thr Phe Lys Trp Thr Thr Leu Leu Ile Pro
 975 980 985

cct acc acc ttg ctt cta ttg aac ttc att ggt gtg gtc gct ggc gtt 3149
 Pro Thr Thr Leu Leu Leu Leu Asn Phe Ile Gly Val Val Ala Gly Val
 990 995 1000

tca aat gcg atc aat aac gga tat gag tca tgg ggc ccc ctc ttt ggg 3197
 Ser Asn Ala Ile Asn Asn Gly Tyr Glu Ser Trp Gly Pro Leu Phe Gly
 1005 1010 1015

aag cta ttc ttt gca ttt tgg gtg att gtc cat ctt tat ccc ttt ctc 3245
 Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu
 1020 1025 1030

aaa ggt ttg gtt gga agg caa aac agg aca cca acg att gtc atc gtc 3293
 Lys Gly Leu Val Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val
 1035 1040 1045 1050

tgg tcc att ctg ctg gct tca atc ttc tcg ctc ctt tgg gtt cgg att 3341
 Trp Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Ile
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gat cct ttc ctt gcg aag gat gat ggt ccg ctt ctt gag gag tgt ggt 3389
 Asp Pro Phe Leu Ala Lys Asp Asp Gly Pro Leu Leu Glu Glu Cys Gly
 1070 1075 1080

ttg gat tgc a actaggatgt cagtgcacatca gctcccccaa tctgcatatg 3439
 Leu Asp Cys
 1085

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<212> PRT

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 Glu Gln Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Asp Val Gly Leu
 35 40 45
 Ala Pro Gly Gly Asp Pro Phe Val Ala Cys Asn Glu Cys Ala Phe Pro
 50 55 60
 Val Cys Arg Asp Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Thr Gln Asn
 65 70 75 80
 Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Leu Lys Gly Cys Gln Arg
 85 90 95

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Phe	Asn	Trp	Asp	Gly	His	Asp	Ser	Gln	Ser	Val	Ala	Glu	Ser	Met	Leu
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Tyr	Gly	His	Met	Ser	Tyr	Gly	Arg	Gly	Gly	Asp	Pro	Asn	Gly	Ala	Pro
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Gln	Ala	Phe	Gln	Leu	Asn	Pro	Asn	Val	Pro	Leu	Leu	Thr	Asn	Gly	Gln
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Met	Val	Asp	Asp	Ile	Pro	Pro	Glu	Gln	His	Ala	Leu	Val	Pro	Ser	Phe
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Met	Gly	Gly	Gly	Gly	Lys	Arg	Ile	His	Pro	Leu	Pro	Tyr	Ala	Asp	Pro
			180					185					190		
Ser	Leu	Pro	Val	Gln	Pro	Arg	Ser	Met	Asp	Pro	Ser	Lys	Asp	Leu	Ala
		195					200					205			
Ala	Tyr	Gly	Tyr	Gly	Ser	Val	Ala	Trp	Lys	Glu	Arg	Met	Glu	Asn	Trp
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Lys	Gln	Arg	Gln	Glu	Arg	Met	His	Gln	Thr	Gly	Asn	Asp	Gly	Gly	Gly
225					230					235					240
Asp	Asp	Gly	Asp	Asp	Ala	Asp	Leu	Pro	Leu	Met	Asp	Glu	Ala	Arg	Gln
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Gln	Leu	Ser	Arg	Lys	Ile	Pro	Leu	Pro	Ser	Ser	Gln	Ile	Asn	Pro	Tyr
			260					265					270		
Arg	Met	Ile	Ile	Ile	Ile	Arg	Leu	Val	Val	Leu	Gly	Phe	Phe	Phe	His
		275					280					285			
Tyr	Arg	Val	Met	His	Pro	Val	Asn	Asp	Ala	Phe	Ala	Leu	Trp	Leu	Ile
	290					295					300				
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Phe	Pro	Lys	Trp	Phe	Pro	Ile	Glu	Arg	Glu	Thr	Tyr	Leu	Asp	Arg	Leu
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Ser	Leu	Arg	Phe	Asp	Lys	Glu	Gly	Gln	Pro	Ser	Gln	Leu	Ala	Pro	Ile
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Asp	Phe	Phe	Val	Ser	Thr	Val	Asp	Pro	Leu	Lys	Glu	Pro	Pro	Leu	Val
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Lys	Val	Ser	Cys	Tyr	Val	Ser	Asp	Asp	Gly	Ala	Ala	Met	Leu	Thr	Phe
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Glu	Ala	Leu	Ser	Glu	Thr	Ser	Glu	Phe	Ala	Lys	Lys	Trp	Val	Pro	Phe
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Cys	Lys	Arg	Tyr	Asn	Ile	Glu	Pro	Arg	Ala	Pro	Glu	Trp	Tyr	Phe	Gln
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Gln	Lys	Ile	Asp	Tyr	Leu	Lys	Asp	Lys	Val	Ala	Ala	Asn	Phe	Val	Arg
		435					440					445			
Glu	Arg	Arg	Ala	Met	Lys	Arg	Glu	Tyr	Glu	Glu	Phe	Lys	Val	Arg	Ile
	450					455					460				
Asn	Ala	Leu	Val	Ala	Lys	Ala	Gln	Lys	Val	Pro	Glu	Glu	Gly	Trp	Thr
465					470					475					480
Met	Gln	Asp	Gly	Thr	Pro	Trp	Pro	Gly	Asn	Asn	Val	Arg	Asp	His	Pro
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Gly	Met	Ile	Gln	Val	Phe	Leu	Gly	Gln	Ser	Gly	Gly	Leu	Asp	Cys	Glu
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Gly	Asn	Glu	Leu	Pro	Arg	Leu	Val	Tyr	Val	Ser	Arg	Glu	Lys	Arg	Pro
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Gly	Tyr	Asn	His	His	Lys	Lys	Ala	Gly	Ala	Met	Asn	Ala	Leu	Val	Arg
	530					535					540				
Val	Ser	Ala	Val	Leu	Thr	Asn	Ala	Pro	Tyr	Leu	Leu	Asn	Leu	Asp	Cys
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Asp	His	Tyr	Ile	Asn	Asn	Ser	Lys	Ala	Ile	Lys	Glu	Ala	Met	Cys	Phe
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Met	Met	Asp	Pro	Leu	Leu	Gly	Lys	Lys	Val	Cys	Tyr	Val	Gln	Phe	Pro

