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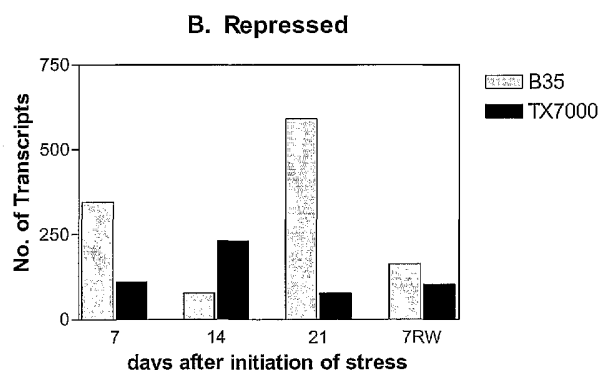
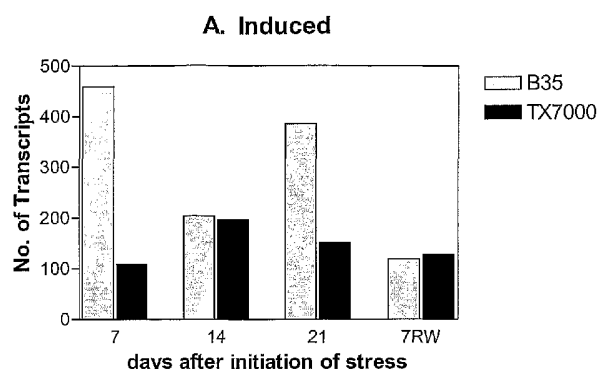
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(54) Title: SORGHUM GENE EXPRESSION PROFILING



(57) Abstract: The present subject matter provides a method for enhancing the drought resistance of a plant. In some embodiments, polynucleotides from *Sorghum bicolor* that hybridize to polynucleotides isolated from rice (*Oryzasativa*) and encode polypeptides for abiotic stress tolerance are also described.

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Description

SORGHUM GENE EXPRESSION PROFILING

Cross Reference To Related Applications

5 This application is based on and claims priority to United States Provisional Application Serial Number 60/548,891, filed March 1, 2004, the entire contents of which is herein incorporated by reference in its entirety.

Technical Field

10 The presently disclosed subject matter pertains to nucleic acid molecules from sorghum [*Sorghum bicolor* (L.) Moench] comprising nucleotide sequences that encode polypeptides for drought resistance. The presently disclosed subject matter also relates to methods of using nucleic acid molecules and/or polypeptides from *Sorghum spp.* in transgenic plants
15 to confer the desired agronomic traits, and to use such nucleic acids to assist germplasm enhancement by breeding.

Table of Abbreviations

	7RW	-	7 days after resuming watering
20	ABA	-	abscisic acid
	ADP	-	adenosine diphosphate
	AMV	-	Alfalfa Mosaic Virus
	ATCC	-	America Type Culture Collection
	ATP	-	adenosine triphosphate
25	BiP	-	binding polypeptide
	bp	-	basepair(s)
	CAB	-	chlorophyll a/b binding
	CaMV	-	cauliflower mosaic virus
	cDNA	-	complementary DNA
30	CDPK	-	calcium-dependent protein kinase
	cM	-	centimorgan(s)
	CMV	-	cytomegalovirus
	CoA	-	coenzyme A

	ColE1	-	a synthetic <i>E. coli</i> origin of replication
	cRNAs	-	complementary RNAs
	DEAE	-	di-ethyl-amino-ethyl
	DH	-	double haploid
5	DHFR	-	dihydrofolate reductase
	dsRNA	-	double-stranded RNA
	EDTA	-	ethylene diamine tetraacetic acid
	ELISA	-	enzyme-linked immunosorbent assay
	EMCV	-	encephalomyocarditis virus
10	EPSP	-	5-enol-pyruvyl shikimate-3-phosphate
	ER	-	endoplasmic reticulum
	EST	-	expressed sequence tag
	FAD	-	flavin adenine dinucleotide
	FMN	-	flavin mononucleotide
15	GS2	-	glutamine synthase 2
	GSH	-	glutathione
	GTP	-	guanosine triphosphate
	GUS	-	β -glucuronidase
	HOADH	-	high osmotic adjusting double haploid
20	HSPs	-	high scoring sequence pairs
	HSV	-	herpes simplex virus
	kb	-	kilobase(s)
	k_{cat}	-	catalytic constant
	K_m	-	Michaelis Constant
25	LEA	-	late embryogenesis abundant
	LOADH	-	low osmotic adjusting double haploid
	MatDB	-	MIPS <i>Arabidopsis thaliana</i> Database
	Mb	-	megabase(s)
	MCMV	-	Maize Chlorotic Mottle Virus
30	MDMV	-	Maize Dwarf Mosaic Virus
	MDS	-	moderate dehydration stress
	MIPS	-	Munich Information Center for Protein Sequences

	MPa	-	megapascal
	MTL	-	metallothionein-like
	NAD(P)	-	nicotinamide adenine dinucleotide (phosphate)
5	NaDS	-	sodium dodecyl sulfate
	OA	-	osmotic adjustment
	ORF	-	open reading frame
	PCR	-	polymerase chain reaction
	PEG	-	polyethylene glycol
10	PEPC	-	phosphoenol carboxylase
	pgk	-	phosphoglycerate kinase
	PMI	-	phosphomannose isomerase
	Protox	-	protoporphyrinogen oxidase
	PTGS	-	post-transcriptional gene silencing
15	QTL	-	quantitative trait locus/loci
	RFLP	-	restriction fragment length polymorphism
	RGP	-	Rice Genome Project
	RI	-	recombinant inbred
	RIL(s)	-	recombinant inbred line(s)
20	RNAi	-	RNA interference
	RT-PCR	-	reverse transcription polymerase chain reaction
	RUBISCO	-	ribulose-1,5-bisphosphate carboxylase/oxygenase
25	RWC	-	relative water content
	SDS	-	severe dehydration stress
	SELEX	-	Systematic Evolution of Ligands by Exponential Enrichment
	Sf9	-	<i>Spodoptera frugiperda</i> cell line
30	SSC	-	standard saline citrate
	STG	-	stay-green
	TBE	-	Tris-borate-EDTA
	tk	-	thymidine kinase

TEV	-	Tobacco Etch Virus
TMTD	-	tetramethylthiuram disulfide
TMV	-	Tobacco Mosaic Virus
WW	-	well watered

5

Amino Acid Abbreviations, Codes, and Functionally Equivalent Codons

	<u>Amino Acid</u>	<u>3-Letter</u>	<u>1-Letter</u>	<u>Codons</u>
	Alanine	Ala	A	GCA; GCC; GCG; GCU
	Arginine	Arg	R	AGA; AGG; CGA; CGC; CGG; CGU
10	Asparagine	Asn	N	AAC; AAU
	Aspartic; Acid	Asp	D	GAC; GAU
	Cysteine	Cys	C	UGC; UGU
	Glutamic; acid	Glu	E	GAA; GAG
	Glutamine	Gln	Q	CAA; CAG
15	Glycine	Gly	G	GGA; GGC; GGG; GGU
	Histidine	His	H	CAC; CAU
	Isoleucine	Ile	I	AUA; AUC; AUU
	Leucine	Leu	L	UUA; UUG; CUA; CUC; CUG; CUU
	Lysine	Lys	K	AAA; AAG
20	Methionine;	Met	M	AUG
	Phenylalanine	Phe	F	UUC; UUU
	Proline	Pro	P	CCA; CCC; CCG; CCU
	Serine	Ser	S	ACG; AGU; UCA; UCC; UCG; UCU
	Threonine	Thr	T	ACA; ACC; ACG; ACU
25	Tryptophan	Trp	W	UGG
	Tyrosine	Tyr	Y	UAC; UAU
	Valine	Val	V	GUA; GUC; GUG; GUU

Background

30 Improvement of the agronomic characteristics of crop plants has been ongoing since the beginning of agriculture. Most of the land suitable for crop production is currently being used. As human populations continue to increase, improved crop varieties will be required to adequately provide food

and feed (Trewavas, 2001). To avoid catastrophic famines and malnutrition, future crop cultivars will need to have improved yields with equivalent farm inputs. These cultivars will need to more effectively withstand adverse conditions such as drought, soil salinity, or disease, which will be especially
5 important as marginal lands are brought into cultivation. Finally, cultivars are needed with altered nutrient composition to enhance human and animal nutrition. To enable more efficient food and feed processing, cultivars can be designed for specific end-uses. For all these traits, identification of the genes controlling phenotypic expression of traits of interest will play a role in
10 accelerating development of superior crop germplasm by conventional or transgenic approaches.

A number of approaches are available to assist identification of genes playing key roles in expression of agronomically important traits. These include genetics, genomics, bioinformatics, and functional genomics.
15 Genetics is the scientific study of the mechanisms of inheritance. By identifying mutations that alter a pathway or response of interest, classical (or forward) genetics can help to identify the genes involved in these pathways or responses. Genetics is also the central component in improvement of germplasm by breeding. Through molecular and phenotypic
20 analysis of genetic crosses, loci controlling traits of interest can be mapped and followed in subsequent generations. Knowledge of the genes underlying phenotypic variation between crop accessions can enable the development of markers that greatly increase efficiency of the germplasm improvement process as well as open avenues for discovery of additional
25 superior alleles.

Genomics is the system-level study of an organism's genome, including genes and the corresponding gene products: RNA and polypeptides. At a first level, genomic approaches have provided large datasets of sequence information from diverse plant species, including full-
30 length and partial cDNA sequences and the complete genomic sequence of a model plant species, *Arabidopsis thaliana*. Recently, the first draft sequence of a crop plant's genome, that of rice (*Oryza sativa*), has also become available (see Goff *et al.*, 2002). The availability of whole genome

sequences makes possible the development of tools for system-level study of other molecular complements, such as arrays and chips for use in determining the complement of expressed genes in an organism under specific conditions. Such data can be used as a first indication of the potential for certain genes to play key roles in expression of different plant phenotypes. Bioinformatics approaches interface directly with first-level genomic datasets in allowing for the uncovering of sequences of interest by annotative or other approaches. Using, for example, similarity searches, alignments, and phylogenetic analyses, bioinformatics can often identify homologs of a gene product of interest. Very similar homologs (for example, greater than about 90% amino acid identity over the entire length of a polypeptide) are likely to be orthologs; that is, they are likely to share the same function in different organisms.

Functional genomics can be defined as the assignment of functions to genes and their products. Functional genomics draws from genetics, genomics, and bioinformatics to derive a path toward identifying genes important in a particular pathway or response of interest. Expression analysis, for example, uses high density DNA microarrays (often derived from genome-scale organismal sequencing) to monitor the mRNA expression of thousands of genes in a single experiment. Experimental treatments can include those eliciting a response of interest, such as the drought resistance response in plants subjected to low water conditions. To give additional examples of the use of microarrays, mRNA expression levels can be monitored in distinct tissues over a developmental time course, or in mutants affected in a response of interest.

Proteomics can also help to assign function by assaying the expression and post-translational modifications of hundreds of polypeptides in a single experiment. Proteomics approaches are in many cases analogous to the approaches taken for monitoring mRNA expression in microarray experiments. Polypeptide-polypeptide interactions can also help to assign polypeptides to a given pathway or response by identifying polypeptides which interact with known components of the pathway or response. For functional genomics, polypeptide-polypeptide interactions are

often studied using large-scale yeast two-hybrid assays. Another approach to assigning gene function is to express the corresponding polypeptide in a heterologous host, for example the bacterium *Escherichia coli*, followed by purification and enzymatic assays of the purified polypeptide.

5 Ultimately, demonstration of the ability of a gene-of-interest to control a given trait is derived from experimental testing in plant species of interest. The generation and analysis of plants that are transgenic for a gene of interest can be used for plant functional genomics, with several advantages. The gene can alternatively be either overexpressed (via transgenesis) or
10 underexpressed ("knocked out"), thereby increasing the chances of observing a phenotype linking the gene to a pathway or response of interest.

 Two aspects of transgenic functional genomics help lend a high level of confidence to functional assignments derived from this approach. First, phenotypic observations are carried out in the context of the living plant.
15 Second, the range of phenotypes observed can be checked and correlated with the observed expression levels of the introduced transgene. Transgenic functional genomics is especially valuable in improved cultivar development. Only genes that function in a pathway or response of interest and that in addition are able to confer a desired trait-based phenotype are
20 promoted as candidate genes for crop improvement efforts. In some cases, transgenic lines developed for functional genomics studies can be directly utilized in the initial stages of product development.

 Another approach towards plant functional genomics involves first identifying plant lines with mutations in specific genes of interest, followed by
25 phenotypic evaluation of the consequences of such gene knockouts on the trait under study. Such an approach reveals genes essential for the expression of specific traits.

 Genes identified through functional genomics can be directly employed in efforts towards germplasm improvement by transgenic
30 approaches as disclosed above, or used to develop markers for identification and tracking of alleles-of-interest in mapping and breeding populations. Knowledge of such genes can also enable the construction by any of a

number of molecular methods of superior alleles that are non-existent in nature.

Therefore, the identification of genes that can be used for crop and germplasm improvement is needed. This and other needs in the art are addressed by the present disclosure.

Summary

This Summary lists several embodiments of the presently disclosed subject matter, and in many cases lists variations and permutations of these embodiments. This Summary is merely exemplary of the numerous and varied embodiments. Mention of one or more representative features of a given embodiment is likewise exemplary. Such an embodiment can typically exist with or without the feature(s) mentioned; likewise, those features can be applied to other embodiments of the presently disclosed subject matter, whether listed in this Summary or not. To avoid excessive repetition, this Summary does not list or suggest all possible combinations of such features.

The presently disclosed subject matter provides nucleic acids and polypeptides from genus *Sorghum*. In some embodiments, the presently disclosed subject matter provides a polypeptide selected from the group consisting of (a) a polypeptide comprising an amino acid sequence encoded by a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl, to a nucleic acid comprising a nucleotide sequence of one of odd numbered SEQ ID NOs: 1-105; and (b) a functional fragment of the polypeptide of (a). In some embodiments, the polypeptide is involved in abiotic stress tolerance. In some embodiments, the abiotic stress tolerance is drought resistance.

The presently disclosed subject matter also provides an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of (a) a nucleotide sequence encoding a polypeptide as disclosed above, or a fragment, domain, or feature thereof; (b) a sequence fully complementary to (a); and (c) a nucleotide sequence which is the full reverse complement of (a).

5 The presently disclosed subject matter also provides an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of (a) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing
step at 50°C in 0.1 M NaCl, to a nucleic acid comprising a nucleotide
sequence of one of odd numbered SEQ ID NOs: 1-105; (b) a nucleotide
sequence fully complementary to (a); and (c) a nucleotide sequence which is
the full reverse complement of (a).

10 The presently disclosed subject matter also provides an expression cassette comprising a promoter operatively linked to one or more of the disclosed nucleic acid molecules. In some embodiments, the presently disclosed subject matter provides a recombinant vector comprising the disclosed expression cassette. In some embodiments, the presently disclosed subject matter also provides a cell comprising an expression
15 cassette.

In some embodiments, the presently disclosed subject matter provides a transgenic plant comprising the disclosed expression cassette. In some embodiments, the expression cassette is expressed in a tissue selected from the group consisting of the epidermis, root, vascular tissue,
20 meristem, cambium, cortex, pith, leaf, flower, and combinations thereof.

In some embodiments, the transgenic plant is a monocot. In some embodiments, the monocot is selected from the group consisting of rice, maize, wheat, barley, oats, rye, millet, sorghum, triticale, secale, einkorn, spelt, emmer, teff, milo, flax, gamma grass, Tripsacum, and teosinte. In
25 some embodiments, the transgenic plant is selected from the group consisting of rice, wheat, barley, rye, corn, potato, canola, soybean, sunflower, carrot, sweet potato, sugarbeet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, squash, pumpkin, cucumber, apple, pear,
30 quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane. In some embodiments, the transgenic plant is sorghum.

In some embodiments of the disclosed transgenic plant, the plant has altered abiotic stress tolerance. In some embodiments, the altered abiotic stress tolerance is enhanced drought resistance.

5 The presently disclosed subject matter also provides a transgenic plant comprising a disclosed nucleic acid molecule.

Additionally, the presently disclosed subject matter provides progeny and seed from the disclosed transgenic plants.

10 The presently disclosed subject matter also provides a method for altering an abiotic stress tolerance of a plant, the method comprising expressing in the plant an expression cassette comprising a nucleic acid molecule encoding a disclosed polypeptide.

15 The presently disclosed subject matter also provides a shuffled nucleic acid comprising a plurality of nucleotide sequence fragments, wherein at least one of the fragments corresponds to a region of a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl, to a nucleic acid comprising a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, and wherein at least two of the plurality of sequence fragments are in an order, from 5' to 3', which is not an order in
20 which the plurality of fragments naturally occur in a nucleic acid.

The presently disclosed subject matter also provides a method for producing a polypeptide encoded by a disclosed nucleic acid molecule, comprising the steps of: (a) growing cells comprising an expression cassette under suitable growth conditions, the expression cassette comprising a
25 nucleic acid molecule encoding a disclosed polypeptide; and (b) isolating the polypeptide from the cells.

The presently disclosed subject matter also provides a method for decreasing the expression of a disclosed nucleic acid molecule in a plant, the method selected from the group consisting of: (a) expressing in said
30 plant a nucleic acid molecule encoding a disclosed polypeptide or a portion thereof in "sense" orientation; (b) expressing in said plant a nucleic acid encoding a disclosed polypeptide molecule or a portion thereof in "antisense" orientation; (c) expressing in said plant a ribozyme capable of

specifically cleaving a messenger RNA transcript encoded by an endogenous gene corresponding to a disclosed nucleic acid molecule; (d) expressing in a plant an aptamer specifically directed to a polypeptide encoded by a disclosed isolated nucleic acid molecule; (e) expressing in a
5 plant a mutated or a truncated form of a disclosed isolated nucleic acid molecule; (f) modifying by homologous recombination in a plant at least one chromosomal copy of the gene corresponding to a disclosed isolated nucleic acid molecule; (g) modifying by homologous recombination in a plant at least one chromosomal copy of the regulatory elements of a gene corresponding
10 to a disclosed isolated nucleic acid molecule; and (h) expressing in said plant a disclosed isolated nucleic acid molecule or a portion thereof in the "sense" and "antisense" orientation.

The presently disclosed subject matter also provides a method for increasing the expression of a disclosed isolated nucleic acid molecule in a
15 plant, the method comprising: (a) inserting into the plant an expression cassette comprising a disclosed isolated nucleic acid molecule; and (b) growing the plant comprising the expression cassette under suitable growth conditions, wherein the expression of the disclosed isolated nucleic acid molecule is increased.

The presently disclosed subject matter also provides a method for enhancing the drought resistance of a plant, the method comprising: (a) inserting into the plant an expression cassette comprising at least one disclosed isolated nucleic acid molecule; and (b) growing the plant comprising the expression cassette under suitable growth conditions,
20 wherein the drought resistance of the plant is enhanced.

The presently disclosed subject matter also provides an antibody that specifically binds to a disclosed polypeptide.

The presently disclosed subject matter also provides a method for altering the expression of a disclosed polypeptide in a plant, the method comprising expressing an expression cassette encoding a disclosed
30 polypeptide in the plant. In some embodiments, the polypeptide is expressed in a predetermined location or tissue of a plant. In some embodiments, the location or tissue is selected from the group consisting of

epidermis, root, vascular tissue, meristem, cambium, cortex, pith, leaf, flower, seed, and combinations thereof.