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Gln	Arg	Phe	Asp	Gly	Ile	Asp	Arg	His	Asp	Arg	Tyr	Ala	Asn	Arg	Asn		
		595					600					605					
Val	Val	Phe	Phe	Asp	Ile	Asn	Met	Lys	Gly	Leu	Asp	Gly	Ile	Gln	Gly		
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Pro	Ile	Tyr	Val	Gly	Thr	Gly	Cys	Val	Phe	Arg	Arg	Gln	Ala	Leu	Tyr		
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			645						650					655			
Cys	Trp	Pro	Lys	Trp	Cys	Phe	Cys	Cys	Cys	Cys	Phe	Gly	Asn	Arg	Lys		
			660					665					670				
Gln	Lys	Lys	Thr	Thr	Lys	Pro	Lys	Thr	Glu	Lys	Lys	Lys	Leu	Leu	Phe		
		675					680					685					
Phe	Lys	Lys	Glu	Glu	Asn	Gln	Ser	Pro	Ala	Tyr	Ala	Leu	Gly	Glu	Ile		
	690				695						700						
Asp	Glu	Ala	Ala	Pro	Gly	Ala	Glu	Asn	Glu	Lys	Ala	Gly	Ile	Val	Asn		
705					710					715					720		
Gln	Gln	Lys	Leu	Glu	Lys	Lys	Phe	Gly	Gln	Ser	Ser	Val	Phe	Val	Thr		
			725					730						735			
Ser	Thr	Leu	Leu	Glu	Asn	Gly	Gly	Thr	Leu	Lys	Ser	Ala	Ser	Pro	Ala		
			740					745						750			
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Lys	Thr	Asp	Trp	Gly	Lys	Glu	Ile	Gly	Trp	Ile	Tyr	Gly	Ser	Val	Thr		
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Glu	Asp	Ile	Leu	Thr	Gly	Phe	Lys	Met	His	Cys	His	Gly	Trp	Arg	Ser		
785					790					795					800		
Ile	Tyr	Cys	Ile	Pro	Lys	Arg	Val	Ala	Phe	Lys	Gly	Ser	Ala	Pro	Leu		
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Asn	Leu	Ser	Asp	Arg	Leu	His	Gln	Val	Leu	Arg	Trp	Ala	Leu	Gly	Ser		
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Ile	Glu	Ile	Phe	Phe	Ser	Asn	His	Cys	Pro	Leu	Trp	Tyr	Gly	Tyr	Gly		
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Gly	Gly	Leu	Lys	Phe	Leu	Glu	Arg	Phe	Ser	Tyr	Ile	Asn	Ser	Ile	Val		
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Tyr	Pro	Trp	Thr	Ser	Ile	Pro	Leu	Leu	Ala	Tyr	Cys	Thr	Leu	Pro	Ala		
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Ala	Ser	Leu	Trp	Phe	Met	Ser	Leu	Phe	Ile	Cys	Ile	Phe	Ala	Thr	Ser		
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Ile	Leu	Glu	Met	Arg	Trp	Ser	Gly	Val	Gly	Ile	Asp	Asp	Trp	Trp	Arg		
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Asn	Glu	Gln	Phe	Trp	Val	Ile	Gly	Gly	Val	Ser	Ser	His	Leu	Phe	Ala		
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Thr	Val	Thr	Ser	Lys	Gly	Gly	Asp	Asp	Glu	Glu	Phe	Ser	Glu	Leu	Tyr		
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Thr	Phe	Lys	Trp	Thr	Thr	Leu	Leu	Ile	Pro	Pro	Thr	Thr	Leu	Leu	Leu		
			980					985						990			
Leu	Asn	Phe	Ile	Gly	Val	Val	Ala	Gly	Val	Ser	Asn	Ala	Ile	Asn	Asn		
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Gly	Tyr	Glu	Ser	Trp	Gly	Pro	Leu	Phe	Gly	Lys	Leu	Phe	Phe	Ala	Phe		
	1010				1015						1020						
Trp	Val	Ile	Val	His	Leu	Tyr	Pro	Phe	Leu	Lys	Gly	Leu	Val	Gly	Arg		
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Gln	Asn	Arg	Thr	Pro	Thr	Ile	Val	Ile	Val	Trp	Ser	Ile	Leu	Leu	Ala		
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Ser	Ile	Phe	Ser	Leu	Leu	Trp	Val	Arg	Ile	Asp	Pro	Phe	Leu	Ala	Lys		
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 cgccggcctc gtcggtgtcg gtggagtgtg aatcggtgtg tgtaggagga gcgcggag 178
 atg gcg gcc aac aag ggg atg gtg gca ggc tct cac aac cgc aac gag 226
 Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu
 1 5 10 15

ttc gtc atg atc cgc cac gac ggc gac gcg cct gtc ccg gct aag ccc 274
 Phe Val Met Ile Arg His Asp Gly Asp Ala Pro Val Pro Ala Lys Pro
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acg aag agt gcg aat ggg cag gtc tgc cag att tgt ggc gac act gtt 322
 Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Thr Val
 35 40 45

ggc gtt tca gcc act ggt gat gtc ttt gtt gcc tgc aat gag tgt gcc 370
 Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala
 50 55 60

ttc cct gtc tgc cgc cct tgc tat gag tac gag cgc aag gaa ggg aac 418
 Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn
 65 70 75 80

caa tgc tgc cct cag tgc aag act aga tac aag aga cag aaa ggt agc 466
 Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly Ser
 85 90 95

cct cga gtt cat ggt gat gat gag gag gaa gat gtt gat gac ctg gac 514
 Pro Arg Val His Gly Asp Asp Glu Glu Glu Asp Val Asp Asp Leu Asp
 100 105 110

aat gaa ttc aac tat aag caa ggc aat ggg aag ggc cca gag tgg cag 562

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Leu	Gln	Gly	Asp	Asp	Ala	Asp	Leu	Ser	Ser	Ser	Ala	Arg	His	Asp	Pro	
	130					135					140					
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His	His	Arg	Ile	Pro	Arg	Leu	Thr	Ser	Gly	Gln	Gln	Ile	Ser	Gly	Glu	
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Ile	Pro	Asp	Ala	Ser	Pro	Asp	Arg	His	Ser	Ile	Arg	Ser	Pro	Thr	Ser	
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Ser	Tyr	Val	Asp	Pro	Ser	Val	Pro	Val	Pro	Val	Arg	Ile	Val	Asp	Pro	
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Ser	Lys	Asp	Leu	Asn	Ser	Tyr	Gly	Leu	Asn	Ser	Val	Asp	Trp	Lys	Glu	
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Arg	Val	Glu	Ser	Trp	Arg	Val	Lys	Gln	Asp	Lys	Asn	Met	Leu	Gln	Val	
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Asn	Gly	Glu	Asp	Met	Gln	Met	Val	Asp	Asp	Ala	Arg	Leu	Pro	Leu	Ser	
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Ile	Ile	Leu	Arg	Leu	Ile	Ile	Leu	Cys	Phe	Phe	Phe	Gln	Tyr	Arg	Ile	
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Ser	His	Pro	Val	Arg	Asn	Ala	Tyr	Gly	Leu	Trp	Leu	Val	Ser	Val	Ile	
	290					295					300					
tgt	gag	gtc	tgg	ttt	gcc	ttg	tcc	tgg	ctt	cta	gat	cag	ttc	cca	aaa	1138
Cys	Glu	Val	Trp	Phe	Ala	Leu	Ser	Trp	Leu	Leu	Asp	Gln	Phe	Pro	Lys	
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Trp	Tyr	Pro	Ile	Asn	Arg	Glu	Thr	Tyr	Leu	Asp	Arg	Leu	Ala	Leu	Arg	
				325					330					335		
tat	gat	aga	gag	gga	gag	cca	tca	cag	ctg	gct	ccc	att	gat	gtc	ttt	1234
Tyr	Asp	Arg	Glu	Gly	Glu	Pro	Ser	Gln	Leu	Ala	Pro	Ile	Asp	Val	Phe	
			340					345					350			
gtc	agt	aca	gtg	gat	cca	ttg	aag	gaa	cct	cca	ctg	atc	aca	gcc	aac	1282
Val	Ser	Thr	Val	Asp	Pro	Leu	Lys	Glu	Pro	Pro	Leu	Ile	Thr	Ala	Asn	

355					360					365						
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Thr	Val	Leu	Ser	Ile	Leu	Ala	Val	Asp	Tyr	Pro	Val	Asp	Lys	Val	Ser	
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Cys	Tyr	Val	Ser	Asp	Asp	Gly	Ser	Ala	Met	Leu	Thr	Phe	Glu	Ser	Leu	
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Ser	Glu	Thr	Ala	Glu	Phe	Ala	Arg	Lys	Trp	Val	Pro	Phe	Cys	Lys	Lys	
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cac	aat	att	gaa	cca	aga	gct	cca	gaa	ttt	tac	ttt	gct	caa	aaa	ata	1474
His	Asn	Ile	Glu	Pro	Arg	Ala	Pro	Glu	Phe	Tyr	Phe	Ala	Gln	Lys	Ile	
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Asp	Tyr	Leu	Lys	Asp	Lys	Ile	Gln	Pro	Ser	Phe	Val	Lys	Glu	Arg	Arg	
		435					440					445				
gca	atg	aag	aga	gag	tat	gaa	gaa	ttc	aaa	ata	aga	atc	aat	gcc	ctt	1570
Ala	Met	Lys	Arg	Glu	Tyr	Glu	Glu	Phe	Lys	Ile	Arg	Ile	Asn	Ala	Leu	
	450					455					460					
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Val	Ala	Lys	Ala	Gln	Lys	Val	Pro	Glu	Glu	Gly	Trp	Thr	Met	Ala	Asp	
465					470					475					480	
gga	act	gct	tgg	cct	ggg	aat	aac	cct	agg	gac	cat	cct	ggc	atg	att	1666
Gly	Thr	Ala	Trp	Pro	Gly	Asn	Asn	Pro	Arg	Asp	His	Pro	Gly	Met	Ile	
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cag	gtg	ttc	ttg	ggg	cac	agt	ggg	ggg	ctt	gac	act	gat	gga	aat	gaa	1714
Gln	Val	Phe	Leu	Gly	His	Ser	Gly	Gly	Leu	Asp	Thr	Asp	Gly	Asn	Glu	
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Leu	Pro	Arg	Leu	Val	Tyr	Val	Ser	Arg	Glu	Lys	Arg	Pro	Gly	Phe	Gln	
		515					520					525				
cat	cac	aag	aag	gct	ggg	gca	atg	aat	gca	ctg	att	cgt	gta	tct	gct	1810
His	His	Lys	Lys	Ala	Gly	Ala	Met	Asn	Ala	Leu	Ile	Arg	Val	Ser	Ala	
		530				535					540					
gtg	ctg	aca	aat	ggg	gcc	tat	ctt	ctc	aat	gtg	gat	tgt	gac	cat	tac	1858
Val	Leu	Thr	Asn	Gly	Ala	Tyr	Leu	Leu	Asn	Val	Asp	Cys	Asp	His	Tyr	
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ttc	aat	agc	agc	aaa	gct	ctt	aga	gaa	gca	atg	tgc	ttc	atg	atg	gat	1906
Phe	Asn	Ser	Ser	Lys	Ala	Leu	Arg	Glu	Ala	Met	Cys	Phe	Met	Met	Asp	
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cca	gct	cta	gga	agg	aaa	act	tgt	tat	gta	caa	ttt	cca	caa	aga	ttt	1954
Pro	Ala	Leu	Gly	Arg	Lys	Thr	Cys	Tyr	Val	Gln	Phe	Pro	Gln	Arg	Phe	
			580					585					590			
gat	ggc	att	gac	ttg	cac	gat	cga	tat	gct	aat	agg	aac	ata	gtc	ttc	2002
Asp	Gly	Ile	Asp	Leu	His	Asp	Arg	Tyr	Ala	Asn	Arg	Asn	Ile	Val	Phe	
		595					600					605				

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Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro Val Tyr	
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Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala Leu Tyr Gly Tyr Asp	
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cct gtt ttg act gaa gct gat ctg gaa cct aac att gtt gtt aag agc	2146
Pro Val Leu Thr Glu Ala Asp Leu Glu Pro Asn Ile Val Val Lys Ser	
645 650 655	
tgc tgt ggt aga agg aag aga aag aac aag agt tat atg gat agt caa	2194
Cys Cys Gly Arg Arg Lys Arg Lys Asn Lys Ser Tyr Met Asp Ser Gln	
660 665 670	
agc cgt att atg aag aga aca gaa tct tca gct ccc atc ttt aac atg	2242
Ser Arg Ile Met Lys Arg Thr Glu Ser Ser Ala Pro Ile Phe Asn Met	
675 680 685	
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Glu Asp Ile Glu Glu Gly Ile Glu Gly Tyr Glu Asp Glu Arg Ser Val	
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Leu Met Ser Gln Arg Lys Leu Glu Lys Arg Phe Gly Gln Ser Pro Ile	
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Phe Ile Ala Ser Thr Phe Met Thr Gln Gly Gly Ile Pro Pro Ser Thr	
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Tyr Glu Asp Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly	
755 760 765	
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Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Ala Arg Gly	
770 775 780	
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Trp Gln Ser Ile Tyr Cys Met Pro Pro Arg Pro Cys Phe Lys Gly Ser	
785 790 795 800	
gca cca atc aat ctt tct gat cgt ctt aat cag gtg ctc cgt tgg gct	2626
Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp Ala	
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ctt ggg tca gtg gaa att ctg ctt agc aga cat tgt cct ata tgg tat	2674
Leu Gly Ser Val Glu Ile Leu Leu Ser Arg His Cys Pro Ile Trp Tyr	
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Gly Tyr Asn Gly Arg Leu Lys Leu Leu Glu Arg Leu Ala Tyr Ile Asn	
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acc att gtt tat cca atc aca tct gtt ccg ctt atc gcc tat tgt gtg Thr Ile Val Tyr Pro Ile Thr Ser Val Pro Leu Ile Ala Tyr Cys Val 850 855 860	2770
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tgg tgg aga aat gag cag ttt tgg gtt att ggt ggc acc tct gcc cat Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Thr Ser Ala His 915 920 925	2962
ctc ttc gcg gtg ttc cag ggt ctg ctg aaa gtg ttg gct ggg att gat Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala Gly Ile Asp 930 935 940	3010
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act gtt ctt gtc att aac ctg gtc gga atg gtg gca gga att tcg tat Thr Val Leu Val Ile Asn Leu Val Gly Met Val Ala Gly Ile Ser Tyr 980 985 990	3154
gcc att aac agc ggc tac caa tcc tgg ggt ccg ctc ttt gga aag ctg Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys Leu 995 1000 1005	3202
ttc ttc tcg atc tgg gtg atc ctc cat ctc tac ccc ttc ctc aag ggt Phe Phe Ser Ile Trp Val Ile Leu His Leu Tyr Pro Phe Leu Lys Gly 1010 1015 1020	3250
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atc ctc ctt gcg tct atc ttc tcc ttg ctg tgg gtg aag atc gat cct Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys Ile Asp Pro 1045 1050 1055	3346
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<212> PRT

<213> Zea mays

<400> 42

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Thr	Lys	Ser	Ala	Asn	Gly	Gln	Val	Cys	Gln	Ile	Cys	Gly	Asp	Thr	Val
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Gly	Val	Ser	Ala	Thr	Gly	Asp	Val	Phe	Val	Ala	Cys	Asn	Glu	Cys	Ala
	50					55					60				
Phe	Pro	Val	Cys	Arg	Pro	Cys	Tyr	Glu	Tyr	Glu	Arg	Lys	Glu	Gly	Asn
65					70					75					80
Gln	Cys	Cys	Pro	Gln	Cys	Lys	Thr	Arg	Tyr	Lys	Arg	Gln	Lys	Gly	Ser
				85					90					95	
Pro	Arg	Val	His	Gly	Asp	Asp	Glu	Glu	Glu	Asp	Val	Asp	Asp	Leu	Asp
			100					105					110		
Asn	Glu	Phe	Asn	Tyr	Lys	Gln	Gly	Asn	Gly	Lys	Gly	Pro	Glu	Trp	Gln
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Leu	Gln	Gly	Asp	Asp	Ala	Asp	Leu	Ser	Ser	Ser	Ala	Arg	His	Asp	Pro
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His	His	Arg	Ile	Pro	Arg	Leu	Thr	Ser	Gly	Gln	Gln	Ile	Ser	Gly	Glu
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Ile	Pro	Asp	Ala	Ser	Pro	Asp	Arg	His	Ser	Ile	Arg	Ser	Pro	Thr	Ser
				165					170					175	
Ser	Tyr	Val	Asp	Pro	Ser	Val	Pro	Val	Pro	Val	Arg	Ile	Val	Asp	Pro
			180					185					190		
Ser	Lys	Asp	Leu	Asn	Ser	Tyr	Gly	Leu	Asn	Ser	Val	Asp	Trp	Lys	Glu
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Thr	Asn	Lys	Tyr	Pro	Glu	Ala	Arg	Gly	Asp	Met	Glu	Gly	Thr	Gly	Ser
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Asn	Gly	Glu	Asp	Met	Gln	Met	Val	Asp	Asp	Ala	Arg	Leu	Pro	Leu	Ser
				245					250					255	
Arg	Ile	Val	Pro	Ile	Ser	Ser	Asn	Gln	Leu	Asn	Leu	Tyr	Arg	Ile	Val
			260					265					270		
Ile	Ile	Leu	Arg	Leu	Ile	Ile	Leu	Cys	Phe	Phe	Phe	Gln	Tyr	Arg	Ile
		275					280					285			
Ser	His	Pro	Val	Arg	Asn	Ala	Tyr	Gly	Leu	Trp	Leu	Val	Ser	Val	Ile
	290					295					300				
Cys	Glu	Val	Trp	Phe	Ala	Leu	Ser	Trp	Leu	Leu	Asp	Gln	Phe	Pro	Lys
305					310					315					320
Trp	Tyr	Pro	Ile	Asn	Arg	Glu	Thr	Tyr	Leu	Asp	Arg	Leu	Ala	Leu	Arg
				325					330					335	
Tyr	Asp	Arg	Glu	Gly	Glu	Pro	Ser	Gln	Leu	Ala	Pro	Ile	Asp	Val	Phe
			340					345					350		
Val	Ser	Thr	Val	Asp	Pro	Leu	Lys	Glu	Pro	Pro	Leu	Ile	Thr	Ala	Asn
		355					360					365			
Thr	Val	Leu	Ser	Ile	Leu	Ala	Val	Asp	Tyr	Pro	Val	Asp	Lys	Val	Ser
	370					375					380				
Cys	Tyr	Val	Ser	Asp	Asp	Gly	Ser	Ala	Met	Leu	Thr	Phe	Glu	Ser	Leu
385					390					395					400