5 The presently disclosed subject matter also provides an isolated product from a plant comprising an expression cassette comprising a promoter sequence operatively linked to a nucleic acid, the nucleic acid comprising a nucleotide sequence selected from the group consisting of (a) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl, to a nucleic acid comprising a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof; 10 (b) a nucleotide sequence fully complementary to (a); and (c) a nucleotide sequence that is the full reverse complement of (a).

The presently disclosed subject matter also provides a method for producing a recombinant polypeptide, the method comprising (a) growing 15 recombinant cells comprising a nucleic acid construct under suitable growth conditions, the construct comprising an expression vector and a nucleic acid comprising a nucleotide sequence selected from the group consisting of: (i) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl, to 20 a nucleic acid comprising a nucleotide sequence listed in odd numbered nucleotide sequences of SEQ ID NOs: 1-105; and (ii) a subsequence of (i); and (b) isolating from the recombinant cells the recombinant polypeptide expressed therein. In some embodiments, the expression vector comprises one or more elements selected from the group consisting of a promoter- 25 enhancer sequence, a selection marker sequence, an origin of replication, an epitope tag-encoding sequence, an affinity purification tag-encoding sequence, a polyamino acid binding substance, and chitin-binding domain, and combinations thereof.

30 Brief Description of the Drawings

Figures 1A and 1B depict a comparison of the transcriptional response of the parental plant lines to dehydration stress over time. Figure 1A depicts the number of transcripts induced in the parental lines at various

time points, and Figure 1B depicts the number of transcripts repressed at these same time points.

Figures 2A-2E depict graphical representations of the expression patterns presented in Table 6. In each of Figures 2A-2E, the first four points on the x-axis correspond to 7, 14, 21, and 7RW treatments for Tx7000, and points 5 to 8 corresponds to 7, 14, 21, and 7RW treatments for B35 respectively. The Y-axis corresponds to the level of expression.

Figure 2A depicts a graphical representation of an expression pattern that is seen in a transient and early response in B35. Figure 2B depicts a graphical representation of an expression pattern that is seen in a sustained response to dehydration. Figure 2C depicts a graphical representation of an expression pattern that is seen in a transient and early response in Tx7000. Figure 2D depicts a graphical representation of an expression pattern that is seen in a sustained response to dehydration. Figure 2E depicts a graphical representation of a bi-modal expression pattern that is seen in certain genes that are induced in B35 at 7 and 21 days after drought.

Brief Description of the Sequence Listing

Odd numbered SEQ ID NOs: 1-105 are nucleotide sequences from *Oryza sativa* that have been identified using the methods and compositions disclosed herein.

Even numbered SEQ ID NOs: 2-106 are polypeptide sequences encoded by the immediately preceding nucleotide sequence. For example, SEQ ID NO: 2 is the polypeptide encoded by the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 4 is the polypeptide encoded by the nucleotide sequence of SEQ ID NO: 3, etc.

SEQ ID NO: 107 is the amino acid sequence of the V5 epitope from paramyxovirus.

SEQ ID NO: 108 is the amino acid sequence of an epitope tag.

Detailed Description

5 The presently disclosed subject matter will be now be described more fully hereinafter with reference to the accompanying Examples, in which representative embodiments of the presently disclosed subject matter are shown. The presently disclosed subject matter can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the presently disclosed subject matter to those skilled in the art.

10 All publications, patent applications, patents, and other references cited herein are incorporated by reference in their entirety.

I. General Considerations

15 A goal of functional genomics is to identify genes controlling expression of organismal phenotypes, and functional genomics employs a variety of methodologies including, but not limited to, bioinformatics, gene expression studies, gene and gene product interactions, genetics, biochemistry, and molecular genetics. For example, bioinformatics can assign function to a given gene by identifying genes in heterologous organisms with a high degree of similarity (homology) at the amino acid or nucleotide level. Studies of the expression of a gene at the mRNA or polypeptide levels can assign function by linking expression of the gene to an environmental response, a developmental process, or a genetic (mutational) or molecular genetic (gene overexpression or underexpression) perturbation. Expression of a gene at the mRNA level can be ascertained either alone (for example, by Northern analysis) or in concert with other genes (for example, by microarray analysis), whereas expression of a gene at the polypeptide level can be ascertained either alone (for example, by native or denatured polypeptide gel or immunoblot analysis) or in concert with other genes (for example, by proteomic analysis). Knowledge of polypeptide/polypeptide and polypeptide/DNA interactions can assign function by identifying polypeptides and nucleic acid sequences acting together in the same biological process. Genetics can assign function to a

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gene by demonstrating that DNA lesions (mutations) in the gene have a quantifiable effect on the organism, including, but not limited to, its development; hormone biosynthesis and response; growth and growth habit (plant architecture); mRNA expression profiles; polypeptide expression profiles; ability to resist diseases; tolerance of abiotic stresses (for example, drought conditions); ability to acquire nutrients; photosynthetic efficiency; altered primary and secondary metabolism; and the composition of various plant organs. Biochemistry can assign function by demonstrating that the polypeptide(s) encoded by the gene, typically when expressed in a heterologous organism, possesses a certain enzymatic activity, either alone or in combination with other polypeptides. Molecular genetics can assign function by overexpressing or underexpressing the gene in the native plant or in heterologous organisms, and observing quantifiable effects as disclosed in functional assignment by genetics above. In functional genomics, any or all of these approaches are utilized, often in concert, to assign functions to genes across any of a number of organismal phenotypes.

It is recognized by those skilled in the art that these different methodologies can each provide data as evidence for the function of a particular gene, and that such evidence is stronger with increasing amounts of data used for functional assignment: in some embodiments from a single methodology, in some embodiments from two methodologies, and in some embodiments from more than two methodologies. In addition, those skilled in the art are aware that different methodologies can differ in the strength of the evidence provided for the assignment of gene function. Typically, but not always, a datum of biochemical, genetic, or molecular genetic evidence is considered stronger than a datum of bioinformatic or gene expression evidence. Finally, those skilled in the art recognize that, for different genes, a single datum from a single methodology can differ in terms of the strength of the evidence provided by each distinct datum for the assignment of the function of these different genes.

The objective of crop trait functional genomics is to identify crop trait genes of interest, for example, genes capable of conferring useful agronomic

traits in crop plants. Such agronomic traits include, but are not limited to, enhanced yield, whether in quantity or quality; enhanced nutrient acquisition and metabolic efficiency; enhanced or altered nutrient composition of plant tissues used for food, feed, fiber, or processing; enhanced utility for
5 agricultural or industrial processing; enhanced resistance to plant diseases; enhanced tolerance of adverse environmental conditions (abiotic stresses) including, but not limited to, drought, excessive cold, excessive heat, or excessive soil salinity or extreme acidity or alkalinity; and alterations in plant architecture or development, including changes in developmental timing.
10 The deployment of such identified trait genes by either transgenic or non-transgenic approaches can materially improve crop plants for the benefit of agriculture.

Cereals are the most important crop plants on the planet in terms of both human and animal consumption. Genomic synteny (conservation of
15 gene order within large chromosomal segments) is observed in rice, maize, wheat, barley, rye, oats, and other agriculturally important monocots including sorghum (see e.g., Kellogg, 1998; Song *et al.*, 2001, and references therein), which facilitates the mapping and isolation of orthologous genes from diverse cereal species based on the sequence of a
20 single cereal gene. Rice has the smallest (about 420 Mb) genome among the cereal grains, and has recently been a major focus of public and private genomic and EST sequencing efforts. See Goff *et al.*, 2002.

The mechanisms of plant adaptations to drought include both avoidance and tolerance. Drought avoidance mechanisms are generally
25 constitutive phenotypic properties such as epicuticular wax structure and root thickness or root depth (Svenningson, 1988, Price *et al.*, 2002), which are expressed in the presence or absence of stress conditions. On the other hand, drought tolerance is an adaptive response that enables plants to maintain metabolism even at low water potential (Ingram & Bartels, 1996).
30 These plants respond to water stress through wide spread changes in cellular metabolism which involves changes in thousands of genes (Hazen *et al.*, 2003b). The functions of those gene products modulated in response to drought define the plant drought response phenotype. Two well-known

traits influencing drought tolerance are antioxidant capacity and osmotic adjustment. Osmotic adjustment results from the accumulation of compatible solutes within cells, which lowers the osmotic potential and helps, maintain turgor as plants experience water stress (Sanchez *et al.*,
5 2002). Antioxidant capacity refers to ability of plants to detoxify reactive oxygen species that cause cellular injury such as lipid peroxidation or protein and nucleic acid modifications (McKersie & Leshem, 1994).

Sorghum has a strong pre-flowering and post-flowering drought response which makes it an excellent crop model for evaluating mechanisms
10 of drought resistance (Rosenow *et al.*, 1983; Rosenow, 1987). Post-flowering drought response is expressed when dehydration occurs during grain development where rapid and premature leaf senescence can occur. Consequently, chlorophyll loss and a progressive decline in photosynthetic capacity lead to reduced crop yield. Some sorghum accessions are able to
15 resist premature leaf senescence by maintaining greater functional photosynthetic leaf area under moisture stress. This capacity is known as the "stay-green" (stg) trait and is an important component of post-flowering drought tolerance in sorghum (Rosenow & Clark, 1981). A recent study has highlighted the contribution of both onset and rate of leaf senescence
20 relative to the nitrogen dynamics of the plant or leaf in sorghum to explain the mechanism leading to the stg phenotype in sorghum (Borrel *et al.*, 2000a, 2000b; Borrel & Hammer, 2000). Several quantitative trait loci (QTL) for the stg trait were identified, but the exact cellular basis of the biochemical and/or physiological mechanism by which the stay-green trait leads to
25 drought tolerance remains unclear (Sanchez *et al.*, 2002; Tuinstra *et al.*, 1997a, 1997b, 1998; Tao *et al.*, 2000). Given the massive alteration of gene expression in response to drought (Kreps *et al.*, 2002; Seki *et al.*, 2002; Hazen *et al.*, 2003b), the analysis of the transcriptome associated with the stay-green phenotype may reveal important insights on the transcriptional
30 response of sorghum to drought stress.

The sorghum accessions B35 and Tx7000 differ phenotypically with respect to post-flowering drought tolerance and were used to develop a recombinant inbred line (RIL) population (Sanchez *et al.*, 2002). B35 is a stg

inbred line and highly tolerant to post-flowering drought stress. Tx7000 is an elite, non-stg line and susceptible to post-flowering drought. A rice GENECHIP® array was used to monitor the gene expression profile of B35, Tx7000, and four derived RILs. This is the first report of 1) hybridization of sorghum to a GENECHIP®; 2) heterologous microarray hybridization in plants; and 3) parallel expression profiling of two contrasting genotypes and their recombinant progeny under gradual dehydration stress.

In some embodiments, the presently disclosed subject matter relates to the screening of a rice GENECHIP® microarray (Affymetrix, Santa Clara, California, United States of America; see Zhu *et al.*, 2003) with cDNAs isolated from sorghum (*Sorghum bicolor* (L.) Moench) to identify changes in gene expression in sorghum when exposed to drought conditions. Using this approach, rice genes that exhibit different levels of hybridization to sorghum sequences isolated from plants under various conditions of water availability likely correspond to orthologs of sorghum genes that play a role in drought resistance. Using standard molecular biological techniques, the identified rice orthologs can be used to isolate the corresponding sorghum genes. Once the sorghum genes are isolated, they can be used for the construction of vectors designed for altering expression of these genes in transgenic plants using plant molecular genetic methodologies, which are disclosed in detail below. Alteration of plant phenotype through overexpression or underexpression of key trait genes in transgenic plants is a robust and established method for assigning functions to plant genes. Assays to identify transgenic plants with alterations in traits of interest are to be used to unambiguously assign the usefulness of these genes for the improvement of rice, and by extension, other cereals, either by transgenic or classical breeding methods.

II. Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the presently disclosed subject matter pertains. For

clarity of the present specification, certain definitions are presented hereinbelow.

Following long-standing patent law convention, the terms "a" and "an" mean "one or more" when used in this application, including in the claims.

5 As used herein, the phrase "altered abiotic stress tolerance" refers to a state wherein a plant exhibits a different response to abiotic stress (for example, low water conditions) than does another plant of the same species. In some embodiments, altered abiotic stress tolerance comprises enhanced drought resistance, wherein "enhanced drought resistance" is defined as an
10 increased ability of a plant (for example, a recombinant or transgenic plant) to withstand periods of low water abundance as compared to a native plant of the same species. As used herein, the phrase "altering an abiotic stress tolerance" refers in some embodiments to a manipulation of a plant's genome to produce a recombinant or transgenic plant in which the
15 manipulation results in a change in the plant's abiotic stress tolerance. In some embodiments, altering an abiotic stress tolerance comprises enhancing a plant's resistance to drought.

 As used herein, the terms "associated with" and "operatively linked" refer to two nucleic acid sequences that are related physically or functionally.
20 For example, a promoter or regulatory DNA sequence is said to be "associated with" a DNA sequence that encodes an RNA or a polypeptide if the two sequences are operatively linked, or situated such that the regulator DNA sequence will affect the expression level of the coding or structural DNA sequence.

25 As used herein, the term "chimera" refers to a polypeptide that comprises domains or other features that are derived from different polypeptides or are in a position relative to each other that is not naturally occurring.

 As used herein, the term "chimeric construct" refers to a recombinant
30 nucleic acid molecule in which a promoter or regulatory nucleic acid sequence is operatively linked to, or associated with, a nucleic acid sequence that codes for an mRNA or which is expressed as a polypeptide, such that the regulatory nucleic acid sequence is able to regulate

transcription or expression of the associated nucleic acid sequence. The regulatory nucleic acid sequence of the chimeric construct is not normally operatively linked to the associated nucleic acid sequence as found in nature.

5 As used herein, the term "co-factor" refers to a natural reactant, such as an organic molecule or a metal ion, required in an enzyme-catalyzed reaction. A co-factor can be, for example, NAD(P), riboflavin (including FAD and FMN), folate, molybdopterin, thiamin, biotin, lipoic acid, pantothenic acid and coenzyme A, S-adenosylmethionine, pyridoxal phosphate, ubiquinone,
10 and menaquinone. In some embodiments, a co-factor can be regenerated and reused.

 As used herein, the terms "coding sequence" and "open reading frame" (ORF) are used interchangeably and refer to a nucleic acid sequence that is transcribed into RNA such as mRNA, rRNA, tRNA, snRNA, sense
15 RNA, or antisense RNA. In some embodiments, the RNA is then translated *in vivo* or *in vitro* to produce a polypeptide.

 As used herein, the term "complementary" refers to two nucleotide sequences that comprise antiparallel nucleotide sequences capable of pairing with one another upon formation of hydrogen bonds between the
20 complementary base residues in the antiparallel nucleotide sequences. As is known in the art, the nucleic acid sequences of two complementary strands are the reverse complement of each other when each is viewed in the 5' to 3' direction.

 As is also known in the art, two sequences that hybridize to each other under a given set of conditions do not necessarily have to be 100%
25 fully complementary. As used herein, the terms "fully complementary" and "100% complementary" refer to sequences for which the complementary regions are 100% in Watson-Crick base-pairing, *i.e.*, that no mismatches occur within the complementary regions. However, as is often the case with
30 recombinant molecules (for example, cDNAs) that are cloned into cloning vectors, certain of these molecules can have non-complementary overhangs on either the 5' or 3' ends that result from the cloning event. In such a situation, it is understood that the region of 100% or full complementarity

excludes any sequences that are added to the recombinant molecule (typically at the ends) solely as a result of, or to facilitate, the cloning event. Such sequences are, for example, polylinker sequences, linkers with restriction enzyme recognition sites, etc.

5 As used herein, the terms "domain" and "feature", when used in reference to a polypeptide or amino acid sequence, refers to a subsequence of an amino acid sequence that has a particular biological function. Domains and features that have a particular biological function include, but are not limited to, ligand binding, nucleic acid binding, catalytic activity, substrate binding, and polypeptide-polypeptide interacting domains. 10 Similarly, when used herein in reference to a nucleic acid sequence, a "domain", or "feature" is that subsequence of the nucleic acid sequence that encodes a domain or feature of a polypeptide.

 As used herein, the term "enzyme activity" refers to the ability of an 15 enzyme to catalyze the conversion of a substrate into a product. A substrate for the enzyme can comprise the natural substrate of the enzyme but also can comprise analogues of the natural substrate, which can also be converted by the enzyme into a product or into an analogue of a product. The activity of the enzyme is measured for example by determining the 20 amount of product in the reaction after a certain period of time, or by determining the amount of substrate remaining in the reaction mixture after a certain period of time. The activity of the enzyme can also be measured by determining the amount of an unused co-factor of the reaction remaining in the reaction mixture after a certain period of time or by determining the 25 amount of used co-factor in the reaction mixture after a certain period of time. The activity of the enzyme can also be measured by determining the amount of a donor of free energy or energy-rich molecule (e.g. ATP, phosphoenolpyruvate, acetyl phosphate, or phosphocreatine) remaining in the reaction mixture after a certain period of time or by determining the 30 amount of a used donor of free energy or energy-rich molecule (e.g. ADP, pyruvate, acetate, or creatine) in the reaction mixture after a certain period of time.

As used herein, the term "expression cassette" refers to a nucleic acid molecule capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operatively linked to the nucleotide sequence of interest which is operatively linked to termination signals. It also typically comprises sequences required for proper translation of the nucleotide sequence. The coding region usually encodes a polypeptide of interest but can also encode a functional RNA of interest, for example antisense RNA or a non-translated RNA, in the sense or antisense direction. The expression cassette comprising the nucleotide sequence of interest can be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette can also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Typically, however, the expression cassette is heterologous with respect to the host; *i.e.*, the particular DNA sequence of the expression cassette does not occur naturally in the host cell and was introduced into the host cell or an ancestor of the host cell by a transformation event. The expression of the nucleotide sequence in the expression cassette can be under the control of a constitutive promoter or of an inducible promoter that initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism such as a plant, the promoter can also be specific to a particular tissue, organ, or stage of development.

As used herein, the term "fragment" refers to a sequence that comprises a subset of another sequence. When used in the context of a nucleic acid or amino acid sequence, the terms "fragment" and "subsequence" are used interchangeably. A fragment of a nucleic acid sequence can be any number of nucleotides that is less than that found in another nucleic acid sequence, and thus includes, but is not limited to, the sequences of an exon or intron, a promoter, an enhancer, an origin of replication, a 5' or 3' untranslated region, a coding region, and a polypeptide binding domain. It is understood that a fragment or subsequence can also comprise less than the entirety of a nucleic acid sequence, for example, a portion of an exon or intron, promoter, enhancer, etc. Similarly, a fragment

or subsequence of an amino acid sequence can be any number of residues that is less than that found in a naturally occurring polypeptide, and thus includes, but is not limited to, domains, features, repeats, etc. Also similarly, it is understood that a fragment or subsequence of an amino acid sequence need not comprise the entirety of the amino acid sequence of the domain, feature, repeat, etc. A fragment can also be a "functional fragment", in which the fragment retains a specific biological function of the nucleic acid sequence or amino acid sequence of interest. For example, a functional fragment of a transcription factor can include, but is not limited to, a DNA binding domain, a transactivating domain, or both. Similarly, a functional fragment of a receptor tyrosine kinase includes, but is not limited to a ligand binding domain, a kinase domain, an ATP binding domain, and combinations thereof.

As used herein, the term "gene" is used broadly to refer to any segment of DNA associated with a biological function. Thus, genes include, but are not limited to, coding sequences and/or the regulatory sequences required for their expression. Genes can also include non-expressed DNA segments that, for example, form recognition sequences for a polypeptide. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and can include sequences designed to have desired parameters.