Ser	Glu	Thr	Ala	Glu	Phe	Ala	Arg	Lys	Trp	Val	Pro	Phe	Cys	Lys	Lys	405	410	415
His	Asn	Ile	Glu	Pro	Arg	Ala	Pro	Glu	Phe	Tyr	Phe	Ala	Gln	Lys	Ile	420	425	430
Asp	Tyr	Leu	Lys	Asp	Lys	Ile	Gln	Pro	Ser	Phe	Val	Lys	Glu	Arg	Arg	435	440	445
Ala	Met	Lys	Arg	Glu	Tyr	Glu	Glu	Phe	Lys	Ile	Arg	Ile	Asn	Ala	Leu	450	455	460
Val	Ala	Lys	Ala	Gln	Lys	Val	Pro	Glu	Glu	Gly	Trp	Thr	Met	Ala	Asp	465	470	475
Gly	Thr	Ala	Trp	Pro	Gly	Asn	Asn	Pro	Arg	Asp	His	Pro	Gly	Met	Ile	485	490	495
Gln	Val	Phe	Leu	Gly	His	Ser	Gly	Gly	Leu	Asp	Thr	Asp	Gly	Asn	Glu	500	505	510
Leu	Pro	Arg	Leu	Val	Tyr	Val	Ser	Arg	Glu	Lys	Arg	Pro	Gly	Phe	Gln	515	520	525
His	His	Lys	Lys	Ala	Gly	Ala	Met	Asn	Ala	Leu	Ile	Arg	Val	Ser	Ala	530	535	540
Val	Leu	Thr	Asn	Gly	Ala	Tyr	Leu	Leu	Asn	Val	Asp	Cys	Asp	His	Tyr	545	550	555
Phe	Asn	Ser	Ser	Lys	Ala	Leu	Arg	Glu	Ala	Met	Cys	Phe	Met	Met	Asp	565	570	575
Pro	Ala	Leu	Gly	Arg	Lys	Thr	Cys	Tyr	Val	Gln	Phe	Pro	Gln	Arg	Phe	580	585	590
Asp	Gly	Ile	Asp	Leu	His	Asp	Arg	Tyr	Ala	Asn	Arg	Asn	Ile	Val	Phe	595	600	605
Phe	Asp	Ile	Asn	Met	Lys	Gly	Leu	Asp	Gly	Ile	Gln	Gly	Pro	Val	Tyr	610	615	620
Val	Gly	Thr	Gly	Cys	Cys	Phe	Asn	Arg	Gln	Ala	Leu	Tyr	Gly	Tyr	Asp	625	630	635
Pro	Val	Leu	Thr	Glu	Ala	Asp	Leu	Glu	Pro	Asn	Ile	Val	Val	Lys	Ser	645	650	655
Cys	Cys	Gly	Arg	Arg	Lys	Arg	Lys	Asn	Lys	Ser	Tyr	Met	Asp	Ser	Gln	660	665	670
Ser	Arg	Ile	Met	Lys	Arg	Thr	Glu	Ser	Ser	Ala	Pro	Ile	Phe	Asn	Met	675	680	685
Glu	Asp	Ile	Glu	Glu	Gly	Ile	Glu	Gly	Tyr	Glu	Asp	Glu	Arg	Ser	Val	690	695	700
Leu	Met	Ser	Gln	Arg	Lys	Leu	Glu	Lys	Arg	Phe	Gly	Gln	Ser	Pro	Ile	705	710	715
Phe	Ile	Ala	Ser	Thr	Phe	Met	Thr	Gln	Gly	Gly	Ile	Pro	Pro	Ser	Thr	725	730	735
Asn	Pro	Ala	Ser	Leu	Leu	Lys	Glu	Ala	Ile	His	Val	Ile	Ser	Cys	Gly	740	745	750
Tyr	Glu	Asp	Lys	Thr	Glu	Trp	Gly	Lys	Glu	Ile	Gly	Trp	Ile	Tyr	Gly	755	760	765
Ser	Val	Thr	Glu	Asp	Ile	Leu	Thr	Gly	Phe	Lys	Met	His	Ala	Arg	Gly	770	775	780
Trp	Gln	Ser	Ile	Tyr	Cys	Met	Pro	Pro	Arg	Pro	Cys	Phe	Lys	Gly	Ser	785	790	795
Ala	Pro	Ile	Asn	Leu	Ser	Asp	Arg	Leu	Asn	Gln	Val	Leu	Arg	Trp	Ala	805	810	815
Leu	Gly	Ser	Val	Glu	Ile	Leu	Leu	Ser	Arg	His	Cys	Pro	Ile	Trp	Tyr	820	825	830
Gly	Tyr	Asn	Gly	Arg	Leu	Lys	Leu	Leu	Glu	Arg	Leu	Ala	Tyr	Ile	Asn	835	840	845
Thr	Ile	Val	Tyr	Pro	Ile	Thr	Ser	Val	Pro	Leu	Ile	Ala	Tyr	Cys	Val	850	855	860
Leu	Pro	Ala	Ile	Cys	Leu	Leu	Thr	Asn	Lys	Phe	Ile	Ile	Pro	Glu	Ile	865	870	875
Ser	Asn	Tyr	Ala	Gly	Met	Phe	Phe	Ile	Leu	Leu	Phe	Ala	Ser	Ile	Phe			

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Arg Glu Ser Gly Ala Ala Gly Gly Gly Ala Ala Arg Arg Ala Glu Ala			
25	30	35	
ccg tgc cag ata tgc ggc gac gag gtc ggg gtg ggc ttc gac ggg gag			379
Pro Cys Gln Ile Cys Gly Asp Glu Val Gly Val Gly Phe Asp Gly Glu			
40	45	50	55
ccc ttc gtg gcg tgc aac gag tgc gcc ttc ccc gtc tgc cgc gcc tgc			427
Pro Phe Val Ala Cys Asn Glu Cys Ala Phe Pro Val Cys Arg Ala Cys			
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Tyr Glu Tyr Glu Arg Arg Glu Gly Ser Gln Ala Cys Pro Gln Cys Arg			
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Glu Glu Asp Gly Val Asp Asp Leu Glu Gly Glu Phe Gly Leu Gln Asp			
105	110	115	
ggc gcc gcc cac gag gac gac ccg cag tac gtc gcc gag tcc atg ctc			619
Gly Ala Ala His Glu Asp Asp Pro Gln Tyr Val Ala Glu Ser Met Leu			
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Arg Ala Gln Met Ser Tyr Gly Arg Gly Gly Asp Ala His Pro Gly Phe			
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Ser Pro Val Pro Asn Val Pro Leu Leu Thr Asn Gly Gln Met Val Asp			
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Asp Ile Pro Pro Glu Gln His Ala Leu Val Pro Ser Tyr Met Ser Gly			
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Asn Leu Pro Val Gln Pro Arg Ser Met Asp Pro Ser Lys Asp Leu Ala			
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gcc tac gga tat ggc agc gtg gcc tgg aag gag aga atg gag ggc tgg			907
Ala Tyr Gly Tyr Gly Ser Val Ala Trp Lys Glu Arg Met Glu Gly Trp			
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Lys Gln Lys Gln Glu Arg Leu Gln His Val Arg Ser Glu Gly Gly Gly			
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Asp Trp Asp Gly Asp Asp Ala Asp Leu Pro Leu Met Asp Glu Ala Arg			
250	255	260	

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His Tyr Arg Val Met His Pro Ala Lys Asp Ala Phe Ala Leu Trp Leu	
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Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro Val	
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gca tca aca ctt ctt gag aac gga ggg acc ctg aag agc gca agt cca	2491

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gct	tct	ctt	ctg	aag	gaa	gct	ata	cat	gtt	atc	agc	tgc	ggc	tac	gaa	2539
Ala	Ser	Leu	Leu	Lys	Glu	Ala	Ile	His	Val	Ile	Ser	Cys	Gly	Tyr	Glu	
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Asp	Lys	Thr	Asp	Trp	Gly	Lys	Glu	Ile	Gly	Trp	Ile	Tyr	Gly	Ser	Ile	
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Thr	Glu	Asp	Ile	Leu	Thr	Gly	Phe	Lys	Met	His	Cys	His	Gly	Trp	Arg	
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Ser	Ile	Tyr	Cys	Ile	Pro	Lys	Arg	Pro	Ala	Phe	Lys	Gly	Ser	Ala	Pro	
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Leu	Asn	Leu	Ser	Asp	Arg	Leu	His	Gln	Val	Leu	Arg	Trp	Ala	Leu	Gly	
	825					830					835					
tcc	gtc	gaa	att	ttc	ttc	agc	aag	cac	tgc	cca	ctt	tgg	tac	gga	tac	2779
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Gly	Gly	Gly	Leu	Lys	Phe	Leu	Glu	Arg	Phe	Ser	Tyr	Ile	Asn	Ser	Ile	
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gtt	tat	ccc	tgg	acg	tcc	att	cct	ctc	ctg	gct	tac	tgt	acc	ttg	cct	2875
Val	Tyr	Pro	Trp	Thr	Ser	Ile	Pro	Leu	Leu	Ala	Tyr	Cys	Thr	Leu	Pro	
			875					880					885			
gcc	atc	tgc	ctg	ctc	acg	ggg	aag	ttt	atc	aca	cca	gag	ctt	acc	aat	2923
Ala	Ile	Cys	Leu	Leu	Thr	Gly	Lys	Phe	Ile	Thr	Pro	Glu	Leu	Thr	Asn	
		890					895					900				
gtc	gcc	agt	atc	tgg	ttc	atg	gca	ctt	ttc	atc	tgc	atc	tcc	gtg	acc	2971
Val	Ala	Ser	Ile	Trp	Phe	Met	Ala	Leu	Phe	Ile	Cys	Ile	Ser	Val	Thr	
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Arg	Asn	Glu	Gln	Phe	Trp	Val	Ile	Gly	Gly	Val	Ser	Ala	His	Leu	Phe	
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gcg	gtg	ttc	cag	ggc	ctg	ctg	aag	gtg	ttc	gcc	ggc	atc	gac	acg	agc	3115
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Phe	Thr	Val	Thr	Ser	Lys	Ala	Gly	Asp	Asp	Glu	Glu	Phe	Ser	Glu	Leu	
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Tyr	Thr	Phe	Lys	Trp	Thr	Thr	Leu	Leu	Ile	Pro	Pro	Thr	Thr	Leu	Leu	

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Leu Leu Asn Phe Ile Gly Val Val Ala Gly Ile Ser Asn Ala Ile Asn			
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aac ggg tac gag tcg tgg ggc ccc ctg ttc ggg aag ctc ttc ttc gcc			3307
Asn Gly Tyr Glu Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala			
	1020	1025	1030
ttc tgg gtg atc gtc cac ctg tac ccg ttc ctc aag ggt ctg gtg ggg			3355
Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys Gly Leu Val Gly			
	1035	1040	1045
agg cag aac agg acg ccg acg atc gtc atc gtc tgg tcc atc ctg ctg			3403
Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp Ser Ile Leu Leu			
	1050	1055	1060
gcc tcg atc ttc tcg ctc ctg tgg gtc cgc gtc gac ccg ttc ctc gcc			3451
Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Val Asp Pro Phe Leu Ala			
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Lys Ser Asn Gly Pro Leu Leu Glu Glu Cys Gly Leu Asp Cys			
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Gly Val Gly Phe Asp Gly Glu Pro Phe Val Ala Cys Asn Glu Cys Ala
50 55 60
Phe Pro Val Cys Arg Ala Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Ser
65 70 75 80
Gln Ala Cys Pro Gln Cys Arg Thr Arg Tyr Lys Arg Leu Lys Gly Cys
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Pro Arg Val Ala Gly Asp Glu Glu Glu Asp Gly Val Asp Asp Leu Glu
100 105 110
Gly Glu Phe Gly Leu Gln Asp Gly Ala Ala His Glu Asp Asp Pro Gln
115 120 125
Tyr Val Ala Glu Ser Met Leu Arg Ala Gln Met Ser Tyr Gly Arg Gly
130 135 140
Gly Asp Ala His Pro Gly Phe Ser Pro Val Pro Asn Val Pro Leu Leu
145 150 155 160
Thr Asn Gly Gln Met Val Asp Asp Ile Pro Pro Glu Gln His Ala Leu
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Pro	Leu	Pro	Phe	Ala	Asp	Pro	Asn	Leu	Pro	Val	Gln	Pro	Arg	Ser	Met
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Asp	Pro	Ser	Lys	Asp	Leu	Ala	Ala	Tyr	Gly	Tyr	Gly	Ser	Val	Ala	Trp
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Lys	Glu	Arg	Met	Glu	Gly	Trp	Lys	Gln	Lys	Gln	Glu	Arg	Leu	Gln	His
225					230					235					240
Val	Arg	Ser	Glu	Gly	Gly	Gly	Asp	Trp	Asp	Gly	Asp	Asp	Ala	Asp	Leu
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Pro	Leu	Met	Asp	Glu	Ala	Arg	Gln	Pro	Leu	Ser	Arg	Lys	Val	Pro	Ile
			260					265					270		
Ser	Ser	Ser	Arg	Ile	Asn	Pro	Tyr	Arg	Met	Ile	Ile	Val	Ile	Arg	Leu
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Ala	Met	Ser	Trp	Ile	Leu	Asp	Gln	Phe	Pro	Lys	Trp	Leu	Pro	Ile	Glu
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Asp	Gly	Ala	Ala	Met	Leu	Thr	Phe	Glu	Ala	Leu	Ser	Glu	Thr	Ser	Glu
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Phe	Ala	Lys	Lys	Trp	Val	Pro	Phe	Ser	Lys	Lys	Phe	Asn	Ile	Glu	Pro
			420				425						430		
Arg	Ala	Pro	Glu	Trp	Tyr	Phe	Gln	Gln	Lys	Ile	Asp	Tyr	Leu	Lys	Asp
		435					440					445			
Lys	Val	Ala	Ala	Ser	Phe	Val	Arg	Glu	Arg	Arg	Ala	Met	Lys	Arg	Glu
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Tyr	Glu	Glu	Phe	Lys	Val	Arg	Ile	Asn	Ala	Leu	Val	Ala	Lys	Ala	Gln
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Lys	Val	Pro	Glu	Glu	Gly	Trp	Thr	Met	Gln	Asp	Gly	Ser	Pro	Trp	Pro
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Gly	Asn	Asn	Val	Arg	Asp	His	Pro	Gly	Met	Ile	Gln	Val	Phe	Leu	Gly
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Gln	Ser	Gly	Gly	Arg	Asp	Val	Glu	Gly	Asn	Glu	Leu	Pro	Arg	Leu	Val
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Tyr	Val	Ser	Arg	Glu	Lys	Arg	Pro	Gly	Tyr	Asn	His	His	Lys	Lys	Ala
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Gly	Ala	Met	Asn	Ala	Leu	Val	Arg	Val	Ser	Ala	Val	Leu	Ser	Asn	Ala
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Ala	Tyr	Leu	Leu	Asn	Leu	Asp	Cys	Asp	His	Tyr	Ile	Asn	Asn	Ser	Lys
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Ala	Ile	Lys	Glu	Ala	Met	Cys	Phe	Met	Met	Asp	Pro	Leu	Val	Gly	Lys
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Lys	Val	Cys	Tyr	Val	Gln	Phe	Pro	Gln	Arg	Phe	Asp	Gly	Ile	Asp	Lys
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Asn	Asp	Arg	Tyr	Ala	Asn	Arg	Asn	Val	Val	Phe	Phe	Asp	Ile	Asn	Met
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Val	Phe	Arg	Arg	Gln	Ala	Leu	Tyr	Gly	Tyr	Asp	Ala	Pro	Lys	Thr	Lys
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Lys	Pro	Pro	Ser	Arg	Thr	Cys	Asn	Cys	Trp	Pro	Lys	Trp	Cys	Leu	Ser