The terms "heterologous", "recombinant", and "exogenous", when used herein to refer to a nucleic acid sequence (e.g. a DNA sequence) or a gene, refer to a sequence that originates from a source foreign to the particular host cell or, if from the same source, is modified from its original form. Thus, a heterologous gene in a host cell includes a gene that is endogenous to the particular host cell but has been modified through, for example, the use of DNA shuffling or other recombinant techniques. The terms also include non-naturally occurring multiple copies of a naturally occurring DNA sequence. Thus, the terms refer to a DNA segment that is foreign or heterologous to the cell, or homologous to the cell but in a position or form within the host cell in which the element is not ordinarily found.

Similarly, when used in the context of a polypeptide or amino acid sequence, an exogenous polypeptide or amino acid sequence is a polypeptide or amino acid sequence that originates from a source foreign to the particular host cell or, if from the same source, is modified from its original form. Thus, exogenous DNA segments can be expressed to yield exogenous polypeptides.

A "homologous" nucleic acid (or amino acid) sequence is a nucleic acid (or amino acid) sequence naturally associated with a host cell into which it is introduced.

The phrase "hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA. The phrase "bind(s) substantially" refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target nucleic acid sequence.

As used herein, the term "inhibitor" refers to a chemical substance that inactivates or decreases the biological activity of a polypeptide such as a biosynthetic and catalytic activity, receptor, signal transduction polypeptide, structural gene product, or transport polypeptide. The term "herbicide" (or "herbicidal compound") is used herein to define an inhibitor applied to a plant at any stage of development, whereby the herbicide inhibits the growth of the plant or kills the plant.

As used herein, the term "isolated", when used in the context of an isolated DNA molecule or an isolated polypeptide, is a DNA molecule or polypeptide that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated DNA molecule or polypeptide can exist in a purified form or can exist in a non-native environment such as, for example, in a transgenic host cell.

As used herein, the term "mature polypeptide" refers to a polypeptide from which the transit peptide, signal peptide, and/or propeptide portions have been removed.

As used herein, the term "minimal promoter" refers to the smallest piece of a promoter, such as a TATA element, that can support any transcription. A minimal promoter typically has greatly reduced promoter activity in the absence of upstream or downstream activation. In the presence of a suitable transcription factor, a minimal promoter can function to permit transcription.

As used herein, the term "modified enzyme activity" refers to enzyme activity that is different from that which naturally occurs in a plant (*i.e.* enzyme activity that occurs naturally in the absence of direct or indirect manipulation of such activity by man). In some embodiments, a modified enzyme activity is displayed by a non-naturally occurring enzyme that is tolerant to inhibitors that inhibit the cognate naturally occurring enzyme activity.

As used herein, the term "native" refers to a gene that is naturally present in the genome of an untransformed plant cell. Similarly, when used in the context of a polypeptide, a "native polypeptide" is a polypeptide that is encoded by a native gene of an untransformed plant cell's genome.

As used herein, the term "naturally occurring" refers to an object that is found in nature as distinct from being artificially produced by man. For example, a polypeptide or nucleotide sequence that is present in an organism (including a virus) in its natural state, which has not been intentionally modified or isolated by man in the laboratory, is naturally occurring. As such, a polypeptide or nucleotide sequence is considered "non-naturally occurring" if it is encoded by or present within a recombinant molecule, even if the amino acid or nucleic acid sequence is identical to an amino acid or nucleic acid sequence found in nature.

As used herein, the term "nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively

modified variants thereof (e.g. degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, 1991; Ohtsuka *et al.*, 1985; Rossolini *et al.*, 1994). The terms “nucleic acid” or “nucleic acid sequence” can also be used interchangeably with gene, cDNA, and mRNA encoded by a gene.

As used herein, the term “orthologs” refers to genes in different species that encode protein that perform the same biological function. For example, the glucose-6-phosphate dehydrogenase genes from, for example, sorghum and rice, are orthologs. Typically, orthologous nucleic acid sequences are characterized by a high degree of sequence similarity (for example, at least about 90% sequence identity). A nucleic acid sequence of an ortholog in one species (for example, rice) can be used to isolate the nucleic acid sequence of the ortholog in another species (for example, sorghum) using standard molecular biology techniques. This can be accomplished, for example, using techniques described in more detail below (see *also* Sambrook & Russell, 2001 for a discussion of hybridization conditions that can be used to isolate closely related sequences).

As used herein, the phrase “percent identical,” in the context of two nucleic acid or polypeptide sequences, refers to two or more sequences or subsequences that have in some embodiments 60%, in some embodiments 70%, in some embodiments 80%, in some embodiments 90%, in some embodiments 95%, and in some embodiments at least 99% nucleotide or amino acid residue identity, respectively, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. The percent identity exists in some embodiments over a region of the sequences that is at least about 50 residues in length, in some embodiments over a region of at least about 100 residues, and in some embodiments, the percent identity exists over at least about 150 residues. In some embodiments, the percent identity exists over the entire length of the sequences.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and
5 sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted,
10 for example, by the local homology algorithm disclosed in Smith & Waterman, 1981, by the homology alignment algorithm disclosed in Needleman & Wunsch, 1970, by the search for similarity method disclosed in Pearson & Lipman, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the GCG®
15 WISCONSIN PACKAGE®, available from Accelrys, Inc., San Diego, California, United States of America), or by visual inspection. See *generally*, Ausubel *et al.*, 1994.

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is
20 described in Altschul *et al.*, 1990. Software for performing BLAST analysis is publicly available through the website of the National Center for Biotechnology Information. 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
25 threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. See *generally*, Altschul *et al.*, 1990. 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
30 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

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
5 or more negative-scoring residue alignments, or the end of 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
10 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 scoring matrix. See Henikoff & Henikoff, 1992.

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two
15 sequences (see e.g., Karlin & Altschul, 1993). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference
20 sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is in some embodiments less than about 0.1, in some embodiments less than about 0.01, and in some embodiments less than about 0.001.

As used herein, the term "shuffled nucleic acid" refers to a
25 recombinant nucleic acid molecule in which the nucleotide sequence comprises a plurality of nucleotide sequence fragments, wherein at least one of the fragments corresponds to a region of a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, and wherein at least two of the plurality of sequence fragments are in an order, from 5' to 3', which is
30 not an order in which the plurality of fragments naturally occur in a nucleic acid

The term "substantially identical", in the context of two nucleotide or amino acid sequences, refers to two or more sequences or subsequences

that have in some embodiments at least about 60% nucleotide or amino acid identity, in some embodiments at least about 65% nucleotide or amino acid identity, in some embodiments at least about 70% nucleotide or amino acid identity, in some embodiments at least about 75% nucleotide or amino acid identity, in some embodiments at least about 80% nucleotide or amino acid identity, in some embodiments at least about 85% nucleotide or amino acid identity, in some embodiments at least about 90% nucleotide or amino acid identity, in some embodiments at least about 95% nucleotide or amino acid identity, in some embodiments at least about 97% nucleotide or amino acid identity, and in some embodiments at least about 99% nucleotide or amino acid identity, when compared and aligned for maximum correspondence, as measured using one of the above-referenced sequence comparison algorithms or by visual inspection. In some embodiments, the substantial identity exists in nucleotide or amino acid sequences of at least 50 residues, in some embodiments in nucleotide or amino acid sequence of at least about 100 residues, in some embodiments in nucleotide or amino acid sequences of at least about 150 residues, and in some embodiments in nucleotide or amino acid sequences comprising complete coding sequences or complete amino acid sequences.

In one aspect, polymorphic sequences can be substantially identical sequences. The term "polymorphic" refers to the two or more genetically determined alternative sequences or alleles in a population. An allelic difference can be as small as one base pair. Nonetheless, one of ordinary skill in the art would recognize that the polymorphic sequences correspond to the same gene.

Another indication that two nucleotide sequences are substantially identical is that the two molecules specifically or substantially hybridize to each other under conditions of medium or high stringency. In the context of nucleic acid hybridization, two nucleic acid sequences being compared can be designated a "probe sequence" and a "target sequence". A "probe sequence" is a reference nucleic acid molecule, and a "target sequence" is a test nucleic acid molecule, often found within a heterogeneous population

of nucleic acid molecules. A "target sequence" is synonymous with a "test sequence".

5 An exemplary nucleotide sequence employed for hybridization studies or assays includes probe sequences that are complementary to or mimic in some embodiments at least an about 14 to 40 nucleotide sequence of a nucleic acid molecule of the presently disclosed subject matter. In some
10 embodiments, probes comprise 14 to 20 nucleotides, or even longer where desired, such as 30, 40, 50, 60, 100, 200, 300, or 500 nucleotides or up to the full length (for example, the full complement) of any of the nucleic acid sequence set forth in the odd numbered SEQ ID NOs: 1-105. Such fragments can be readily prepared by, for example, directly synthesizing the fragment by chemical synthesis, by application of nucleic acid amplification technology, or by introducing selected sequences into recombinant vectors for recombinant production.

15 The phrase "hybridizing substantially to" refers to complementary hybridization between a probe nucleic acid molecule and a target nucleic acid molecule and embraces minor mismatches (for example, polymorphisms) that can be accommodated by reducing the stringency of the hybridization and/or wash media to achieve the desired hybridization.

20 "Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern blot analysis are both sequence- and environment-dependent. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen,
25 1993. Generally, high stringency hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Typically, under "highly stringent conditions" a probe will hybridize specifically to its target subsequence, but to no other sequences. Similarly, medium stringency
30 hybridization and wash conditions are selected to be more than about 5°C lower than the T_m for the specific sequence at a defined ionic strength and pH. Exemplary medium stringency conditions include hybridizations and washes as for high stringency conditions, except that the temperatures for

the hybridization and washes are in some embodiments 8°C, in some embodiments 10°C, in some embodiments 12°C, and in some embodiments 15°C lower than the T_m for the specific sequence at a defined ionic strength and pH.

5 The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of highly stringent hybridization conditions for Southern or Northern Blot analysis of complementary nucleic acids having more than
10 about 100 complementary residues is overnight hybridization in 50% formamide with 1 mg of heparin at 42°C. An example of highly stringent wash conditions is 15 minutes in 0.1x standard saline citrate (SSC), 0.1% (w/v) SDS at 65°C. Another example of highly stringent wash conditions is 15 minutes in 0.2x SSC buffer at 65°C (see Sambrook and Russell, 2001 for
15 a description of SSC buffer and other stringency conditions). Often, a high stringency wash is preceded by a lower stringency wash to remove background probe signal. An example of medium stringency wash conditions for a duplex of more than about 100 nucleotides is 15 minutes in 1X SSC at 45°C. Another example of medium stringency wash for a duplex
20 of more than about 100 nucleotides is 15 minutes in 4-6X SSC at 40°C. For short probes (e.g., about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1M Na^+ ion, typically about 0.01 to 1M Na^+ ion concentration (or other salts) at pH 7.0-8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be
25 achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2-fold (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

30 The following are examples of hybridization and wash conditions that can be used to clone homologous nucleotide sequences that are substantially identical to reference nucleotide sequences of the presently disclosed subject matter: a probe nucleotide sequence hybridizes in some embodiments to a target nucleotide sequence in 7% sodium dodecyl sulfate

(NaDS), 0.5M NaPO₄, 1 mm ethylene diamine tetraacetic acid (EDTA) at 50°C followed by washing in 2X SSC, 0.1% NaDS at 50°C; in some embodiments, a probe and target sequence hybridize in 7% NaDS, 0.5 M NaPO₄, 1 mm EDTA at 50°C followed by washing in 1X SSC, 0.1% NaDS at 50°C; in some embodiments, a probe and target sequence hybridize in 7% NaDS, 0.5 M NaPO₄, 1 mm EDTA at 50°C followed by washing in 0.5X SSC, 0.1% NaDS at 50°C; in some embodiments, a probe and target sequence hybridize in 7% NaDS, 0.5 M NaPO₄, 1 mm EDTA at 50°C followed by washing in 0.1X SSC, 0.1% NaDS at 50°C; in some embodiments, a probe and target sequence hybridize in 7% NaDS, 0.5 M NaPO₄, 1 mm EDTA at 50°C followed by washing in 0.1X SSC, 0.1% NaDS at 65°C. In some embodiments, hybridization conditions comprise hybridization in a roller tube for at least 12 hours at 42°C.

As used herein, the term "pre-polypeptide" refers to a polypeptide that is normally targeted to a cellular organelle, such as a chloroplast, and still comprises a transit peptide.

As used herein, the term "purified", when applied to a nucleic acid or polypeptide, denotes that the nucleic acid or polypeptide is essentially free of other cellular components with which it is associated in the natural state. It can be in a homogeneous state although it can be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A polypeptide that is the predominant species present in a preparation is substantially purified. The term "purified" denotes that a nucleic acid or polypeptide gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or polypeptide is in some embodiments at least about 50% pure, in some embodiments at least about 85% pure, and in some embodiments at least about 99% pure.

Two nucleic acids are "recombined" when sequences from each of the two nucleic acids are combined in a progeny nucleic acid. Two sequences are "directly" recombined when both of the nucleic acids are substrates for recombination. Two sequences are "indirectly recombined"

when the sequences are recombined using an intermediate such as a cross-over oligonucleotide. For indirect recombination, no more than one of the sequences is an actual substrate for recombination, and in some cases, neither sequence is a substrate for recombination.

5 As used herein, the term "regulatory elements" refers to nucleotide sequences involved in controlling the expression of a nucleotide sequence. Regulatory elements can comprise a promoter operatively linked to the nucleotide sequence of interest and termination signals. Regulatory sequences also include enhancers and silencers. They also typically
10 encompass sequences required for proper translation of the nucleotide sequence.

 As used herein, the term "significant increase" refers to an increase in activity (for example, enzymatic activity) that is larger than the margin of error inherent in the measurement technique, in some embodiments an
15 increase by about 2-fold or greater over a baseline activity (for example, the activity of the wild-type enzyme in the presence of the inhibitor), in some embodiments an increase by about 5-fold or greater, and in some embodiments an increase by about 10-fold or greater.

 As used herein, the terms "significantly less" and "significantly
20 reduced" refer to a result (for example, an amount of a product of an enzymatic reaction) that is reduced by more than the margin of error inherent in the measurement technique, in some embodiments a decrease by about 2-fold or greater with respect to a baseline activity (for example, the activity of the wild-type enzyme in the absence of the inhibitor), In some
25 embodiments, a decrease by about 5-fold or greater, and in some embodiments a decrease by about 10-fold or greater.

 As used herein, the terms "specific binding" and "immunological cross-reactivity" refer to an indicator that two molecules are substantially identical. An indication that two nucleic acid sequences or polypeptides are
30 substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with, or specifically binds to, the polypeptide encoded by the second nucleic acid. Thus, a polypeptide is

typically substantially identical to a second polypeptide, for example, where the two polypeptides differ only by conservative substitutions.

5 The phrase "specifically (or selectively) binds to an antibody," or "specifically (or selectively) immunoreactive with," when referring to a polypeptide or peptide, refers to a binding reaction which is determinative of the presence of the polypeptide in the presence of a heterogeneous population of polypeptides and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular polypeptide and do not bind in a significant amount to other polypeptides present in the sample. Specific binding to an antibody under such conditions can require an antibody that is selected for its specificity for a particular polypeptide. For example, antibodies raised to the polypeptide with the amino acid sequence encoded by any of the nucleic acid sequences of the presently disclosed subject matter can be selected to obtain antibodies specifically immunoreactive with that polypeptide and not with other polypeptides except for polymorphic variants. A variety of immunoassay formats can be used to select antibodies, specifically immunoreactive with a particular polypeptide. For example, solid-phase ELISA immunoassays, Western blots, or immunohistochemistry are routinely used to select monoclonal antibodies specifically immunoreactive with a polypeptide. See Harlow & Lane, 1988, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

25 As used herein, the term "subsequence" refers to a sequence of nucleic acids or amino acids that comprises a part of a longer sequence of nucleic acids or amino acids (e.g., polypeptide), respectively.

30 As used herein, the term "substrate" refers to a molecule that an enzyme naturally recognizes and converts to a product in the biochemical pathway in which the enzyme naturally carries out its function; or is a modified version of the molecule, which is also recognized by the enzyme and is converted by the enzyme to a product in an enzymatic reaction similar to the naturally-occurring reaction.

As used herein, the term "suitable growth conditions" refers to growth conditions that are suitable for a certain desired outcome, for example, the production of a recombinant polypeptide or the expression of a nucleic acid molecule.

5 As used herein, the term "transformation" refers to a process for introducing heterologous DNA into a plant cell, plant tissue, or plant. Transformed plant cells, plant tissue, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof.

10 As used herein, the terms "transformed", "transgenic", and "recombinant" refer to a host organism such as a bacterium or a plant into which a heterologous nucleic acid molecule has been introduced. The nucleic acid molecule can be stably integrated into the genome of the host or the nucleic acid molecule can also be present as an extrachromosomal molecule. Such an extrachromosomal molecule can be auto-replicating. Transformed cells, tissues, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof. A "non-transformed," "non-transgenic", or "non-recombinant" host refers to a wild-type organism, e.g., a bacterium or plant, which does not
15
20 contain the heterologous nucleic acid molecule.

As used herein, the term "viability" refers to a fitness parameter of a plant. Plants are assayed for their homozygous performance of plant development, indicating which polypeptides are essential for plant growth.

25 III. Nucleic Acid Molecules and Polypeptides

The presently disclosed subject matter encompasses the identification and isolation of cDNAs encoding genes of interest in the expression of abiotic stress tolerance. Abiotic stresses include, but are not limited to, cold, heat, drought, and salt stress, and can significantly affect the growth and/or yield of plants. In some embodiments, an abiotic stress is drought. Altering the expression of genes related to these traits can be used
30 to improve or modify the rice plants, grain, or both plants and grain, as desired. Examples describe the isolated genes of interest and methods of

analyzing the alteration of expression and their effects on the plant characteristics.

III.A. Nucleic Acid Molecules

Embodiments of the presently disclosed subject matter encompass
5 isolated nucleic acid molecules corresponding to genes that are differentially regulated in sorghum in response to abiotic stress (for example, drought). In some embodiments, an isolated nucleic acid molecule of the presently disclosed subject matter comprises a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final
10 washing step at 50°C in 0.1 M NaCl to a nucleotide sequence as set forth in odd numbered sequences SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof. In some embodiments, an isolated nucleic acid molecule of the presently disclosed subject matter comprises a nucleotide sequence having substantial identity to a nucleotide sequence that hybridizes under
15 conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence as set forth in odd numbered sequences SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof. Another embodiment of the presently disclosed subject matter encompasses an isolated nucleic acid molecule comprising a nucleotide
20 sequence that is complementary to, or the reverse complement of, a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof. Some embodiments of the
25 presently disclosed subject matter encompass an isolated nucleic acid molecule comprising a nucleotide sequence that is complementary to, or the reverse complement of, a nucleotide sequence that has substantial identity to, or is capable of hybridizing to, a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final
30 washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof.

In some embodiments, the substantial identity is at least about 60% identity, in some embodiments at least about 65% identity, in some embodiments at least about 70% identity, in some embodiments at least about 75% identity, in some embodiments about 80% identity, in some
5 embodiments at least about 85% identity, in some embodiments about 90% identity, and in some embodiments at least about 95% identity to the nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof.

In some embodiments, the nucleotide sequence having substantial
10 identity comprises an allelic variant of the nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof. In some embodiments, the nucleotide sequence having
15 substantial identity comprises a naturally occurring variant. In some embodiments, the nucleotide sequence having substantial identity comprises a polymorphic variant of the nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered
20 sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof.