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Cys	Cys	Cys	Ser	Arg	Asn	Lys	Asn	Lys	Lys	Lys	Thr	Thr	Lys	Pro	Lys		
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Thr	Glu	Lys	Lys	Lys	Arg	Leu	Phe	Phe	Lys	Lys	Ala	Glu	Asn	Pro	Ser		
	690					695					700						
Pro	Ala	Tyr	Ala	Leu	Gly	Glu	Ile	Asp	Glu	Gly	Ala	Pro	Gly	Ala	Asp		
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Ile	Glu	Lys	Ala	Gly	Ile	Val	Asn	Gln	Gln	Lys	Leu	Glu	Lys	Lys	Phe		
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Met	His	Cys	His	Gly	Trp	Arg	Ser	Ile	Tyr	Cys	Ile	Pro	Lys	Arg	Pro		
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Ala	Phe	Lys	Gly	Ser	Ala	Pro	Leu	Asn	Leu	Ser	Asp	Arg	Leu	His	Gln		
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Val	Leu	Arg	Trp	Ala	Leu	Gly	Ser	Val	Glu	Ile	Phe	Phe	Ser	Lys	His		
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Cys	Pro	Leu	Trp	Tyr	Gly	Tyr	Gly	Gly	Gly	Leu	Lys	Phe	Leu	Glu	Arg		
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Val	Ala	Ile	Asp	Asp	Trp	Trp	Arg	Asn	Glu	Gln	Phe	Trp	Val	Ile	Gly		
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Ile	Pro	Pro	Thr	Thr	Leu	Leu	Leu	Leu	Asn	Phe	Ile	Gly	Val	Val	Ala		
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			1060					1065					1070				
Arg	Val	Asp	Pro	Phe	Leu	Ala	Lys	Ser	Asn	Gly	Pro	Leu	Leu	Glu	Glu		
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Arg Arg Gly Gly Gly Gln Val Cys Gln Ile Cys Gly Asp Gly Val Gly
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Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln
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95 100 105

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Arg Lys Trp Val Pro Phe Val Lys Lys Tyr Asn Ile Glu Pro Arg Ala	
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Pro Glu Trp Tyr Phe Ser Gln Lys Ile Asp Tyr Leu Lys Asp Lys Val	
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His Pro Ser Phe Val Lys Asp Arg Arg Ala Met Lys Arg Glu Tyr Glu	
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Glu Phe Lys Val Arg Val Asn Gly Leu Val Ala Lys Ala Gln Lys Val	
460 465 470 475	
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Pro Glu Glu Gly Trp Ile Met Gln Asp Gly Thr Pro Trp Pro Gly Asn	
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Asn Thr Xaa Asp His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser	
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ggt ggc ctt gat act gag ggc aat gag cta ccc cgt ttg gtc tat gtt	1889
Gly Gly Leu Asp Thr Glu Gly Asn Glu Leu Pro Arg Leu Val Tyr Val	
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Ser Arg Glu Lys Arg Pro Gly Phe Gln His His Lys Lys Ala Gly Ala	
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Met Leu Asn Leu Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Leu	
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Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg Asn Asp	
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Arg Tyr Ala Asn Arg Asn Thr Val Phe Phe Asp Ile Asn Leu Arg Gly	
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Ser	Lys	Lys	Gly 670	Ser	Asp	Lys	Lys	Lys	Ser 675	Gln	Lys	His	Val	Asp	Ser	
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Ser	Val	Pro	Val	Phe	Asn	Leu 690	Glu	Asp	Ile	Glu	Glu	Gly	Val	Glu	Gly	
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Ala	Gly	Phe	Asp	Asp	Glu	Lys 705	Ser	Leu	Leu	Met	Ser	Gln	Met	Ser	Leu	
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Glu	Lys	Arg	Phe	Gly 720	Gln	Ser	Ala	Ala	Phe	Val	Ala	Ser	Thr	Leu	Met	
gag	tat	ggg	ggg	gtt	cct	cag	tcc	gca	act	ccg	gag	tct	ctt	ctg	aaa	2561
Glu	Tyr	Gly	Gly 735	Val	Pro	Gln	Ser	Ala	Thr 740	Pro	Glu	Ser	Leu	Leu	Lys	
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Glu	Ala	Ile	His	Val	Ile	Ser	Cys 755	Gly	Tyr	Glu	Asp	Lys	Thr	Glu	Trp	
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Pro	Lys	Arg	Pro	Ala 800	Phe	Lys	Gly	Ser	Ala 805	Pro	Ile	Asn	Leu	Ser	Asp	
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Thr Gly Lys Phe Ile Ile Pro Glu Ile Ser Asn Phe Ala Ser Ile Trp	880	885	890	
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Phe Ile Ser Leu Phe Ile Ser Ile Phe Ala Thr Gly Ile Leu Glu Met	895	900	905	
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Arg Trp Ser Gly Val Gly Ile Asp Glu Trp Trp Arg Asn Glu Gln Phe	910	915	920	
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Trp Val Ile Gly Gly Ile Ser Ala His Leu Phe Ala Val Phe Gln Gly	925	930	935	
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Leu Leu Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser	940	945	950	955
aag gcc tcg gac gag gac ggc gac ttc gcg gag ctg tac atg ttc aag				3233
Lys Ala Ser Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Met Phe Lys	960	965	970	
tgg acg acg ctc ctg atc ccg ccc acc acc atc ctg atc atc aac ctg				3281
Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Ile Leu Ile Ile Asn Leu	975	980	985	
gtc ggc gtc gtc gcc ggc atc tcc tac gcc atc aac agc gga tac cag				3329
Val Gly Val Val Ala Gly Ile Ser Tyr Ala Ile Asn Ser Gly Tyr Gln	990	995	1000	
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Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile	1005	1010	1015	
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Val His Leu Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg	1020	1025	1030	1035
acc ccg acc atc gtc gtc gtc tgg gccatcctgc tggcgtccat cttctccttg				3479
Thr Pro Thr Ile Val Val Val Trp	1040			
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<212> PRT

<213> Zea mays

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Asp	Val	Phe	Ala	Ala	Cys	Asp	Val	Cys	Gly	Phe	Pro	Val	Cys	Arg	Pro	
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Cys	Tyr	Glu	Tyr	Glu	Arg	Lys	Asp	Gly	Thr	Gln	Ala	Cys	Pro	Gln	Cys	
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Lys	Thr	Lys	Tyr	Lys	Arg	His	Lys	Gly	Ser	Pro	Ala	Ile	Arg	Gly	Glu	
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Glu	Gly	Asp	Asp	Thr	Asp	Ala	Asp	Ser	Asp	Phe	Asn	Tyr	Leu	Ala	Ser	
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Gly	Asn	Glu	Asp	Gln	Lys	Gln	Lys	Ile	Ala	Asp	Arg	Met	Arg	Ser	Trp	
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Arg	Met	Asn	Val	Gly	Gly	Ser	Gly	Asp	Val	Gly	Arg	Pro	Lys	Tyr	Asp	
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Ser	Gly	Glu	Ile	Gly	Leu	Thr	Lys	Tyr	Asp	Ser	Gly	Glu	Ile	Pro	Arg	
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Gly	Tyr	Ile	Pro	Ser	Val	Thr	Asn	Ser	Gln	Ile	Ser	Gly	Glu	Ile	Pro	
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Gly	Ala	Ser	Pro	Asp	His	His	Met	Met	Ser	Pro	Thr	Gly	Asn	Ile	Gly	
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Lys	Arg	Ala	Pro	Phe	Pro	Tyr	Val	Asn	His	Ser	Pro	Asn	Pro	Ser	Arg	
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Glu	Phe	Ser	Gly	Ser	Ile	Gly	Asn	Val	Ala	Trp	Lys	Glu	Arg	Val	Asp	
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Gln	Pro	Leu	Ser	Arg	Lys	Val	Pro	Leu	Pro	Ser	Ser	Arg	Ile	Asn	Pro	
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His	Tyr	Arg	Ile	Thr	Asn	Pro	Val	Arg	Asn	Ala	Tyr	Pro	Leu	Trp	Leu	
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Gln	Phe	Pro	Lys	Trp	Phe	Pro	Ile	Asn	Arg	Glu	Thr	Tyr	Leu	Asp	Arg	
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Phe	Asp	Ala	Leu	Ala	Glu	Thr	Ser	Glu	Phe	Ala	Arg	Lys	Trp	Val	Pro	
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Phe	Val	Lys	Lys	Tyr	Asn	Ile	Glu	Pro	Arg	Ala	Pro	Glu	Trp	Tyr	Phe	
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Ser	Gln	Lys	Ile	Asp	Tyr	Leu	Lys	Asp	Lys	Val	His	Pro	Ser	Phe	Val	
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Lys	Asp	Arg	Arg	Ala	Met	Lys	Arg	Glu	Tyr	Glu	Glu	Phe	Lys	Val	Arg	
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Val	Asn	Gly	Leu	Val	Ala	Lys	Ala	Gln	Lys	Val	Pro	Glu	Glu	Gly	Trp	
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Ile	Met	Gln	Asp	Gly	Thr	Pro	Trp	Pro	Gly	Asn	Asn	Thr	Xaa	Asp	His	
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Pro	Gly	Met	Ile	Gln	Val	Phe	Leu	Gly	His	Ser	Gly	Gly	Leu	Asp	Thr	
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Glu	Gly	Asn	Glu	Leu	Pro	Arg	Leu	Val	Tyr	Val	Ser	Arg	Glu	Lys	Arg
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Pro	Gly	Phe	Gln	His	His	Lys	Lys	Ala	Gly	Ala	Met	Asn	Ala	Leu	Val
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Arg	Val	Ser	Ala	Val	Leu	Thr	Asn	Gly	Gln	Tyr	Met	Leu	Asn	Leu	Asp
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Cys	Asp	His	Tyr	Ile	Asn	Asn	Ser	Lys	Ala	Leu	Arg	Glu	Ala	Met	Cys
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Phe	Leu	Met	Asp	Pro	Asn	Leu	Gly	Arg	Ser	Val	Cys	Tyr	Val	Gln	Phe
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Pro	Gln	Arg	Phe	Asp	Gly	Ile	Asp	Arg	Asn	Asp	Arg	Tyr	Ala	Asn	Arg
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Gly	Pro	Val	Tyr	Val	Gly	Thr	Gly	Cys	Val	Phe	Asn	Arg	Thr	Ala	Leu
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Tyr	Gly	Tyr	Glu	Pro	Pro	Ile	Lys	Gln	Lys	Lys	Gly	Gly	Phe	Leu	Ser
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Ser	Leu	Cys	Gly	Gly	Arg	Lys	Lys	Ala	Ser	Lys	Ser	Lys	Lys	Gly	Ser
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Glu	Lys	Ser	Leu	Leu	Met	Ser	Gln	Met	Ser	Leu	Glu	Lys	Arg	Phe	Gly
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Gln	Ser	Ala	Ala	Phe	Val	Ala	Ser	Thr	Leu	Met	Glu	Tyr	Gly	Gly	Val
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Pro	Gln	Ser	Ala	Thr	Pro	Glu	Ser	Leu	Leu	Lys	Glu	Ala	Ile	His	Val
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Ile	Ser	Cys	Gly	Tyr	Glu	Asp	Lys	Thr	Glu	Trp	Gly	Thr	Glu	Ile	Gly
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Trp	Ile	Tyr	Gly	Ser	Val	Thr	Glu	Asp	Ile	Leu	Thr	Gly	Phe	Lys	Met
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His	Ala	Arg	Gly	Trp	Arg	Ser	Ile	Tyr	Cys	Met	Pro	Lys	Arg	Pro	Ala
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Phe	Lys	Gly	Ser	Ala	Pro	Ile	Asn	Leu	Ser	Asp	Arg	Leu	Asn	Gln	Val
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Pro	Leu	Trp	Tyr	Gly	Tyr	Gly	Gly	Arg	Leu	Lys	Phe	Leu	Glu	Arg	Phe
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Ala	Tyr	Ile	Asn	Thr	Thr	Ile	Tyr	Pro	Leu	Thr	Ser	Ile	Pro	Leu	Leu
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Ile	Tyr	Cys	Ile	Leu	Pro	Ala	Ile	Cys	Leu	Leu	Thr	Gly	Lys	Phe	Ile
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Ile	Pro	Glu	Ile	Ser	Asn	Phe	Ala	Ser	Ile	Trp	Phe	Ile	Ser	Leu	Phe
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 Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn
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 Glu Phe Val Met Ile Arg His Asp Gly Asp Val Pro Gly Ser Ala Lys
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ccc aca aag agt gcg aat gga cag gtc tgc cag att tgc ggt gac tct 324
 Pro Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Ser
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gtg ggt gtt tca gcc act ggt gat gtc ttt gtt gcc tgc aat gag tgt 372
 Val Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys
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gcc ttc cct gtc tgc cgc cca tgc tat gag tat gag cgc aag gag ggg 420
 Ala Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly
 65 70 75

aac caa tgc tgc ccc cag tgc aag act aga tac aag aga cag aaa ggt 468
 Asn Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly
 80 85 90 95