In some embodiments, the nucleic acid having substantial identity comprises a deletion or insertion of at least one nucleotide. In some embodiments, the deletion or insertion comprises less than about thirty nucleotides. In some embodiments, the deletion or insertion comprises less
25 than about five nucleotides. In some embodiments, the sequence of the isolated nucleic acid having substantial identity comprises a substitution in at least one codon. In some embodiments, the substitution is conservative.

In some embodiments, the isolated nucleic acid comprises a plurality of regions having a nucleotide sequence that hybridizes under conditions of
30 hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or an exon, domain, or feature thereof.

In some embodiments, the sequence having substantial identity to the nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof, is from a plant. In some
5 embodiments, the plant is a dicot. In some embodiments, the plant is a gymnosperm. In some embodiments, the plant is a monocot. In some embodiments, the monocot is a cereal. In some embodiments, the cereal can be, for example, maize, wheat, barley, oats, rye, millet, sorghum, triticale, secale, einkorn, spelt, emmer, teff, milo, flax, gramma grass,
10 *Tripsacum* sp., or *teosinte*. In some embodiments, the cereal is rice.

In some embodiments, the nucleic acid is expressed in a specific location or tissue of a plant. In some embodiments, the location or tissue includes, but is not limited to, epidermis, root, vascular tissue, meristem, cambium, cortex, pith, leaf, flower, and combinations thereof. In some
15 embodiments, the location or tissue is a seed.

In some embodiments, the nucleic acid encodes a polypeptide involved in a function including, but not limited to, carbon metabolism, photosynthesis, signal transduction, cell growth, reproduction, disease
20 processes, gene regulation, and differentiation. In some embodiments, the nucleic acid encodes a polypeptide involved in abiotic stress tolerance, enhanced yield, disease resistance, or nutritional content.

Embodiments of the presently disclosed subject matter further relate to an isolated polynucleotide comprising a nucleotide sequence of at least
25 10 bases, which sequence is identical, complementary (for example, fully complementary), or substantially identical to a region of any sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence of odd numbered sequences of SEQ ID NOs: 1-105, and wherein the
30 polynucleotide is adapted for any of numerous uses.

In some embodiments, the polynucleotide is used as a chromosomal marker. In some embodiments, the polynucleotide is used as a marker for restriction fragment length polymorphism (RFLP) analysis. In some

embodiments, the polynucleotide is used as a marker for quantitative trait-linked breeding. In some embodiments, the polynucleotide is used as a marker for marker-assisted breeding. In some embodiments, the polynucleotide is used as a bait sequence in a two-hybrid system to identify sequence-encoding polypeptides interacting with the polypeptide encoded by the bait sequence. In some embodiments, the polynucleotide is used as a diagnostic indicator for genotyping or identifying an individual or population of individuals. In some embodiments, the polynucleotide is used for genetic analysis to identify boundaries of genes or exons.

Embodiments of the presently disclosed subject matter also relate to a shuffled nucleic acid molecule comprising a plurality of nucleotide sequence fragments, wherein at least one of the fragments corresponds to a region of a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, and wherein at least two of the plurality of sequence fragments are in an order, from 5' to 3', which is not an order in which the plurality of fragments naturally occur. In some embodiments, all of the fragments in a shuffled nucleic acid comprising a plurality of nucleotide sequence fragments are from a single gene. In some embodiments, the plurality of fragments is derived from at least two different genes. In some embodiments, the shuffled nucleic acid is operatively linked to a promoter sequence. In some embodiments, the shuffled nucleic acid comprises a chimeric polynucleotide comprising a promoter sequence operatively linked to the shuffled nucleic acid. In some embodiments, the shuffled nucleic acid is contained within a host cell.

III.B. Identifying, Cloning, and Sequencing cDNAs

The cloning and sequencing of the cDNAs of the presently disclosed subject matter is accomplished using techniques known in the art. See generally, Sambrook & Russell, 2001; Silhavy *et al.*, 1984; Ausubel *et al.*, 1994; Reiter *et al.*, 1992; Schultz *et al.*, 1998.

The isolated nucleic acids and polypeptides of the presently disclosed subject matter are usable over a range of plants – monocots and dicots – in

particular monocots such as sorghum, rice, wheat, barley, and maize. In some embodiments, the monocot is a cereal. In some embodiments, the cereal can be, for example, maize, wheat, barley, oats, rye, millet, sorghum, triticale, secale, einkorn, spelt, emmer, teff, milo, flax, gramma grass, *Tripsacum* sp., or *teosinte*. In some embodiments, the cereal is sorghum. Other plant genera relevant to the presently disclosed subject matter include, but are not limited to, *Cucurbita*, *Rosa*, *Vitis*, *Juglans*, *Gragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *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*, *Allium*, and *Triticum*.

The presently disclosed subject matter also provides a method for genotyping a plant or plant part comprising a nucleic acid molecule of the presently disclosed subject matter. Optionally, the plant is a monocot such as, but not limited to, sorghum, rice or wheat. Genotyping provides a methodology for distinguishing homologs of a chromosome pair and can be used to differentiate segregants in a plant population. Molecular marker methods can be used in phylogenetic studies, characterizing genetic relationships among crop varieties, identifying crosses or somatic hybrids, localizing chromosomal segments affecting monogenic traits, mapping based cloning, and the study of quantitative inheritance (see Clark, 1997; Paterson, 1996).

The method for genotyping can employ any number of molecular marker analytical techniques including, but not limited to, restriction length polymorphisms (RFLPs). As is well known in the art, RFLPs are produced by differences in the DNA restriction fragment lengths resulting from nucleotide differences between alleles of the same gene. Thus, the presently disclosed subject matter provides a method for following segregation of a gene or nucleic acid of the presently disclosed subject

matter or chromosomal sequences genetically linked by using RFLP analysis. Linked chromosomal sequences are in some embodiments within 50 centimorgans (cM), in some embodiments within 40 cM, in some embodiments within 30 cM, in some embodiments within 20 cM, in some
5 embodiments within 10 cM, and in some embodiments within 5, 3, 2, or 1 cM of the nucleic acid of the presently disclosed subject matter.

Embodiments of the presently disclosed subject matter also relate to an isolated nucleic acid molecule comprising a nucleotide sequence, its complement (for example, its full complement), or its reverse complement
10 (for example, its full reverse complement), the nucleotide sequence encoding a polypeptide (for example, a biologically active polypeptide or biologically active fragment). In some embodiments, the nucleotide sequence encodes a polypeptide that is an ortholog of a polypeptide comprising a polypeptide sequence listed in even numbered sequences of
15 SEQ ID NOs: 2-106, or a fragment, domain, repeat, feature, or chimera thereof. In some embodiments, the nucleotide sequence encodes a polypeptide that is an ortholog of a polypeptide comprising a polypeptide sequence having substantial identity to a polypeptide sequence listed in even numbered sequences of SEQ ID NOs: 2-106, or a fragment, domain,
20 repeat, feature, or chimera thereof. In some embodiments, the nucleotide sequence encodes a polypeptide that is an ortholog of a polypeptide comprising a polypeptide sequence encoded by a nucleotide sequence identical to or having substantial identity to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or
25 feature thereof, or a sequence complementary thereto. In some embodiments, the nucleotide sequence encodes a polypeptide comprising a polypeptide sequence encoded by a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd
30 numbered sequences of SEQ ID NOs: 1-105, or to a sequence complementary thereto. In some embodiments, the nucleotide sequence encodes a functional fragment of a polypeptide of the presently disclosed subject matter.

In some embodiments, the isolated nucleic acid comprises a polypeptide-encoding sequence. In some embodiments, the polypeptide-encoding sequence encodes a polypeptide that is an ortholog of a polypeptide comprising a polypeptide sequence listed in even numbered sequences of SEQ ID NOs: 2-106, or a fragment thereof. In some
5 embodiments, the polypeptide is an ortholog of a polypeptide disclosed in Table 4 or 5. In some embodiments, the polypeptide is a plant polypeptide. In some embodiments, the plant is a dicot. In some embodiments, the plant is a gymnosperm. In some embodiments, the plant is a monocot. In some
10 embodiments, the monocot is a cereal. In some embodiments, the cereal includes, but is not limited to, maize, wheat, barley, oats, rye, millet, sorghum, triticale, secale, einkorn, spelt, emmer, teff, miloflax, gramma grass, *Tripsacum*, and *teosinte*. In some embodiments, the cereal is sorghum.

15 In some embodiments, the polypeptide is involved in a function such as abiotic stress tolerance, enhanced yield, disease resistance, or nutritional content. In some embodiments, the polypeptide is involved in drought resistance.

Embodiments of the presently disclosed subject matter also relate to
20 an isolated nucleic acid molecule comprising a nucleotide sequence, its complement (for example, its full complement), or its reverse complement (for example, its full reverse complement), encoding a polypeptide selected from a group comprising one or more of:

- 25 (a) a polypeptide sequence encoded by a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof, or a sequence complementary thereto; and
30 (b) a functional fragment of (a).

In some embodiments, the polypeptide having substantial identity comprises an allelic variant of a polypeptide that is an ortholog of a polypeptide having an amino acid sequence listed in even numbered

sequences of SEQ ID NOs: 2-106, or a fragment, domain, repeat, feature, or chimera thereof. In some embodiments, the isolated nucleic acid comprises a plurality of regions from the polypeptide sequence encoded by a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof, or a sequence complementary thereto.

In some embodiments, the sequence of the isolated nucleic acid encodes a polypeptide useful for generating an antibody having immunoreactivity against a polypeptide encoded by a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof.

III.C. Polypeptides

The presently disclosed subject matter further relates to isolated polypeptides that are orthologs of the polypeptides comprising the amino acid sequences set forth in even numbered SEQ ID NOs: 2-106, including biologically active polypeptides. In some embodiments, the polypeptide comprises a polypeptide sequence of an ortholog of a polypeptide listed in even numbered sequences of SEQ ID NOs: 2-106. In some embodiments, the polypeptide comprises a functional fragment or domain of an ortholog of a polypeptide comprising a polypeptide sequence listed in even numbered sequences of SEQ ID NOs: 2-106. In some embodiments, the polypeptide comprises a chimera of an ortholog of the polypeptide sequence listed in even numbered sequences of SEQ ID NOs: 2-106, where the chimera can comprise functional polypeptide motifs, including domains, repeats, post-translational modification sites, or other features. In some embodiments, the polypeptide is a plant polypeptide. In some embodiments, the plant is a dicot. In some embodiments, the plant is a gymnosperm. In some embodiments, the plant is a monocot. In some embodiments, the monocot is a cereal. In some embodiments, the cereal is, for example, maize, wheat,

barley, oats, rye, millet, sorghum, triticale, secale, einkorn, spelt, emmer, teff, milo, flax, gramma grass, *Tripsacum*, or *teosinte*. In some embodiments, the cereal is sorghum.

5 In some embodiments, the polypeptide is expressed in a specific location or tissue of a plant. In some embodiments, the location or tissue includes, but is not limited to, epidermis, root, vascular tissue, meristem, cambium, cortex, pith, leaf, flower, and combinations thereof. In some embodiments, the location or tissue is a seed.

10 In some embodiments, the polypeptide is involved in a function such as abiotic stress tolerance, disease resistance, enhanced yield or nutritional quality or composition. In some embodiments, the polypeptide is involved in drought resistance.

15 In some embodiments, isolated polypeptides comprise the amino acid sequences of orthologs of the polypeptides comprising the amino acid sequences set forth in even numbered SEQ ID NOs: 2-106, and variants having conservative amino acid modifications. The term "conservative modified variants" refers to polypeptides that can be encoded by nucleic acid sequences having degenerate codon substitutions wherein at least one position of one or more selected (or all) codons is substituted with mixed-
20 base and/or deoxyinosine residues (Batzner *et al.*, 1991; Ohtsuka *et al.*, 1985; Rossolini *et al.*, 1994). Additionally, one skilled in the art will recognize that individual substitutions, deletions, or additions to a nucleic acid, peptide, polypeptide, or polypeptide sequence that alters, adds, or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is
25 a "conservative modification" where the modification results in the substitution of an amino acid with a chemically similar amino acid. Conservative modified variants provide similar biological activity as the unmodified polypeptide. Conservative substitution tables listing functionally similar amino acids are known in the art. See Creighton, 1984.

30 The term "conservatively modified variant" also refers to a peptide having an amino acid residue sequence substantially identical to a sequence of a polypeptide of the presently disclosed subject matter in which one or more residues have been conservatively substituted with a functionally

similar residue. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another; the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine; the substitution of one basic residue such as lysine, arginine or histidine for another; or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

Amino acid substitutions, such as those which might be employed in modifying the polypeptides described herein, are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. An analysis of the size, shape and type of the amino acid side-chain substituents reveals that arginine, lysine and histidine are all positively charged residues; that alanine, glycine and serine are all of similar size; and that phenylalanine, tryptophan and tyrosine all have a generally similar shape. Therefore, based upon these considerations, arginine, lysine and histidine; alanine, glycine and serine; and phenylalanine, tryptophan and tyrosine; are defined herein as biologically functional equivalents. Other biologically functionally equivalent changes will be appreciated by those of skill in the art.

In making biologically functional equivalent amino acid substitutions, the hydropathic index of amino acids can be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte & Doolittle, 1982, incorporated herein by reference). It is known that certain amino acids can be substituted for other amino acids having a similar

hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, substitutions of amino acids can involve amino acids for which the hydropathic indices are in some embodiments within ± 2 of the original value, in some embodiments within ± 1 of the original value, and in some embodiments within ± 0.5 of the original value.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent No. 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.* with a biological property of the protein. It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent protein.

As detailed in U.S. Patent No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 $\pm$ 1); glutamate (+3.0 $\pm$ 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 $\pm$ 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

In making changes based upon similar hydrophilicity values, substitutions of amino acids can involve amino acids for which the hydrophilicity values are in some embodiments within ± 2 of the original value, in some embodiments within ± 1 of the original value, and in some embodiments within ± 0.5 of the original value.

While discussion has focused on functionally equivalent polypeptides arising from amino acid changes, it will be appreciated that these changes can be effected by alteration of the encoding DNA, taking into consideration also that the genetic code is degenerate and that two or more codons can code for the same amino acid.

In some embodiments, the sequence having substantial identity contains a deletion or insertion of at least one amino acid. In some

embodiments, the deletion or insertion is of less than about ten amino acids. In some embodiments, the deletion or insertion is of less than about three amino acids.

5 In some embodiments, the sequence having substantial identity encodes a substitution in at least one amino acid.

Embodiments of the presently disclosed subject matter also provide an isolated polypeptide comprising a polypeptide sequence selected from the group consisting of:

- 10 (a) a polypeptide sequence having substantial identity to a polypeptide sequence listed in even numbered SEQ ID NO: 2-106, or a domain or feature thereof;
- (b) a polypeptide sequence encoded by a nucleotide sequence identical to or having substantial identity to a nucleotide sequence that hybridizes under conditions of hybridization of
15 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl listed in odd numbered SEQ ID NO: 1-105, or an exon, domain, or feature thereof, or a sequence complementary thereto;
- (c) a polypeptide sequence encoded by a nucleotide sequence
20 capable of hybridizing under medium stringency conditions to a nucleotide sequence listed in odd numbered SEQ ID NO: 1-105, or to a sequence complementary thereto; and
- (d) a functional fragment of (a), (b), or (c).

25 In some embodiments, a polypeptide having substantial identity to a polypeptide sequence listed in even numbered SEQ ID NO: 2-106, or a domain or feature thereof, is an allelic variant of the polypeptide sequence listed in even numbered SEQ ID NO: 2-106. In some embodiments, a polypeptide having substantial identity to a polypeptide sequence listed in even numbered SEQ ID NO: 2-106, or a domain or feature thereof, is a
30 naturally occurring variant of the polypeptide sequence listed in even numbered SEQ ID NO: 2-106. In some embodiments, a polypeptide having substantial identity to a polypeptide sequence listed in even numbered SEQ

ID NO: 2-106, or a domain or feature thereof, is a polymorphic variant of the polypeptide sequence listed in even numbered SEQ ID NO: 2-106.

5 In some embodiments, the polypeptide is an ortholog of a polypeptide comprising one of the amino acid sequences listed in even numbered SEQ ID NO: 2-106. In some embodiments, the polypeptide is a functional fragment or domain of an ortholog of a polypeptide comprising one of the amino acid sequences listed in even numbered SEQ ID NOs: 2-106. In some embodiments, the polypeptide is a chimera, where the chimera comprises a functional polypeptide domain, including, but not limited to, a domain, a repeat, a post-translational modification site, and combinations thereof. In some embodiments, the polypeptide is a plant polypeptide. In some embodiments, the plant is a dicot. In some embodiments, the plant is a gymnosperm. In some embodiments, the plant is a monocot. In some embodiments, the monocot is a cereal. In some embodiments, the cereal can be, for example, maize, wheat, barley, oats, rye, millet, sorghum, 10 triticale, secale, einkorn, spelt, emmer, teff, milo, flax, gamma grass, *Tripsacum*, or *teosinte*. In some embodiments, the cereal is sorghum.

In some embodiments, the polypeptide is expressed in a specific location or tissue of a plant. In some embodiments, the location or tissue includes, but is not limited to, epidermis, vascular tissue, meristem, 20 cambium, cortex, or pith. In some embodiments, the location or tissue is leaf or sheath, root, flower, and developing ovule or seed. In some embodiments, the location or tissue can be, for example, epidermis, root, vascular tissue, meristem, cambium, cortex, pith, leaf, or flower. In some 25 embodiments, the location or tissue is a seed.

In some embodiments, the polypeptide sequence is encoded by a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered SEQ ID NO: 1-105 or a 30 fragment, domain, or feature thereof or a sequence complementary thereto, wherein the nucleotide sequence includes a deletion or insertion of at least one nucleotide. In some embodiments, the deletion or insertion is of less than about thirty nucleotides. In some embodiments, the deletion or

insertion is of less than about five nucleotides. In some embodiments, the polypeptide sequence encoded by a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered SEQ ID NO: 1-105, or a fragment, domain, or feature thereof or a sequence complementary thereto, includes a substitution of at least one codon. In some embodiments, the substitution is conservative. In some embodiments, the polypeptide sequences having substantial identity to the polypeptide sequence listed in even numbered SEQ ID NO: 2-106, or a fragment, domain, repeat, feature, or chimera thereof, includes a deletion or insertion of at least one amino acid.

The polypeptides of the presently disclosed subject matter, fragments thereof, or variants thereof, can comprise any number of contiguous amino acid residues from a polypeptide of the presently disclosed subject matter, wherein the number of residues is selected from the group of integers consisting of from 10 to the number of residues in a full-length polypeptide of the presently disclosed subject matter. In some embodiments, the portion or fragment of the polypeptide is a functional polypeptide. The presently disclosed subject matter includes active polypeptides having specific activity of at least in some embodiments 20%, in some embodiments 30%, in some embodiments 40%, in some embodiments 50%, in some embodiments 60%, in some embodiments 70%, in some embodiments 80%, in some embodiments 90%, and in some embodiments 95% that of the native (non-synthetic) endogenous polypeptide. Further, the substrate specificity (k_{cat}/K_m) can be substantially identical to the native (non-synthetic), endogenous polypeptide. Typically the K_m will be at least in some embodiments 30%, in some embodiments 40%, in some embodiments 50% of the native, endogenous polypeptide; and in some embodiments at least 60%, in some embodiments 70%, in some embodiments 80%, and in some embodiments 90% of the native, endogenous polypeptide. Methods of assaying and quantifying measures of activity and substrate specificity are well known to those of skill in the art.

The isolated polypeptides of the presently disclosed subject matter can elicit production of an antibody specifically reactive to a polypeptide of the presently disclosed subject matter when presented as an immunogen. Therefore, the polypeptides of the presently disclosed subject matter can be employed as immunogens for constructing antibodies immunoreactive to a polypeptide of the presently disclosed subject matter for such purposes including, but not limited to, immunoassays or polypeptide purification techniques. Immunoassays for determining binding are well known to those of skill in the art and include, but are not limited to enzyme-linked immunosorbent assays (ELISA) and competitive immunoassays.

Embodiments of the presently disclosed subject matter also relate to chimeric polypeptides encoded by the isolated nucleic acid molecules of the present disclosure including a chimeric polypeptide containing a polypeptide sequence encoded by an isolated nucleic acid containing a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered SEQ ID NO: 1-105, or an exon, domain, or feature thereof;
- (b) a nucleotide sequence complementary (for example, fully complementary) to (a); and
- (c) a nucleotide sequence which is the reverse complement (for example, full reverse complement) of (a);
- (d) or a functional fragment thereof.

IV. Controlling and Altering the Expression of Nucleic Acid Molecules

IV.A. General Considerations

One aspect of the presently disclosed subject matter provides compositions and methods for altering (*i.e.* increasing or decreasing) the level of nucleic acid molecules and/or polypeptides of the presently disclosed subject matter in plants. In particular, the nucleic acid molecules and polypeptides of the presently disclosed subject matter are expressed

constitutively, temporally, or spatially (*e.g.* at developmental stages), in certain tissues, and/or quantities, which are uncharacteristic of non-recombinantly engineered plants. Therefore, the presently disclosed subject matter provides utility in such exemplary applications as altering the
5 specified characteristics identified above.

The isolated nucleic acid molecules of the presently disclosed subject matter are useful for expressing a polypeptide of the presently disclosed subject matter in a recombinantly engineered cell such as a bacterial, yeast, insect, mammalian, or plant cell. Expressing cells can produce the
10 polypeptide in a non-natural condition (*e.g.* in quantity, composition, location and/or time) because they have been genetically altered to do so. Those skilled in the art are knowledgeable in the numerous expression systems available for expression of nucleic acids encoding a polypeptide of the presently disclosed subject matter.

15 Embodiments of the presently disclosed subject matter provide an expression cassette comprising a promoter sequence operatively linked to an isolated nucleic acid, the isolated nucleic acid comprising:

- (a) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing
20 step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof;
- (b) a nucleotide sequence complementary (for example, fully complementary) to (a); and
- 25 (c) a nucleotide sequence that is the reverse complement (for example, the full reverse complement) of (a).

Further encompassed within the presently disclosed subject matter is a recombinant vector comprising an expression cassette according to the embodiments of the presently disclosed subject matter. Also encompassed
30 are plant cells comprising expression cassettes according to the present disclosure, and plants comprising these plant cells. In some embodiments, the plant is a dicot. In some embodiments, the plant is a gymnosperm. In some embodiments, the plant is a monocot. In some embodiments, the

monocot is a cereal. In some embodiments, the cereal is, for example, maize, wheat, barley, oats, rye, millet, sorghum, triticale, secale, einkorn, spelt, emmer, teff, milo, flax, gramma grass, *Tripsacum* or *teosinte*. In some embodiments, the cereal is sorghum.

5 In some embodiments, the expression cassette is expressed throughout the plant. In some embodiments, the expression cassette is expressed in a specific location or tissue of a plant. In some embodiments, the location or tissue includes, but is not limited to, epidermis, root, vascular tissue, meristem, cambium, cortex, pith, leaf, flower, and combinations
10 thereof. In some embodiments, the location or tissue is a seed.

 In some embodiments, the expression cassette is involved in a function including, but not limited to, disease resistance, yield, abiotic stress resistance, nutritional quality, carbon metabolism, photosynthesis, signal transduction, cell growth, reproduction, disease processes (for example,
15 pathogen resistance), gene regulation, and differentiation. In some embodiments, the chimeric polypeptide is involved in a function such as abiotic stress tolerance, enhanced yield, disease resistance, or nutritional composition. In some embodiments, the abiotic stress tolerance is drought resistance.

20 Embodiments of the presently disclosed subject matter also relate to an expression vector comprising a nucleic acid molecule selected from the group consisting of:

- 25 (a) a nucleic acid encoding a polypeptide as listed in even numbered sequences of SEQ ID NOs: 2-106, or ortholog thereof;
- (b) a fragment, domain, or featured region of a nucleic acid sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered
30 sequences of SEQ ID NOs: 1-105; and
- (c) a complete nucleic acid sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide

sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment thereof, in combination with a heterologous sequence.

5 In some embodiments, the expression vector comprises one or more elements including, but not limited to, a promoter-enhancer sequence, a selection marker sequence, an origin of replication, an epitope tag-encoding sequence, and an affinity purification tag-encoding sequence. In some
10 embodiments, the promoter-enhancer sequence comprises, for example, the cauliflower mosaic virus (CaMV) 35S promoter, the CaMV 19S promoter, the tobacco PR-1a promoter, the ubiquitin promoter, or the phaseolin promoter. In some embodiments, the promoter is operable in plants, and in some
15 embodiments, the promoter is a constitutive or inducible promoter. In some embodiments, the selection marker sequence encodes an antibiotic resistance gene. In some embodiments, the epitope tag sequence encodes V5 (GKPIP NPLLGLDST; SEQ ID NO: 107; Southern *et al.*, 1991), the peptide Phe-His-His-Thr-Thr (SEQ ID NO: 108), hemagglutinin, or
20 glutathione-S-transferase. In some embodiments the affinity purification tag sequence encodes a polyamino acid sequence or a polypeptide. In some embodiments, the polyamino acid sequence comprises polyhistidine. In
some embodiments, the polypeptide is chitin-binding domain or glutathione-S-transferase. In some embodiments, the affinity purification tag sequence
comprises an intein encoding sequence.

25 In some embodiments, the expression vector comprises a eukaryotic expression vector, and in some embodiments, the expression vector comprises a prokaryotic expression vector. In some embodiments, the eukaryotic expression vector comprises a tissue-specific promoter. In some
embodiments, the expression vector is operable in plants.

30 Embodiments of the presently disclosed subject matter also relate to a cell comprising a nucleic acid construct comprising an expression vector and a nucleic acid comprising a nucleic acid encoding a polypeptide that is an ortholog of a polypeptide as listed in even numbered sequences of SEQ ID NOs: 2-106, or a nucleic acid sequence that hybridizes under conditions
of hybridization of 45°C in 1 M NaCl, followed by a final washing step at

50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a subsequence thereof, in combination with a heterologous sequence.

5 In some embodiments, the cell is a bacterial cell, a fungal cell, a plant cell, or an animal cell. In some embodiments, the polypeptide is expressed in a specific location or tissue of a plant. In some embodiments, the location or tissue includes, but is not limited to, epidermis, root, vascular tissue, meristem, cambium, cortex, pith, leaf, flower, and combinations thereof. In some embodiments, the location or tissue is a seed. In some embodiments, 10 the polypeptide is involved in a function such as, for example, carbon metabolism, photosynthesis, signal transduction, cell growth, reproduction, disease processes, gene regulation, and differentiation. In some embodiments, the polypeptide is involved in a function such as abiotic stress tolerance, enhanced yield, disease resistance, or nutritional composition.

15 Prokaryotic cells including, but not limited to, *Escherichia coli* and other microbial strains known to those in the art, can be used as host cells. Methods for expressing polypeptides in prokaryotic cells are well known to those in the art and can be found in many laboratory manuals such as Sambrook & Russell, 2001. A variety of promoters, ribosome binding sites, 20 and operators to control expression are available to those skilled in the art, as are selectable markers such as antibiotic resistance genes. The type of vector is chosen to allow for optimal growth and expression in the selected cell type.

25 A variety of eukaryotic expression systems are available such as, for example, yeast, insect cell lines, plant cells, and mammalian cells. Expression and synthesis of heterologous polypeptides in yeast is well known (see Sherman *et al.*, 1982). Yeast strains widely used for production of eukaryotic polypeptides are *Saccharomyces cerevisiae* and *Pichia pastoris*, and vectors, strains, and protocols for expression are available 30 from commercial suppliers (e.g., Invitrogen Corp., Carlsbad, California, United States of America).

Mammalian cell systems can be transformed with expression vectors for production of polypeptides. Suitable host cell lines available to those in

the art include, but are not limited to, the HEK293, BHK21 and CHO cells lines. Expression vectors for these cells can include expression control sequences such as an origin of replication, a promoter, (e.g., the CMV promoter, a Herpes Simplex Virus thymidine kinase (HSV-tk) promoter or phosphoglycerate kinase (pgk) promoter), an enhancer, and polypeptide processing sites such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcription terminator sequences. Other animal cell lines useful for the production of polypeptides are available commercially or from depositories such as the American Type Culture Collection (Manassas, Virginia, United States of America).

Expression vectors for expressing polypeptides in insect cells are usually derived from baculovirus or other viruses known in the art. A number of suitable insect cell lines are available including, but not limited to, mosquito larvae, silkworm, armyworm (for example, *Spodoptera frugiperda*), moth, and *Drosophila* cell lines.

Methods of transforming animal and lower eukaryotic cells are known. Numerous methods can be used to introduce exogenous DNA into eukaryotic cells including, but not limited to, calcium phosphate precipitation, fusion of the recipient cell with bacterial protoplasts containing the DNA, treatment of the recipient cells with liposomes containing the DNA, DEAE dextran, electroporation, biolistics, and microinjection of the DNA directly into the cells. Transformed cells are cultured using means well known in the art (see Kuchler, 1997).

Once a polypeptide of the presently disclosed subject matter is expressed it can be isolated and purified from the expressing cells using methods known to those skilled in the art. The purification process can be monitored using Western blot techniques, radioimmunoassay, or other standard immunoassay techniques. Polypeptide purification techniques are commonly known and used by those skilled in the art (see Scopes, 1982; Deutscher, 1990).

Embodiments of the presently disclosed subject matter provide a method for producing a recombinant polypeptide in which the expression vector comprise one or more elements including, but not limited to, a

promoter-enhancer sequence, a selection marker sequence, an origin of replication, an epitope tag-encoding sequence, and an affinity purification tag-encoding sequence. In some embodiments, the nucleic acid construct comprises an epitope tag-encoding sequence and the isolating step employs
5 an antibody specific for the epitope tag. In some embodiments, the nucleic acid construct comprises a polyamino acid-encoding sequence and the isolating step employs a resin comprising a polyamino acid binding substance, in some embodiments where the polyamino acid is polyhistidine and the polyamino acid binding resin is nickel-charged agarose resin. In
10 some embodiments, the nucleic acid construct comprises a polypeptide-encoding sequence and the isolating step employs a resin comprising a polypeptide binding substance. In some embodiments, the polypeptide is a chitin-binding domain and the resin contains chitin-sepharose.

The polypeptides of the presently disclosed subject matter can be
15 synthesized using non-cellular synthetic methods known to those in the art. Techniques for solid phase synthesis are disclosed in Barany & Merrifield, 1980; Merrifield *et al.*, 1963; Stewart & Young, 1984.

The presently disclosed subject matter further provides a method for modifying (*i.e.* increasing or decreasing) the concentration or composition of
20 a polypeptide of the presently disclosed subject matter in a plant or part thereof. Modification can be effected by increasing or decreasing the concentration and/or the composition (*i.e.* the ration of the polypeptides of the presently disclosed subject matter) in a plant. The method comprises introducing into a plant cell an expression cassette comprising a nucleic acid
25 molecule of the presently disclosed subject matter as disclosed above to obtain a transformed plant cell or tissue, and culturing the transformed plant cell or tissue. The nucleic acid molecule can be under the regulation of a constitutive or inducible promoter. The method can further comprise inducing or repressing expression of a nucleic acid molecule of a sequence
30 in the plant for a time sufficient to modify the concentration and/or composition in the plant or plant part.

A plant or plant part having modified expression of a nucleic acid molecule of the presently disclosed subject matter can be analyzed and

selected using methods known to those skilled in the art including, but not limited to, Southern blotting, DNA sequencing, or PCR analysis using primers specific to the nucleic acid molecule and detecting amplicons produced therefrom.

5 In general, a concentration or composition is increased or decreased by at least in some embodiments 5%, in some embodiments 10%, in some embodiments 20%, in some embodiments 30%, in some embodiments 40%, in some embodiments 50%, in some embodiments 60%, in some
10 embodiments 70%, in some embodiments 80%, and in some embodiments 90% relative to a native control plant, plant part, or cell lacking the expression cassette.

IV.B. Alteration of Expression of Nucleic Acid Molecules

15 The alteration in expression of the nucleic acid molecules of the presently disclosed subject matter can be achieved, for example, in one of the following ways:

IV.B.1. "Sense" Suppression

20 Alteration of the expression of a nucleotide sequence of the presently disclosed subject matter, in some embodiments reduction of its expression, is obtained by "sense" suppression (referenced in *e.g.* Jorgensen *et al.*, 1996). In this case, the entirety or a portion of a nucleotide sequence of the presently disclosed subject matter is comprised in a DNA molecule. The DNA molecule can be operatively linked to a promoter functional in a cell comprising the target gene, in some embodiments a plant cell, and introduced into the cell, in which the nucleotide sequence is expressible.
25 The nucleotide sequence is inserted in the DNA molecule in the "sense orientation", meaning that the coding strand of the nucleotide sequence can be transcribed. In some embodiments, the nucleotide sequence is fully translatable and all the genetic information comprised in the nucleotide sequence, or portion thereof, is translated into a polypeptide. In some
30 embodiments, the nucleotide sequence is partially translatable and a short peptide is translated. In some embodiments, this is achieved by inserting at least one premature stop codon in the nucleotide sequence, which brings translation to a halt. In some embodiments, the nucleotide sequence is

transcribed but no translation product is made. This is usually achieved by removing the start codon, *i.e.* the "ATG", of the polypeptide encoded by the nucleotide sequence. In some embodiments, the DNA molecule comprising the nucleotide sequence, or a portion thereof, is stably integrated in the genome of the plant cell. In some embodiments, the DNA molecule comprising the nucleotide sequence, or a portion thereof, is comprised in an extrachromosomally replicating molecule.

In transgenic plants containing one of the DNA molecules disclosed immediately above, the expression of the nucleotide sequence corresponding to the nucleotide sequence comprised in the DNA molecule can be reduced. The nucleotide sequence in the DNA molecule in some embodiments is at least 70% identical to the nucleotide sequence the expression of which is reduced, in some embodiments is at least 80% identical, in some embodiments is at least 90% identical, in some embodiments is at least 95% identical, and in some embodiments is at least 99% identical.

IV.B.2. "Antisense" Suppression

In some embodiments, the alteration of the expression of a nucleotide sequence of the presently disclosed subject matter, for example the reduction of its expression, is obtained by "antisense" suppression. The entirety or a portion of a nucleotide sequence of the presently disclosed subject matter is comprised in a DNA molecule. The DNA molecule can be operatively linked to a promoter functional in a plant cell, and introduced in a plant cell, in which the nucleotide sequence is expressible. The nucleotide sequence is inserted in the DNA molecule in the "antisense orientation", meaning that the reverse complement (also called sometimes non-coding strand) of the nucleotide sequence can be transcribed. In some embodiments, the DNA molecule comprising the nucleotide sequence, or a portion thereof, is stably integrated in the genome of the plant cell. In some embodiments the DNA molecule comprising the nucleotide sequence, or a portion thereof, is comprised in an extrachromosomally replicating molecule. Several publications describing this approach are cited for further illustration

(Green *et al.*, 1986; van der Krol *et al.*, 1991; Powell *et al.*, 1989; Ecker & Davis, 1986).

5 In transgenic plants containing one of the DNA molecules disclosed immediately above, the expression of the nucleotide sequence corresponding to the nucleotide sequence comprised in the DNA molecule can be reduced. The nucleotide sequence in the DNA molecule is in some
10 embodiments at least 70% identical to the nucleotide sequence the expression of which is reduced, in some embodiments at least 80% identical, in some embodiments at least 90% identical, in some embodiments at least 95% identical, and in some embodiments at least 99% identical.

IV.B.3. Homologous Recombination

15 In some embodiments, at least one genomic copy corresponding to a nucleotide sequence of the presently disclosed subject matter is modified in the genome of the plant by homologous recombination as further illustrated in Paszkowski *et al.*, 1988. This technique uses the ability of homologous
20 sequences to recognize each other and to exchange nucleotide sequences between respective nucleic acid molecules by a process known in the art as homologous recombination. Homologous recombination can occur between the chromosomal copy of a nucleotide sequence in a cell and an incoming copy of the nucleotide sequence introduced in the cell by transformation. Specific modifications are thus accurately introduced in the chromosomal
25 copy of the nucleotide sequence. In some embodiments, the regulatory elements of the nucleotide sequence of the presently disclosed subject matter are modified. Such regulatory elements are easily obtainable by screening a genomic library using the nucleotide sequence of the presently disclosed subject matter, or a portion thereof, as a probe. The existing
30 regulatory elements are replaced by different regulatory elements, thus altering expression of the nucleotide sequence, or they are mutated or deleted, thus abolishing the expression of the nucleotide sequence. In some embodiments, the nucleotide sequence is modified by deletion of a part of the nucleotide sequence or the entire nucleotide sequence, or by mutation. Expression of a mutated polypeptide in a plant cell is also provided in the

presently disclosed subject matter. Recent refinements of this technique to disrupt endogenous plant genes have been disclosed (Kempin *et al.*, 1997 and Miao & Lam, 1995).

5 In some embodiments, a mutation in the chromosomal copy of a nucleotide sequence is introduced by transforming a cell with a chimeric oligonucleotide composed of a contiguous stretch of RNA and DNA residues in a duplex conformation with double hairpin caps on the ends. An additional feature of the oligonucleotide is for example the presence of 2'-O-methylation at the RNA residues. The RNA/DNA sequence is
10 designed to align with the sequence of a chromosomal copy of a nucleotide sequence of the presently disclosed subject matter and to contain the desired nucleotide change. For example, this technique is further illustrated in U.S. Patent No. 5,501,967 and Zhu *et al.*, 1999.

IV.B.4. Ribozymes

15 In some embodiments, an RNA coding for a polypeptide of the presently disclosed subject matter is cleaved by a catalytic RNA, or ribozyme, specific for such RNA. The ribozyme is expressed in transgenic plants and results in reduced amounts of RNA coding for the polypeptide of the presently disclosed subject matter in plant cells, thus leading to
20 reduced amounts of polypeptide accumulated in the cells. This method is further illustrated in U.S. Patent No. 4,987,071.

IV.B.5. Dominant-Negative Mutants

In some embodiments, the activity of a polypeptide encoded by the nucleotide sequences of the presently disclosed subject matter is changed.
25 This is achieved by expression of dominant negative mutants of the polypeptides in transgenic plants, leading to the loss of activity of the endogenous polypeptide.

IV.B.6. Aptamers

30 In some embodiments, the activity of polypeptide of the presently disclosed subject matter is inhibited by expressing in transgenic plants nucleic acid ligands, so-called aptamers, which specifically bind to the polypeptide. Aptamers can be obtained by the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) method. In the SELEX

method, a candidate mixture of single stranded nucleic acids having regions of randomized sequence is contacted with the polypeptide and those nucleic acids having an increased affinity to the target are partitioned from the remainder of the candidate mixture. The partitioned nucleic acids are amplified to yield a ligand-enriched mixture. After several iterations a nucleic acid with optimal affinity to the polypeptide is obtained and is used for expression in transgenic plants. This method is further illustrated in U.S. Patent No. 5,270,163.