agc cct cga gtt cat ggt gat gag gat gag gaa gat gtt gat gac cta	516
Ser Pro Arg Val His Gly Asp Glu Asp Glu Glu Asp Val Asp Asp Leu	
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gac aat gaa ttc aac tac aag caa ggc agt ggg aaa ggc cca gag tgg	564
Asp Asn Glu Phe Asn Tyr Lys Gln Gly Ser Gly Lys Gly Pro Glu Trp	
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Gln Leu Gln Gly Asp Asp Ala Asp Leu Ser Ser Ser Ala Arg His Glu	
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Glu Ile Pro Asp Ala Ser Pro Asp Arg His Ser Ile Arg Ser Pro Thr	
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Ser Ser Tyr Val Asp Pro Ser Val Pro Val Pro Val Arg Ile Val Asp	
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Val Ile Cys Glu Val Trp Phe Ala Leu Ser Trp Leu Leu Asp Gln Phe	
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Val	Ser	Cys	Tyr	Val	Ser	Asp	Asp	Gly	Ser	Ala	Met	Leu	Thr	Phe	Glu	
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Ser	Leu	Ser	Glu	Thr	Ala	Glu	Phe	Ala	Arg	Lys	Trp	Val	Pro	Phe	Cys	
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Lys	Lys	His	Asn	Ile	Glu	Pro	Arg	Ala	Pro	Glu	Phe	Tyr	Phe	Ala	Gln	
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aaa	ata	gat	tac	ctg	aag	gac	aaa	att	caa	cct	tca	ttt	gtt	aag	gaa	1524
Lys	Ile	Asp	Tyr	Leu	Lys	Asp	Lys	Ile	Gln	Pro	Ser	Phe	Val	Lys	Glu	
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Asn	Met	Glu	Asp	Ile	Glu	Glu	Gly	Ile	Glu	Gly	Tyr	Glu	Asp	Glu	Arg	
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Ser	Thr	Asn	Pro	Ala	Ser	Leu	Leu	Lys	Glu	Ala	Ile	His	Val	Ile	Ser	
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Cys	Gly	Tyr	Glu	Asp	Lys	Thr	Glu	Trp	Gly	Lys	Glu	Ile	Gly	Trp	Ile	
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Cys Val Leu Pro Ala Ile Cys Leu Leu Thr Asn Lys Phe Ile Ile Pro	
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Asp Phe Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser Leu Leu Ile Pro	
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Trp Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys Ile	
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Asp Pro Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala Leu Gly Gln Cys	
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Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn
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Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly Ser
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Pro Arg Val His Gly Asp Glu Asp Glu Glu Asp Val Asp Asp Leu Asp
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Ile Pro Asp Ala Ser Pro Asp Arg His Ser Ile Arg Ser Pro Thr Ser
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Asp Trp Trp Arg Asn Glu	Gln Phe Trp Val Ile	Gly Gly Thr Ser Ala			
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His Leu Phe Ala Val Phe	Gln Gly Leu Leu Lys	Val Leu Ala Gly Ile			
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Asp Thr Asn Phe Thr Val	Thr Ser Lys Ala Ser	Asp Glu Asp Gly Asp			
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Phe Ala Glu Leu Tyr Val	Phe Lys Trp Thr Ser	Leu Leu Ile Pro Pro			
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Ser Ile Leu Leu Ala Ser	Ile Phe Ser Leu Leu	Trp Val Lys Ile Asp			
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His Ser Gln Ile Ser Gly Glu Ile Pro Gly Ala Ser Pro Asp His Met	
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Val Asn His Ser Pro Asn Pro Ser Arg Glu Phe Ser Gly Ser Leu Gly	
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Asn Val Ala Trp Lys Glu Arg Val Asp Gly Trp Lys Met Lys Asp Lys	
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Gly Ala Ile Pro Met Thr Asn Gly Thr Ser Ile Ala Pro Ser Glu Gly	
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Arg Gly Val Ala Asp Ile Asp Ala Ser Thr Asp Tyr Asn Met Glu Asp	
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WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a polynucleotide encoding a functional cellulose synthase polypeptide, wherein the polypeptide comprises a cellulose synthase substrate binding and/or catalysis motif, and having at least 90% sequence identity, as determined by the BLAST 2.0 algorithm under default parameters wherein wordlength is 11, expectation value is 10, cutoff is 100, reward score for a pair of matching residues is 5 and penalty score for mismatching residues is -4, to the full length nucleotide sequence selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 25, and 57.
2. An isolated nucleic acid molecule encoding a functional cellulose synthase comprising a polynucleotide encoding a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 26, or 58.
3. An isolated nucleic acid molecule comprising a polynucleotide encoding a functional cellulose synthase polypeptide, wherein the polypeptide comprises a cellulose synthase substrate binding and/or catalysis motif, and having at least 60 contiguous nucleotides of any of SEQ ID NOS: 1, 5, 9, 13, 17, 25, and 57, and which polynucleotide selectively hybridizes, under stringent hybridization conditions and a wash in 2X SSC at 50°C, along the full length to the complement of a polynucleotide comprising SEQ ID NOS: 1, 5, 9, 13, 17, 25, or 57.
4. An isolated nucleic acid molecule encoding a functional cellulose synthase comprising a polynucleotide of SEQ ID NOS: 1, 5, 9, 13, 17, 25, or 57 .
5. An isolated nucleic acid molecule comprising a polynucleotide which is complementary to the nucleic acid of claim 1, 2, 3, or 4.
6. An isolated nucleic acid probe for detecting a nucleic acid molecule encoding a cellulose synthase, comprising at least 60 contiguous nucleotides of a nucleic acid molecule of claim 1, 2, 3, 4, or 5.

7. An isolated nucleic acid primer for amplifying a nucleic acid molecule encoding a cellulose synthase, comprising at least 60 contiguous nucleotides of a nucleic acid molecule of claim 1, 2, 3, 4, or 5.
8. A recombinant expression cassette, comprising a nucleic acid molecule of claim 1, 2, 3, or 4 operably linked, in sense or anti-sense orientation, to a promoter.
9. A host cell transformed with the recombinant expression cassette of claim 8.
10. The host cell of claim 9, wherein said host cell is from a monocot.
11. The host cell of claim 9, wherein said host cell is selected from the group consisting of: maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, and millet.
12. A method of modulating the level of cellulose synthase in a plant cell capable of plant regeneration, comprising:
 - (a) transforming the plant cell with a recombinant expression cassette comprising a cellulose synthase polynucleotide of claim 1, 2, 3, or 4, operably linked to a promoter,
 - (b) culturing the transformed plant cell; and
 - (c) inducing expression of said polynucleotide for a time sufficient to modulate the level of cellulose synthase in said transformed plant cell.
13. The method of claim 12, wherein a plant is regenerated from the transformed plant cell.
14. The method of claim 13, wherein the plant is selected from the group consisting of: maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley and millet.
15. The method of claim 12, wherein the promoter is a tissue-preferred promoter.

16. The method of claim 12, wherein the level of cellulose synthase is increased.
17. The method of claim 12 wherein the cellulose synthase polynucleotide is amplified from a *Zea mays* nucleic acid library using primers which selectively hybridize, under stringent hybridization conditions, to loci within a polynucleotide selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 25, and 57.
18. The method of claim 12 wherein the cellulose synthase polynucleotide is selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 25, and 57.
19. An isolated protein for use as an immunogen for eliciting the production of antibodies to a cellulose synthase protein, wherein said isolated protein comprises a polypeptide of at least 60 contiguous amino acids from a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 26, or 58.
20. An isolated cellulose synthase protein comprising a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 26, or 58.
21. An isolated protein comprising a functional cellulose synthase polypeptide comprising a cellulose synthase substrate binding and/or catalysis motif, and having at least one linear epitope in common with, and having at least 90% sequence identity to, the full length amino acid sequence of SEQ ID NO: 2, 6, 10, 14, 18, 26, or 58, wherein said sequence identity is determined using BLAST 2.0 under default parameters wherein wordlength is 3, expectation value is 10, and matrix is BLOSUM62.
22. An isolated cellulose synthase protein comprising a polypeptide encoded by the nucleic acid of claim 1, 2, 3, or 4.
23. Use of the polynucleotide of any of claims 1 to 5 for modulating the expression of cellulose synthase in a plant.
24. The use of claim 23 wherein the level of cellulose synthase is increased in the plant.