IV.B.7. Zinc Finger Polypeptides

A zinc finger polypeptide that binds a nucleotide sequence of the presently disclosed subject matter or to its regulatory region can also be used to alter expression of the nucleotide sequence. In alternative embodiments, transcription of the nucleotide sequence is reduced or increased. Zinc finger polypeptides are disclosed in, for example, Beerli *et al.*, 1998, or in WO 95/19431, WO 98/54311, or WO 96/06166, all incorporated herein by reference in their entirety.

IV.B.8. dsRNA

Alteration of the expression of a nucleotide sequence of the presently disclosed subject matter can also be obtained by double stranded RNA (dsRNA) interference (RNAi) as disclosed, for example, in WO 99/32619, WO 99/53050, or WO 99/61631, all incorporated herein by reference in their entirety. In some embodiments, the alteration of the expression of a nucleotide sequence of the presently disclosed subject matter, in some embodiments the reduction of its expression, is obtained by dsRNA interference. The entirety, or in some embodiments a portion, of a nucleotide sequence of the presently disclosed subject matter, can be comprised in a DNA molecule. The size of the DNA molecule is in some embodiments from 100 to 1000 nucleotides or more; the optimal size to be determined empirically. Two copies of the identical DNA molecule are linked, separated by a spacer DNA molecule, such that the first and second copies are in opposite orientations. In some embodiments, the first copy of the DNA molecule is the reverse complement (also known as the non-coding strand) and the second copy is the coding strand; In some embodiments, the

first copy is the coding strand, and the second copy is the reverse complement. The size of the spacer DNA molecule is in some embodiments 200 to 10,000 nucleotides, in some embodiments 400 to 5000 nucleotides, and in some embodiments 600 to 1500 nucleotides in length. The spacer is
5 in some embodiments a random piece of DNA, in some embodiments a random piece of DNA without homology to the target organism for dsRNA interference, and in some embodiments a functional intron that is effectively spliced by the target organism. The two copies of the DNA molecule separated by the spacer are operatively linked to a promoter functional in a
10 plant cell, and introduced in a plant cell in which the nucleotide sequence is expressible. In some embodiments, the DNA molecule comprising the nucleotide sequence, or a portion thereof, is stably integrated in the genome of the plant cell. In some embodiments, the DNA molecule comprising the nucleotide sequence, or a portion thereof, is comprised in an
15 extrachromosomally replicating molecule. Several publications describing this approach are cited for further illustration (Waterhouse *et al.*, 1998; Chuang & Meyerowitz, 2000; Smith *et al.*, 2000).

In transgenic plants containing one of the DNA molecules disclosed immediately above, the expression of the nucleotide sequence
20 corresponding to the nucleotide sequence comprised in the DNA molecule is in some embodiments reduced. In some embodiments, the nucleotide sequence in the DNA molecule is at least 70% identical to the nucleotide sequence the expression of which is reduced, in some embodiments it is at least 80% identical, in some embodiments it is at least 90% identical, in
25 some embodiments it is at least 95% identical, and in some embodiments it is at least 99% identical.

IV.B.9. Insertion of a DNA Molecule (Insertional Mutagenesis)

In some embodiments, a DNA molecule is inserted into a chromosomal copy of a nucleotide sequence of the presently disclosed
30 subject matter, or into a regulatory region thereof. In some embodiments, such DNA molecule comprises a transposable element capable of transposition in a plant cell, such as, for example, Ac/Ds, Em/Spm, mutator. Alternatively, the DNA molecule comprises a T-DNA border of an

5 *Agrobacterium* T-DNA. The DNA molecule can also comprise a recombinase or integrase recognition site that can be used to remove part of the DNA molecule from the chromosome of the plant cell. Methods of insertional mutagenesis using T-DNA, transposons, oligonucleotides, or other methods known to those skilled in the art are also encompassed. Methods of using T-DNA and transposon for insertional mutagenesis are disclosed in Winkler & Feldmann, 1989, and Martienssen, 1998, incorporated herein by reference in their entireties.

10 IV.B.10. Deletion Mutagenesis

In some embodiments, a mutation of a nucleic acid molecule of the presently disclosed subject matter is created in the genomic copy of the sequence in the cell or plant by deletion of a portion of the nucleotide sequence or regulator sequence. Methods of deletion mutagenesis are known to those skilled in the art. See e.g., Miao & Lam, 1995.

15 In some embodiments, a deletion is created at random in a large population of plants by chemical mutagenesis or irradiation and a plant with a deletion in a gene of the presently disclosed subject matter is isolated by forward or reverse genetics. Irradiation with fast neutrons or gamma rays is known to cause deletion mutations in plants (Silverstone *et al.*, 1998; Bruggemann *et al.*, 1996; Redei & Koncz, 1992). Deletion mutations in a gene of the presently disclosed subject matter can be recovered in a reverse genetics strategy using PCR with pooled sets of genomic DNAs as has been shown in *C. elegans* (Liu *et al.*, 1999). A forward genetics strategy involves mutagenesis of a line bearing a trait of interest followed by screening the M2 progeny for the absence of the trait. Among these mutants would be expected to be some that disrupt a gene of the presently disclosed subject matter. This could be assessed by Southern blotting or PCR using primers designed for a gene of the presently disclosed subject matter with genomic DNA from these mutants.

25 IV.B.11. Overexpression in a Plant Cell

30 In some embodiments, a nucleotide sequence of the presently disclosed subject matter encoding a polypeptide is over-expressed. Examples of nucleic acid molecules and expression cassettes for over-

expression of a nucleic acid molecule of the presently disclosed subject matter are disclosed above. Methods known to those skilled in the art of over-expression of nucleic acid molecules are also encompassed by the presently disclosed subject matter.

5 In some embodiments, the expression of the nucleotide sequence of the presently disclosed subject matter is altered in every cell of a plant. This can be obtained, for example, through homologous recombination or by insertion into a chromosome. This can also be obtained, for example, by expressing a sense or antisense RNA, zinc finger polypeptide or ribozyme
10 under the control of a promoter capable of expressing the sense or antisense RNA, zinc finger polypeptide, or ribozyme in every cell of a plant. Constitutive, inducible, tissue-specific, or developmentally-regulated expression are also within the scope of the presently disclosed subject matter and result in a constitutive, inducible, tissue-specific, or
15 developmentally-regulated alteration of the expression of a nucleotide sequence of the presently disclosed subject matter in the plant cell. Constructs for expression of the sense or antisense RNA, zinc finger polypeptide, or ribozyme, or for over-expression of a nucleotide sequence of the presently disclosed subject matter, can be prepared and transformed
20 into a plant cell according to the teachings of the presently disclosed subject matter, for example, as disclosed herein.

IV.C. Construction of Plant Expression Vectors

 Coding sequences intended for expression in transgenic plants can be first assembled in expression cassettes operatively linked to a suitable
25 promoter expressible in plants. The expression cassettes can also comprise any further sequences required or selected for the expression of the transgene. Such sequences include, but are not limited to, transcription terminators, extraneous sequences to enhance expression such as introns, vital sequences, and sequences intended for the targeting of the gene
30 product to specific organelles and cell compartments. These expression cassettes can then be easily transferred to the plant transformation vectors disclosed below. The following is a description of various components of typical expression cassettes.

IV.C.1. Promoters

The selection of the promoter used in expression cassettes can determine the spatial and temporal expression pattern of the transgene in the transgenic plant. Selected promoters can express transgenes in specific cell types (such as leaf epidermal cells, mesophyll cells, root cortex cells) or in specific tissues or organs (roots, leaves, or flowers, for example) and the selection can reflect the desired location for accumulation of the gene product. Alternatively, the selected promoter can drive expression of the gene under various inducing conditions. Promoters vary in their strength; *i.e.*, their abilities to promote transcription. Depending upon the host cell system utilized, any one of a number of suitable promoters can be used, including the gene's native promoter. The following are non-limiting examples of promoters that can be used in expression cassettes.

IV.C.1.a. Constitutive Expression: the Ubiquitin Promoter

Ubiquitin is a gene product known to accumulate in many cell types and its promoter has been cloned from several species for use in transgenic plants (*e.g.* sunflower - Binet *et al.*, 1991; maize - Christensen & Quail, 1989; and *Arabidopsis* - Callis *et al.*, 1990; Norris *et al.*, 1993). The maize ubiquitin promoter has been developed in transgenic monocot systems and its sequence and vectors constructed for monocot transformation are disclosed in the patent publication EP 0 342 926 (to Lubrizol) which is herein incorporated by reference. Taylor *et al.*, 1993, describes a vector (pAHC25) that comprises the maize ubiquitin promoter and first intron and its high activity in cell suspensions of numerous monocotyledons when introduced via microprojectile bombardment. The *Arabidopsis* ubiquitin promoter is suitable for use with the nucleotide sequences of the presently disclosed subject matter. The ubiquitin promoter is suitable for gene expression in transgenic plants, both monocotyledons and dicotyledons. Suitable vectors are derivatives of pAHC25 or any of the transformation vectors disclosed herein, modified by the introduction of the appropriate ubiquitin promoter and/or intron sequences.

IV.C.1.b. Constitutive Expression: the CaMV 35S Promoter

Construction of the plasmid pCGN1761 is disclosed in the published patent application EP 0 392 225 (Example 23), which is hereby incorporated by reference. pCGN1761 contains the "double" CaMV 35S promoter and the *tml* transcriptional terminator with a unique *EcoRI* site between the promoter and the terminator and has a pUC-type backbone. A derivative of pCGN1761 is constructed which has a modified polylinker that includes *NotI* and *XhoI* sites in addition to the existing *EcoRI* site. This derivative is designated pCGN1761ENX. pCGN1761ENX is useful for the cloning of cDNA sequences or coding sequences (including microbial ORF sequences) within its polylinker for the purpose of their expression under the control of the 35S promoter in transgenic plants. The entire 35S promoter-coding sequence-*tml* terminator cassette of such a construction can be excised by *HindIII*, *SphI*, *Sall*, and *XbaI* sites 5' to the promoter and *XbaI*, *BamHI* and *BglI* sites 3' to the terminator for transfer to transformation vectors such as those disclosed below. Furthermore, the double 35S promoter fragment can be removed by 5' excision with *HindIII*, *SphI*, *Sall*, *XbaI*, or *PstI*, and 3' excision with any of the polylinker restriction sites (*EcoRI*, *NotI* or *XhoI*) for replacement with another promoter. If desired, modifications around the cloning sites can be made by the introduction of sequences that can enhance translation. This is particularly useful when overexpression is desired. For example, pCGN1761ENX can be modified by optimization of the translational initiation site as disclosed in Example 37 of U.S. Patent No. 5,639,949, incorporated herein by reference.

IV.C.1.c. Constitutive Expression: the Actin Promoter

Several isoforms of actin are known to be expressed in most cell types and consequently the actin promoter can be used as a constitutive promoter. In particular, the promoter from the rice *Act1* gene has been cloned and characterized (McElroy *et al.*, 1990). A 1.3 kilobase (kb) fragment of the promoter was found to contain all the regulatory elements required for expression in rice protoplasts. Furthermore, numerous expression vectors based on the *Act1* promoter have been constructed specifically for use in monocotyledons (McElroy *et al.*, 1991). These

incorporate the *Act1*-intron 1, *Adhl* 5' flanking sequence (from the maize alcohol dehydrogenase gene) and *Adhl*-intron 1 and sequence from the CaMV 35S promoter. Vectors showing highest expression were fusions of 35S and *Act1* intron or the *Act1* 5' flanking sequence and the *Act1* intron.

5 Optimization of sequences around the initiating ATG (of the β -glucuronidase (GUS) reporter gene) also enhanced expression. The promoter expression cassettes disclosed in McElroy *et al.*, 1991, can be easily modified for gene expression and are particularly suitable for use in monocotyledonous hosts. For example, promoter-containing fragments are removed from the McElroy

10 constructions and used to replace the double 35S promoter in pCGN1761ENX, which is then available for the insertion of specific gene sequences. The fusion genes thus constructed can then be transferred to appropriate transformation vectors. In a separate report, the rice *Act1* promoter with its first intron has also been found to direct high expression in

15 cultured barley cells (Chibbar *et al.*, 1993).

IV.C.1.d. Inducible Expression: PR-1 Promoters

The double 35S promoter in pCGN1761ENX can be replaced with any other promoter of choice that will result in suitably high expression levels. By way of example, one of the chemically regulatable promoters

20 disclosed in U.S. Patent No. 5,614,395, such as the tobacco PR-1a promoter, can replace the double 35S promoter. Alternately, the *Arabidopsis* PR-1 promoter disclosed in Lebel *et al.*, 1998, can be used. The promoter of choice can be excised from its source by restriction enzymes, but can alternatively be PCR-amplified using primers that carry

25 appropriate terminal restriction sites. Should PCR-amplification be undertaken, the promoter can be re-sequenced to check for amplification errors after the cloning of the amplified promoter in the target vector. The chemically/pathogen regulatable tobacco PR-1a promoter is cleaved from

30 plasmid pCIB1004 (for construction, see example 21 of EP 0 332 104, which is hereby incorporated by reference) and transferred to plasmid pCGN1761ENX (Uknes *et al.*, 1992). pCIB1004 is cleaved with *Nco*I and the resulting 3' overhang of the linearized fragment is rendered blunt by treatment with T4 DNA polymerase. The fragment is then cleaved with

*Hind*III and the resultant PR-1a promoter-containing fragment is gel purified and cloned into pCGN1761ENX from which the double 35S promoter has been removed. This is accomplished by cleavage with *Xho*I and blunting with T4 polymerase, followed by cleavage with *Hind*III, and isolation of the larger vector-terminator containing fragment into which the pCIB1004 promoter fragment is cloned. This generates a pCGN1761ENX derivative with the PR-1a promoter and the *tml* terminator and an intervening polylinker with unique *Eco*RI and *Not*I sites. The selected coding sequence can be inserted into this vector, and the fusion products (*i.e.* promoter-gene-terminator) can subsequently be transferred to any selected transformation vector, including those disclosed herein. Various chemical regulators can be employed to induce expression of the selected coding sequence in the plants transformed according to the presently disclosed subject matter, including the benzothiadiazole, isonicotinic acid, and salicylic acid compounds disclosed in U.S. Patent Nos. 5,523,311 and 5,614,395.

IV.C.1.e. Inducible Expression: an Ethanol-Inducible Promoter

A promoter inducible by certain alcohols or ketones, such as ethanol, can also be used to confer inducible expression of a coding sequence of the presently disclosed subject matter. Such a promoter is for example the *alcA* gene promoter from *Aspergillus nidulans* (Caddick *et al.*, 1998). In *A. nidulans*, the *alcA* gene encodes alcohol dehydrogenase I, the expression of which is regulated by the AlcR transcription factors in presence of the chemical inducer. For the purposes of the presently disclosed subject matter, the CAT coding sequences in plasmid *palcA*:CAT comprising a *alcA* gene promoter sequence fused to a minimal 35S promoter (Caddick *et al.*, 1998) are replaced by a coding sequence of the presently disclosed subject matter to form an expression cassette having the coding sequence under the control of the *alcA* gene promoter. This is carried out using methods known in the art.

IV.C.1.f. Inducible Expression: a Glucocorticoid-Inducible Promoter

Induction of expression of a nucleic acid sequence of the presently disclosed subject matter using systems based on steroid hormones is also

provided. For example, a glucocorticoid-mediated induction system is used (Aoyama & Chua, 1997) and gene expression is induced by application of a glucocorticoid, for example a synthetic glucocorticoid, for example dexamethasone, at a concentration ranging in some embodiments from 0.1 mM to 1 mM, and in some embodiments from 10 mM to 100 mM. For the purposes of the presently disclosed subject matter, the luciferase gene sequences Aoyama & Chua, 1997 are replaced by a nucleic acid sequence of the presently disclosed subject matter to form an expression cassette having a nucleic acid sequence of the presently disclosed subject matter under the control of six copies of the GAL4 upstream activating sequences fused to the 35S minimal promoter. This is carried out using methods known in the art. The trans-acting factor comprises the GAL4 DNA-binding domain (Keegan *et al.*, 1986) fused to the transactivating domain of the herpes viral polypeptide VP16 (Triezenberg *et al.*, 1988) fused to the hormone-binding domain of the rat glucocorticoid receptor (Picard *et al.*, 1988). The expression of the fusion polypeptide is controlled either by a promoter known in the art or disclosed herein. A plant comprising an expression cassette comprising a nucleic acid sequence of the presently disclosed subject matter fused to the 6x GAL4/minimal promoter is also provided. Thus, tissue- or organ-specificity of the fusion polypeptide is achieved leading to inducible tissue- or organ-specificity of the nucleic acid sequence to be expressed.

IV.C.1.g. Root Specific Expression

Another pattern of gene expression is root expression. A suitable root promoter is the promoter of the maize metallothionein-like (MTL) gene disclosed in de Framond, 1991, and also in U.S. Patent No. 5,466,785, each of which is incorporated herein by reference. This "MTL" promoter is transferred to a suitable vector such as pCGN1761ENX for the insertion of a selected gene and subsequent transfer of the entire promoter-gene-terminator cassette to a transformation vector of interest.

IV.C.1.h. Wound-Inducible Promoters

Wound-inducible promoters can also be suitable for gene expression. Numerous such promoters have been disclosed (*e.g.* Xu *et al.*, 1993; Logemann *et al.*, 1989; Rohrmeier & Lehle, 1993; Firek *et al.*, 1993; Warner

et al., 1993) and all are suitable for use with the presently disclosed subject matter. Logemann *et al.* describe the 5' upstream sequences of the dicotyledonous potato *wun1* gene. Xu *et al.* show that a wound-inducible promoter from the dicotyledon potato (*pin2*) is active in the monocotyledon rice. Further, Rohrmeier & Lehle describe the cloning of the maize *Wip1* cDNA that is wound induced and which can be used to isolate the cognate promoter using standard techniques. Similarly, Firek *et al.* and Warner *et al.* have disclosed a wound-induced gene from the monocotyledon *Asparagus officinalis*, which is expressed at local wound and pathogen invasion sites. Using cloning techniques well known in the art, these promoters can be transferred to suitable vectors, fused to the genes pertaining to the presently disclosed subject matter, and used to express these genes at the sites of plant wounding.

IV.C.1.i. Pith-Preferred Expression

PCT International Publication WO 93/07278, which is herein incorporated by reference, describes the isolation of the maize *trpA* gene, which is preferentially expressed in pith cells. The gene sequence and promoter extending up to -1726 basepairs (bp) from the start of transcription are presented. Using standard molecular biological techniques, this promoter, or parts thereof, can be transferred to a vector such as pCGN1761 where it can replace the 35S promoter and be used to drive the expression of a foreign gene in a pith-preferred manner. In fact, fragments containing the pith-preferred promoter or parts thereof can be transferred to any vector and modified for utility in transgenic plants.

IV.C.1.j. Leaf-Specific Expression

A maize gene encoding phosphoenol carboxylase (PEPC) has been disclosed by Hudspeth & Grula, 1989. Using standard molecular biological techniques, the promoter for this gene can be used to drive the expression of any gene in a leaf-specific manner in transgenic plants.

IV.C.1.k. Pollen-Specific Expression

WO 93/07278 describes the isolation of the maize calcium-dependent protein kinase (CDPK) gene that is expressed in pollen cells. The gene sequence and promoter extend up to 1400 bp from the start of transcription.

Using standard molecular biological techniques, this promoter or parts thereof can be transferred to a vector such as pCGN1761 where it can replace the 35S promoter and be used to drive the expression of a nucleic acid sequence of the presently disclosed subject matter in a pollen-specific manner.

IV.C.2. Transcriptional Terminators

A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for termination of transcription and correct mRNA polyadenylation. Appropriate transcriptional terminators are those that are known to function in plants and include the CaMV 35S terminator, the *tm1* terminator, the nopaline synthase terminator, and the pea *rbcS* E9 terminator. These can be used in both monocotyledons and dicotyledons. In addition, a gene's native transcription terminator can be used.

IV.C.3. Sequences for the Enhancement or Regulation of Expression

Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes of the presently disclosed subject matter to increase their expression in transgenic plants.

Various intron sequences have been shown to enhance expression, particularly in monocotyledonous cells. For example, the introns of the maize *Adhl* gene have been found to significantly enhance the expression of the wild-type gene under its cognate promoter when introduced into maize cells. Intron 1 was found to be particularly effective and enhanced expression in fusion constructs with the chloramphenicol acetyltransferase gene (Callis *et al.*, 1987). In the same experimental system, the intron from the maize *bronze1* gene had a similar effect in enhancing expression. Intron sequences have been routinely incorporated into plant transformation vectors, typically within the non-translated leader.

A number of non-translated leader sequences derived from viruses are also known to enhance expression, and these are particularly effective in dicotyledonous cells. Specifically, leader sequences from Tobacco Mosaic

Virus (TMV; the "W-sequence"), Maize Chlorotic Mottle Virus (MCMV), and Alfalfa Mosaic Virus (AMV) have been shown to be effective in enhancing expression (see e.g. Gallie *et al.*, 1987; Skuzeski *et al.*, 1990). Other leader sequences known in the art include, but are not limited to, picornavirus
5 leaders, for example, EMCV (encephalomyocarditis virus) leader (5' noncoding region; see Elroy-Stein *et al.*, 1989); potyvirus leaders, for example, from Tobacco Etch Virus (TEV; see Allison *et al.*, 1986); Maize Dwarf Mosaic Virus (MDMV; see Kong & Steinbiss 1998); human immunoglobulin heavy-chain binding polypeptide (BiP) leader (Macejak &
10 Sarnow, 1991); untranslated leader from the coat polypeptide mRNA of alfalfa mosaic virus (AMV; RNA 4; see Jobling & Gehrke, 1987); tobacco mosaic virus (TMV) leader (Gallie *et al.*, 1989); and Maize Chlorotic Mottle Virus (MCMV) leader (Lommel *et al.*, 1991). See also, Della-Cioppa *et al.*, 1987.

15 In addition to incorporating one or more of the aforementioned elements into the 5' regulatory region of a target expression cassette of the presently disclosed subject matter, other elements can also be incorporated. Such elements include, but are not limited to, a minimal promoter. By minimal promoter it is intended that the basal promoter elements are inactive
20 or nearly so in the absence of upstream or downstream activation. Such a promoter has low background activity in plants when there is no transactivator present or when enhancer or response element binding sites are absent. One minimal promoter that is particularly useful for target genes in plants is the Bz1 minimal promoter, which is obtained from the *bronze1*
25 gene of maize. The Bz1 core promoter is obtained from the "myc" mutant Bz1-luciferase construct pBz1LucR98 via cleavage at the *NheI* site located at positions -53 to -58 (Roth *et al.*, 1991). The derived Bz1 core promoter fragment thus extends from positions -53 to +227 and includes the Bz1 intron-1 in the 5' untranslated region. Also useful for the presently disclosed
30 subject matter is a minimal promoter created by use of a synthetic TATA element. The TATA element allows recognition of the promoter by RNA polymerase factors and confers a basal level of gene expression in the absence of activation (see *generally*, Mukumoto *et al.*, 1993; Green, 2000.

IV.C.4. Targeting of the Gene Product Within the Cell

Various mechanisms for targeting gene products are known to exist in plants and the sequences controlling the functioning of these mechanisms have been characterized in some detail. For example, the targeting of gene products to the chloroplast is controlled by a signal sequence found at the amino terminal end of various polypeptides that is cleaved during chloroplast import to yield the mature polypeptides (see *e.g.*, Comai *et al.*, 1988). These signal sequences can be fused to heterologous gene products to affect the import of heterologous products into the chloroplast (Van den Broeck *et al.*, 1985). DNA encoding for appropriate signal sequences can be isolated from the 5' end of the cDNAs encoding the ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) polypeptide, the chlorophyll a/b binding (CAB) polypeptide, the 5-enol-pyruvyl shikimate-3-phosphate (EPSP) synthase enzyme, the GS2 polypeptide and many other polypeptides which are known to be chloroplast localized. See also, the section entitled "Expression With Chloroplast Targeting" in Example 37 of U.S. Patent No. 5,639,949, herein incorporated by reference.

Other gene products can be localized to other organelles such as the mitochondrion and the peroxisome (*e.g.* Unger *et al.*, 1989). The cDNAs encoding these products can also be manipulated to effect the targeting of heterologous gene products to these organelles. Examples of such sequences are the nuclear-encoded ATPases and specific aspartate amino transferase isoforms for mitochondria. Targeting cellular polypeptide bodies has been disclosed by Rogers *et al.*, 1985.

In addition, sequences have been characterized that control the targeting of gene products to other cell compartments. Amino terminal sequences are responsible for targeting to the endoplasmic reticulum (ER), the apoplast, and extracellular secretion from aleurone cells (Koehler & Ho, 1990). Additionally, amino terminal sequences in conjunction with carboxy terminal sequences are responsible for vacuolar targeting of gene products (Shinshi *et al.*, 1990).

By the fusion of the appropriate targeting sequences disclosed above to transgene sequences of interest it is possible to direct the transgene

product to any organelle or cell compartment. For chloroplast targeting, for example, the chloroplast signal sequence from the RUBISCO gene, the CAB gene, the EPSP synthase gene, or the GS2 gene is fused in frame to the amino terminal ATG of the transgene. The signal sequence selected can include the known cleavage site, and the fusion constructed can take into account any amino acids after the cleavage site that are required for cleavage. In some cases this requirement can be fulfilled by the addition of a small number of amino acids between the cleavage site and the transgene ATG or, alternatively, replacement of some amino acids within the transgene sequence. Fusions constructed for chloroplast import can be tested for efficacy of chloroplast uptake by *in vitro* translation of *in vitro* transcribed constructions followed by *in vitro* chloroplast uptake using techniques disclosed by Bartlett *et al.*, 1982 and Wasmann *et al.*, 1986. These construction techniques are well known in the art and are equally applicable to mitochondria and peroxisomes.

The above-disclosed mechanisms for cellular targeting can be utilized not only in conjunction with their cognate promoters, but also in conjunction with heterologous promoters so as to effect a specific cell-targeting goal under the transcriptional regulation of a promoter that has an expression pattern different from that of the promoter from which the targeting signal derives.

IV.D. Construction of Plant Transformation Vectors

Numerous transformation vectors available for plant transformation are known to those of ordinary skill in the plant transformation art, and the genes pertinent to the presently disclosed subject matter can be used in conjunction with any such vectors. The selection of vector will depend upon the selected transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers might be employed. Selection markers used routinely in transformation include the *nptII* gene, which confers resistance to kanamycin and related antibiotics (Messing & Vieira, 1982; Bevan *et al.*, 1983); the *bar* gene, which confers resistance to the herbicide phosphinothricin (White *et al.*, 1990; Spencer *et al.*, 1990); the *hph* gene, which confers resistance to

the antibiotic hygromycin (Blochinger & Diggelmann, 1984); the *dhfr* gene, which confers resistance to methotrexate (Bourouis & Jarry, 1983); the EPSP synthase gene, which confers resistance to glyphosate (U.S. Patent Nos. 4,940,935 and 5,188,642); and the mannose-6-phosphate isomerase gene, which provides the ability to metabolize mannose (U.S. Patent Nos. 5,767,378 and 5,994,629).

IV.D.1. Vectors Suitable for *Agrobacterium* Transformation

Many vectors are available for transformation using *Agrobacterium tumefaciens*. These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, 1984). Below, the construction of two typical vectors suitable for *Agrobacterium* transformation is disclosed.

IV.D.1.a. pCIB200 and pCIB2001

The binary vectors pCIB200 and pCIB2001 are used for the construction of recombinant vectors for use with *Agrobacterium* and are constructed in the following manner. pTJS75kan is created by *NarI* digestion of pTJS75 (Schmidhauser & Helinski, 1985) allowing excision of the tetracycline-resistance gene, followed by insertion of an *AccI* fragment from pUC4K carrying an NPTII sequence (Messing & Vieira, 1982; Bevan *et al.*, 1983; McBride & Summerfelt, 1990). *XhoI* linkers are ligated to the *EcoRV* fragment of PCIB7 which contains the left and right T-DNA borders, a plant selectable *nos/nptII* chimeric gene and the pUC polylinker (Rothstein *et al.*, 1987), and the *XhoI*-digested fragment are cloned into *Sall*-digested pTJS75kan to create pCIB200 (see also EP 0 332 104, example 19). pCIB200 contains the following unique polylinker restriction sites: *EcoRI*, *SstI*, *KpnI*, *BglII*, *XbaI*, and *Sall*. pCIB2001 is a derivative of pCIB200 created by the insertion into the polylinker of additional restriction sites. Unique restriction sites in the polylinker of pCIB2001 are *EcoRI*, *SstI*, *KpnI*, *BglII*, *XbaI*, *Sall*, *MluI*, *BclI*, *AvrII*, *Apal*, *HpaI*, and *StuI*. pCIB2001, in addition to containing these unique restriction sites, also has plant and bacterial kanamycin selection, left and right T-DNA borders for *Agrobacterium*-mediated transformation, the RK2-derived *trfA* function for mobilization between *E. coli* and other hosts, and the *oriT* and *oriV*

functions also from RK2. The pCIB2001 polylinker is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

IV.D.1.b. pCIB10 and Hygromycin Selection Derivatives Thereof

5 The binary vector pCIB10 contains a gene encoding kanamycin resistance for selection in plants, T-DNA right and left border sequences, and incorporates sequences from the wide host-range plasmid pRK252 allowing it to replicate in both *E. coli* and *Agrobacterium*. Its construction is disclosed by Rothstein *et al.*, 1987. Various derivatives of pCIB10 can be constructed which incorporate the gene for hygromycin B
10 phosphotransferase disclosed by Gritz & Davies, 1983. These derivatives enable selection of transgenic plant cells on hygromycin only (pCIB743), or hygromycin and kanamycin (pCIB715, pCIB717).

IV.D.2. Vectors Suitable for non-*Agrobacterium* Transformation

Transformation without the use of *Agrobacterium tumefaciens*
15 circumvents the requirement for T-DNA sequences in the chosen transformation vector, and consequently vectors lacking these sequences can be utilized in addition to vectors such as the ones disclosed above that contain T-DNA sequences. Transformation techniques that do not rely on *Agrobacterium* include transformation via particle bombardment, protoplast
20 uptake (e.g. polyethylene glycol (PEG) and electroporation), and microinjection. The choice of vector depends largely on the species being transformed. Below, the construction of typical vectors suitable for non-*Agrobacterium* transformation is disclosed.

IV.D.2.a. pCIB3064

25 pCIB3064 is a pUC-derived vector suitable for direct gene transfer techniques in combination with selection by the herbicide BASTA® (glufosinate ammonium or phosphinothricin). The plasmid pCIB246 comprises the CaMV 35S promoter in operational fusion to the *E. coli* β -glucuronidase (GUS) gene and the CaMV 35S transcriptional terminator and
30 is disclosed in the PCT International Publication WO 93/07278. The 35S promoter of this vector contains two ATG sequences 5' of the start site. These sites are mutated using standard PCR techniques in such a way as to remove the ATGs and generate the restriction sites *Ssp*I and *Pvu*II. The

new restriction sites are 96 and 37 bp away from the unique *Sall* site and 101 and 42 bp away from the actual start site. The resultant derivative of pCIB246 is designated pCIB3025. The GUS gene is then excised from pCIB3025 by digestion with *Sall* and *Sacl*, the termini rendered blunt and
5 religated to generate plasmid pCIB3060. The plasmid pJIT82 is obtained from the John Innes Centre, Norwich, England, and the 400 bp *Sma*I fragment containing the *bar* gene from *Streptomyces viridochromogenes* is excised and inserted into the *Hpa*I site of pCIB3060 (Thompson *et al.*, 1987). This generated pCIB3064, which comprises the *bar* gene under the control
10 of the CaMV 35S promoter and terminator for herbicide selection, a gene for ampicillin resistance (for selection in *E. coli*) and a polylinker with the unique sites *Sph*I, *Pst*I, *Hind*III, and *Bam*HI. This vector is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

IV.D.2.b. pSOG19 and pSOG35

15 pSOG35 is a transformation vector that utilizes the *E. coli* dihydrofolate reductase (DHFR) gene as a selectable marker conferring resistance to methotrexate. PCR is used to amplify the 35S promoter (-800 bp), intron 6 from the maize *Adh1* gene (-550 bp), and 18 bp of the GUS untranslated leader sequence from pSOG10. A 250-bp fragment encoding
20 the *E. coli* dihydrofolate reductase type II gene is also amplified by PCR and these two PCR fragments are assembled with a *Sac*I-*Pst*I fragment from pB1221 (BD Biosciences Clontech, Palo Alto, California, United States of America) that comprises the pUC19 vector backbone and the nopaline synthase terminator. Assembly of these fragments generates pSOG19 that
25 contains the 35S promoter in fusion with the intron 6 sequence, the GUS leader, the DHFR gene, and the nopaline synthase terminator. Replacement of the GUS leader in pSOG19 with the leader sequence from Maize Chlorotic Mottle Virus (MCMV) generates the vector pSOG35. pSOG19 and pSOG35 carry the pUC gene for ampicillin resistance and
30 have *Hind*III, *Sph*I, *Pst*I, and *Eco*RI sites available for the cloning of foreign substances.

IV.D.3. Vector Suitable for Chloroplast Transformation

For expression of a nucleotide sequence of the presently disclosed subject matter in plant plastids, plastid transformation vector pPH143 (PCT International Publication WO 97/32011, example 36) is used. The nucleotide sequence is inserted into pPH143 thereby replacing the protoporphyrinogen oxidase (Protox) coding sequence. This vector is then used for plastid transformation and selection of transformants for spectinomycin resistance. Alternatively, the nucleotide sequence is inserted in pPH143 so that it replaces the *aadH* gene. In this case, transformants are selected for resistance to Protox inhibitors.

IV.E. Transformation

Once a nucleic acid sequence of the presently disclosed subject matter has been cloned into an expression system, it is transformed into a plant cell. The receptor and target expression cassettes of the presently disclosed subject matter can be introduced into the plant cell in a number of art-recognized ways. Methods for regeneration of plants are also well known in the art. For example, Ti plasmid vectors have been utilized for the delivery of foreign DNA, as well as direct DNA uptake, liposomes, electroporation, microinjection, and microprojectiles. In addition, bacteria from the genus *Agrobacterium* can be utilized to transform plant cells. Below are descriptions of representative techniques for transforming both dicotyledonous and monocotyledonous plants, as well as a representative plastid transformation technique.

IV.E.1. Transformation of Dicotyledons

Transformation techniques for dicotyledons are well known in the art and include *Agrobacterium*-based techniques and techniques that do not require *Agrobacterium*. Non-*Agrobacterium* techniques involve the uptake of exogenous genetic material directly by protoplasts or cells. This can be accomplished by PEG or electroporation-mediated uptake, particle bombardment-mediated delivery, or microinjection. Examples of these techniques are disclosed in Paszkowski *et al.*, 1984; Potrykus *et al.*, 1985; Reich *et al.*, 1986; and Klein *et al.*, 1987. In each case the transformed cells are regenerated to whole plants using standard techniques known in the art.

Agrobacterium-mediated transformation is a useful technique for transformation of dicotyledons because of its high efficiency of transformation and its broad utility with many different species. *Agrobacterium* transformation typically involves the transfer of the binary vector carrying the foreign DNA of interest (e.g. pCIB200 or pCIB2001) to an appropriate *Agrobacterium* strain which can depend on the complement of *vir* genes carried by the host *Agrobacterium* strain either on a co-resident Ti plasmid or chromosomally (e.g. strain CIB542 for pCIB200 and pCIB2001 (Uknes *et al.*, 1993). The transfer of the recombinant binary vector to *Agrobacterium* is accomplished by a triparental mating procedure using *E. coli* carrying the recombinant binary vector, a helper *E. coli* strain that carries a plasmid such as pRK2013 and which is able to mobilize the recombinant binary vector to the target *Agrobacterium* strain. Alternatively, the recombinant binary vector can be transferred to *Agrobacterium* by DNA transformation (Höfgen & Willmitzer, 1988).

Transformation of the target plant species by recombinant *Agrobacterium* usually involves co-cultivation of the *Agrobacterium* with explants from the plant and follows protocols well known in the art. Transformed tissue is regenerated on selectable medium carrying the antibiotic or herbicide resistance marker present between the binary plasmid T-DNA borders.

Another approach to transforming plant cells with a gene involves propelling inert or biologically active particles at plant tissues and cells. This technique is disclosed in U.S. Patent Nos. 4,945,050; 5,036,006; and 5,100,792; all to Sanford *et al.* Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and afford incorporation within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the desired gene. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried yeast cells, dried bacterium, or a

bacteriophage, each containing DNA sought to be introduced) can also be propelled into plant cell tissue.

IV.E.2. Transformation of Monocotyledons

Transformation of most monocotyledon species has now also become routine. Exemplary techniques include direct gene transfer into protoplasts using PEG or electroporation, and particle bombardment into callus tissue. Transformations can be undertaken with a single DNA species or multiple DNA species (*i.e.* co-transformation), and both these techniques are suitable for use with the presently disclosed subject matter. Co-transformation can have the advantage of avoiding complete vector construction and of generating transgenic plants with unlinked loci for the gene of interest and the selectable marker, enabling the removal of the selectable marker in subsequent generations, should this be regarded as desirable. However, a disadvantage of the use of co-transformation is the less than 100% frequency with which separate DNA species are integrated into the genome (Schocher *et al.*, 1986).

Patent Applications EP 0 292 435, EP 0 392 225, and WO 93/07278 describe techniques for the preparation of callus and protoplasts from an elite inbred line of maize, transformation of protoplasts using PEG or electroporation, and the regeneration of maize plants from transformed protoplasts. Gordon-Kamm *et al.*, 1990 and Fromm *et al.*, 1990 have published techniques for transformation of A188-derived maize line using particle bombardment. Furthermore, WO 93/07278 and Koziel *et al.*, 1993 describe techniques for the transformation of elite inbred lines of maize by particle bombardment. This technique utilizes immature maize embryos of 1.5-2.5 mm length excised from a maize ear 14-15 days after pollination and a PDS-1000He Biolistic particle delivery device (DuPont Biotechnology, Wilmington, Delaware, United States of America) for bombardment.

Transformation of rice can also be undertaken by direct gene transfer techniques utilizing protoplasts or particle bombardment. Protoplast-mediated transformation has been disclosed for *Japonica*-types and *Indica*-types (Zhang *et al.*, 1988; Shimamoto *et al.*, 1989; Datta *et al.*, 1990) of rice. Both types are also routinely transformable using particle bombardment

(Christou *et al.*, 1991). Furthermore, WO 93/21335 describes techniques for the transformation of rice via electroporation. Casas *et al.*, 1993 discloses the production of transgenic sorghum plants by microprojectile bombardment.

5 European Patent Application EP 0 332 581 describes techniques for the generation, transformation, and regeneration of *Pooideae* protoplasts. These techniques allow the transformation of *Dactylis* and wheat. Furthermore, wheat transformation has been disclosed in Vasil *et al.*, 1992 using particle bombardment into cells of type C long-term regenerable
10 callus, and also by Vasil *et al.*, 1993 and Weeks *et al.*, 1993 using particle bombardment of immature embryos and immature embryo-derived callus.

 A representative technique for wheat transformation, however, involves the transformation of wheat by particle bombardment of immature embryos and includes either a high sucrose or a high maltose step prior to
15 gene delivery. Prior to bombardment, embryos (0.75-1 mm in length) are plated onto MS medium with 3% sucrose (Murashige & Skoog, 1962) and 3 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) for induction of somatic embryos, which is allowed to proceed in the dark. On the chosen day of bombardment, embryos are removed from the induction medium and placed
20 onto the osmoticum (*i.e.* induction medium with sucrose or maltose added at the desired concentration, typically 15%). The embryos are allowed to plasmolyze for 2-3 hours and are then bombarded. Twenty embryos per target plate are typical, although not critical. An appropriate gene-carrying plasmid (such as pCIB3064 or pSG35) is precipitated onto micrometer size
25 gold particles using standard procedures. Each plate of embryos is shot with the DuPont BIOLISTICS® helium device using a burst pressure of about 1000 pounds per square inch (psi) using a standard 80 mesh screen. After bombardment, the embryos are placed back into the dark to recover for about 24 hours (still on osmoticum). After 24 hours, the embryos are
30 removed from the osmoticum and placed back onto induction medium where they stay for about a month before regeneration. Approximately one month later the embryo explants with developing embryogenic callus are transferred to regeneration medium (MS + 1 mg/liter NAA, 5 mg/liter GA),

further containing the appropriate selection agent (10 mg/l BASTA® in the case of pCIB3064 and 2 mg/l methotrexate in the case of pSOG35). After approximately one month, developed shoots are transferred to larger sterile containers known as "GA7s" which contain half-strength MS, 2% sucrose, and the same concentration of selection agent.

Transformation of monocotyledons using *Agrobacterium* has also been disclosed. See WO 94/00977 and U.S. Patent No. 5,591,616, both of which are incorporated herein by reference. See also Negrotto *et al.*, 2000, incorporated herein by reference. Zhao *et al.*, 2000 specifically discloses transformation of sorghum with *Agrobacterium*. See also U.S. Patent No. 6,369,298.

Rice (*Oryza sativa*) can be used for generating transgenic plants. Various rice cultivars can be used (Hiei *et al.*, 1994; Dong *et al.*, 1996; Hiei *et al.*, 1997). Also, the various media constituents disclosed below can be either varied in quantity or substituted. Embryogenic responses are initiated and/or cultures are established from mature embryos by culturing on MS-CIM medium (MS basal salts, 4.3 g/liter; B5 vitamins (200 x), 5 ml/liter; Sucrose, 30 g/liter; proline, 500 mg/liter; glutamine, 500 mg/liter; casein hydrolysate, 300 mg/liter; 2,4-D (1 mg/ml), 2 ml/liter; pH adjusted to 5.8 with 1 N KOH; Phytigel, 3 g/liter). Either mature embryos at the initial stages of culture response or established culture lines are inoculated and co-cultivated with the *Agrobacterium tumefaciens* strain LBA4404 (*Agrobacterium*) containing the desired vector construction. *Agrobacterium* is cultured from glycerol stocks on solid YPC medium (plus 100 mg/L spectinomycin and any other appropriate antibiotic) for about 2 days at 28°C. *Agrobacterium* is re-suspended in liquid MS-CIM medium. The *Agrobacterium* culture is diluted to an OD₆₀₀ of 0.2-0.3 and acetosyringone is added to a final concentration of 200 µM. Acetosyringone is added before mixing the solution with the rice cultures to induce *Agrobacterium* for DNA transfer to the plant cells. For inoculation, the plant cultures are immersed in the bacterial suspension. The liquid bacterial suspension is removed and the inoculated cultures are placed on co-cultivation medium and incubated at 22°C for two days. The cultures are then transferred to MS-CIM medium with ticarcillin (400 mg/liter)

to inhibit the growth of *Agrobacterium*. For constructs utilizing the PMI selectable marker gene (Reed *et al.*, 2001), cultures are transferred to selection medium containing mannose as a carbohydrate source (MS with 2% mannose, 300 mg/liter ticarcillin) after 7 days, and cultured for 3-4 weeks
5 in the dark. Resistant colonies are then transferred to regeneration induction medium (MS with no 2,4-D, 0.5 mg/liter IAA, 1 mg/liter zeatin, 200 mg/liter TIMENTIN®, 2% mannose, and 3% sorbitol) and grown in the dark for 14 days. Proliferating colonies are then transferred to another round of regeneration induction media and moved to the light growth room.
10 Regenerated shoots are transferred to GA7 containers with GA7-1 medium (MS with no hormones and 2% sorbitol) for 2 weeks and then moved to the greenhouse when they are large enough and have adequate roots. Plants are transplanted to soil in the greenhouse (T₀ generation) grown to maturity and the T₁ seed is harvested.

15 IV.E.3. Transformation of Plastids

Seeds of *Nicotiana tabacum* c.v. 'Xanthi nc' are germinated seven per plate in a 1" circular array on T agar medium and bombarded 12-14 days after sowing with 1 µm tungsten particles (M10, Bio-Rad Laboratories, Hercules, California, United States of America) coated with DNA from
20 plasmids pPH143 and pPH145 essentially as disclosed (Svab & Maliga, 1993). Bombarded seedlings are incubated on T medium for two days after which leaves are excised and placed abaxial side up in bright light (350-500 µmol photons/m²/s) on plates of RMOP medium (Svab *et al.*, 1990) containing 500 µg/ml spectinomycin dihydrochloride (Sigma, St. Louis,
25 Missouri, United States of America). Resistant shoots appearing underneath the bleached leaves three to eight weeks after bombardment are subcloned onto the same selective medium, allowed to form callus, and secondary shoots isolated and subcloned. Complete segregation of transformed plastid genome copies (homoplasmy) in independent subclones is assessed by
30 standard techniques of Southern blotting (Sambrook & Russell, 2001). *Bam*HI/*Eco*RI-digested total cellular DNA (Mettler, 1987) is separated on 1% Tris-borate-EDTA (TBE) agarose gels, transferred to nylon membranes (Amersham Biosciences, Piscataway, New Jersey, United States of

America) and probed with ^{32}P -labeled random primed DNA sequences corresponding to a 0.7 kb *Bam*HI/*Hind*III DNA fragment from pC8 containing a portion of the *rps7/12* plastid targeting sequence. Homoplasmic shoots are rooted aseptically on spectinomycin-containing MS/IBA medium (McBride *et al.*, 1994) and transferred to the greenhouse.

V. Plants, Breeding, and Seed Production

V.A. Plants

The presently disclosed subject matter also provides plants comprising the disclosed compositions. In some embodiments, the plant is characterized by a modification of a phenotype or measurable characteristic of the plant, the modification being attributable to the expression cassette. In some embodiments, the modification involves, for example, nutritional enhancement, increased nutrient uptake efficiency, enhanced production of endogenous compounds, or production of heterologous compounds. In some embodiments, the modification includes having increased or decreased resistance to an herbicide, an abiotic stress, or a pathogen. In some embodiments, the modification includes having enhanced or diminished requirement for light, water, nitrogen, or trace elements. In some embodiments, the modification includes being enriched for an essential amino acid as a proportion of a polypeptide fraction of the plant. In some embodiments, the polypeptide fraction can be, for example, total seed polypeptide, soluble polypeptide, insoluble polypeptide, water-extractable polypeptide, and lipid-associated polypeptide. In some embodiments, the modification includes overexpression, underexpression, antisense modulation, sense suppression, inducible expression, inducible repression, or inducible modulation of a gene.

V.B. Breeding

The plants obtained via transformation with a nucleic acid sequence of the presently disclosed subject matter can be any of a wide variety of plant species, including monocots and dicots; however, the plants used in the method for the presently disclosed subject matter are selected in some embodiments from the list of agronomically important target crops set forth

hereinabove. The expression of a gene of the presently disclosed subject matter in combination with other characteristics important for production and quality can be incorporated into plant lines through breeding. Breeding approaches and techniques are known in the art. See e.g., Welsh, 1981; 5 Wood, 1983; Mayo, 1987; Singh, 1986; Wricke & Weber, 1986.

The genetic properties engineered into the transgenic seeds and plants disclosed above are passed on by sexual reproduction or vegetative growth and can thus be maintained and propagated in progeny plants. Generally, the maintenance and propagation make use of known agricultural 10 methods developed to fit specific purposes such as tilling, sowing, or harvesting. Specialized processes such as hydroponics or greenhouse technologies can also be applied. As the growing crop is vulnerable to attack and damage caused by insects or infections as well as to competition by weed plants, measures are undertaken to control weeds, plant diseases, 15 insects, nematodes, and other adverse conditions to improve yield. These include mechanical measures such as tillage of the soil or removal of weeds and infected plants, as well as the application of agrochemicals such as herbicides, fungicides, gametocides, nematocides, growth regulants, ripening agents, and insecticides.

20 Use of the advantageous genetic properties of the transgenic plants and seeds according to the presently disclosed subject matter can further be made in plant breeding, which aims at the development of plants with improved properties such as tolerance of pests, herbicides, or abiotic stress, improved nutritional value, increased yield, or improved structure causing 25 less loss from lodging or shattering. The various breeding steps are characterized by well-defined human intervention such as selecting the lines to be crossed, directing pollination of the parental lines, or selecting appropriate progeny plants.

Depending on the desired properties, different breeding measures are 30 taken. The relevant techniques are well known in the art and include, but are not limited to, hybridization, inbreeding, backcross breeding, multiline breeding, variety blend, interspecific hybridization, aneuploid techniques, etc. Hybridization techniques can also include the sterilization of plants to

yield male or female sterile plants by mechanical, chemical, or biochemical means. Cross-pollination of a male sterile plant with pollen of a different line assures that the genome of the male sterile but female fertile plant will uniformly obtain properties of both parental lines. Thus, the transgenic seeds and plants according to the presently disclosed subject matter can be used for the breeding of improved plant lines that, for example, increase the effectiveness of conventional methods such as herbicide or pesticide treatment or allow one to dispense with said methods due to their modified genetic properties. Alternatively new crops with improved stress tolerance can be obtained, which, due to their optimized genetic "equipment", yield harvested product of better quality than products that were not able to tolerate comparable adverse developmental conditions (for example, drought).

V.C. Seed Production

Some embodiments of the presently disclosed subject matter also provide seed and isolated product from plants that comprise an expression cassette comprising a promoter sequence operatively linked to an isolated nucleic acid, the nucleic acid sequence being selected from the group consisting of:

- (a) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or fragment, domain, or feature thereof;
- (b) a nucleotide sequence encoding a polypeptide that is an ortholog of a polypeptide of even numbered sequences of SEQ ID NOs: 2-106, or a fragment, domain or feature thereof;
- (c) a nucleotide sequence complementary (for example, fully complementary) to (a) or (b); and
- (d) a nucleotide sequence that is the reverse complement (for example, its full reverse complement) of (a) or (b) according to the present disclosure.

In some embodiments the isolated product comprises an enzyme, a nutritional polypeptide, a structural polypeptide, an amino acid, a lipid, a fatty acid, a polysaccharide, a sugar, an alcohol, an alkaloid, a carotenoid, a propanoid, a steroid, a pigment, a vitamin, or a plant hormone.

5 Embodiments of the presently disclosed subject matter also relate to isolated products produced by expression of an isolated nucleic acid containing a nucleotide sequence selected from the group consisting of:

- 10 (a) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof;
- 15 (b) a nucleotide sequence encoding a polypeptide that is an ortholog of a polypeptide listed in even numbered sequences of SEQ ID NOs: 2-106, or a fragment, domain, or feature thereof;
- (c) a nucleotide sequence complementary (for example, fully complementary) to (a) or (b); and
- 20 (d) a nucleotide sequence that is the reverse complement (for example, its full reverse complement) of (a) or (b) according to the present disclosure.

 In some embodiments, the product is produced in a plant. In some embodiments, the product is produced in cell culture. In some embodiments, the product is produced in a cell-free system. In some
25 embodiments, the product comprises an enzyme, a nutritional polypeptide, a structural polypeptide, an amino acid, a lipid, a fatty acid, a polysaccharide, a sugar, an alcohol, an alkaloid, a carotenoid, a propanoid, a steroid, a pigment, a vitamin, or a plant hormone. In some embodiments, the product is polypeptide comprising an amino acid sequence listed in even numbered sequences of SEQ ID NOs: 2-106, or ortholog thereof. In some
30 embodiments, the polypeptide comprises an enzyme.

 In seed production, germination quality and uniformity of seeds are essential product characteristics. As it is difficult to keep a crop free from other crop and weed seeds, to control seedborne diseases, and to produce

seed with good germination, fairly extensive and well-defined seed production practices have been developed by seed producers who are experienced in the art of growing, conditioning, and marketing of pure seed. Thus, it is common practice for the farmer to buy certified seed meeting specific quality standards instead of using seed harvested from his own crop. Propagation material to be used as seeds is customarily treated with a protectant coating comprising herbicides, insecticides, fungicides, bactericides, nematocides, molluscicides, or mixtures thereof. Customarily used protectant coatings comprise compounds such as captan, carboxin, thiram (tetramethylthiuram disulfide; TMTD®; available from R. T. Vanderbilt Company, Inc., Norwalk, Connecticut, United States of America), methalaxyl (APRON XL®; available from Syngenta Corp., Wilmington, Delaware, United States of America), and pirimiphos-methyl (ACTELLIC®; available from Agrilience, LLC, St. Paul, Minnesota, United States of America). If desired, these compounds are formulated together with further carriers, surfactants, and/or application-promoting adjuvants customarily employed in the art of formulation to provide protection against damage caused by bacterial, fungal, or animal pests. The protectant coatings can be applied by impregnating propagation material with a liquid formulation or by coating with a combined wet or dry formulation. Other methods of application are also possible such as treatment directed at the buds or the fruit.

VI. Other Applications

The presently disclosed subject matter also provides a method for altering an abiotic stress tolerance of a plant, the method comprising expressing in the plant an expression cassette comprising a nucleic acid molecule encoding a polypeptide as disclosed herein. In some embodiments, the method alters the drought resistance of the plant.

Also provided is a method for enhancing the drought resistance of a plant, the method comprising (a) inserting into the plant an expression cassette comprising at least one isolated nucleic acid molecule of the presently disclosed subject matter; and (b) growing the plant comprising the

expression cassette under suitable growth conditions, wherein the drought resistance of the plant is enhanced.

Also provided is a method for producing a polypeptide as disclosed herein, the method comprising (a) growing cells comprising an expression
5 cassette under suitable growth conditions, the expression cassette comprising a nucleic acid molecule encoding the polypeptide; and (b) isolating the polypeptide from the cells.

Also provided is a method for producing a plant comprising a modification thereto, including the steps of:

- 10 (1) providing a nucleic acid comprising a nucleotide sequence selected from the group consisting of:
- (a) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in
15 odd numbered sequences of SEQ ID NOs: 1-105, or exon, domain, or feature thereof;
 - (b) a nucleotide sequence complementary (for example, fully complementary) to (a); and
 - (c) a nucleotide sequence which is the reverse complement (for
20 example, its full reverse complement) of (a); and
- (2) introducing the nucleic acid into the plant, wherein the nucleic acid is expressible in the plant in an amount effective to effect the modification. In some embodiments, the modification comprises an altered trait in the plant, wherein the trait corresponds to the nucleic
25 acid introduced into the plant. In other embodiments, the altered trait is related to a feature listed in any of Tables 1-6. For example, the trait can correspond to disease resistance, yield, abiotic stress tolerance, nutritional composition, carbon metabolism, photosynthesis, signal transduction, cell growth, reproduction, a
30 disease process, or differentiation.

In some embodiments, the modification comprises an increased or decreased expression or accumulation of a product of the plant. In some embodiments, the product comprises a natural product of the plant. In some

embodiments, the product comprises a new or altered product of the plant. In some embodiments, the product comprises an enzyme, a nutritional polypeptide, a structural polypeptide, an amino acid, a lipid, a fatty acid, a polysaccharide, a sugar, an alcohol, an alkaloid, a carotenoid, a propanoid, a steroid, a pigment, a vitamin, or a plant hormone. In some embodiments, the presently disclosed subject matter provides a method for increasing drought resistance by delivering an effective amount of a product resulting from modification of the plant.

Also encompassed within the presently disclosed subject matter is a method for producing a recombinant polypeptide, the method comprising:

- (a) growing recombinant cells comprising a nucleic acid construct under suitable growth conditions, the construct comprising an expression vector and a nucleic acid comprising a nucleic acid encoding a polypeptide that is an ortholog of a polypeptide as listed in even numbered nucleotide sequences of SEQ ID NOs: 2-106, or a nucleic acid sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl to a nucleotide sequence listed in odd numbered nucleotide sequences of SEQ ID NOs: 1-105, or a subsequence thereof; and
- (b) isolating from the recombinant cells the recombinant polypeptide expressed thereby.

Embodiments of the presently disclosed subject matter provide a method for producing a recombinant polypeptide in which the expression vector comprises one or more elements including, but not limited to, a promoter-enhancer sequence, a selection marker sequence, an origin of replication, an epitope tag-encoding sequence, and an affinity purification tag-encoding sequence. In some embodiments, the nucleic acid construct comprises an epitope tag-encoding sequence and the isolating step employs an antibody specific for the epitope tag. In some embodiments, the nucleic acid construct comprises a polyamino acid-encoding sequence and the isolating step employs a resin comprising a polyamino acid binding substance. In some embodiments, the polyamino acid comprises

polyhistidine and the polyamino acid binding resin comprises nickel-charged agarose resin. In some embodiments, the nucleic acid construct comprises a polypeptide-encoding sequence and the isolating step employs a resin containing a polypeptide binding substance. In some embodiments, the
5 polypeptide comprises a chitin binding domain and the resin comprises chitin-sepharose.

The presently disclosed subject matter also provides a method for altering the expression of a polypeptide of the presently disclosed subject matter in a plant, the method comprising expressing an expression cassette
10 encoding a polypeptide of the presently disclosed subject matter in the plant. In some embodiments, the polypeptide is expressed in a predetermined location or tissue of a plant. In some embodiments, the location or tissue is selected from the group consisting of epidermis, root, vascular tissue, meristem, cambium, cortex, pith, leaf, flower, seed, and combinations
15 thereof.

The presently disclosed subject matter also provides a method for decreasing the expression of an isolated nucleic acid molecule as disclosed herein in a plant, the method selected from the group consisting of (a) expressing in said plant a molecule of the presently disclosed subject matter
20 or a portion thereof in "sense" orientation; (b) expressing in said plant a molecule of the presently disclosed subject matter or a portion thereof in "antisense" orientation; (c) expressing in said plant a ribozyme capable of specifically cleaving a messenger RNA transcript encoded by an endogenous gene corresponding to an isolated nucleic acid molecule of the
25 presently disclosed subject matter; (d) expressing in a plant an aptamer specifically directed to a polypeptide encoded by an isolated nucleic acid molecule of the presently disclosed subject matter; (e) expressing in a plant a mutated or a truncated form of an isolated nucleic acid molecule of the
30 presently disclosed subject matter; (f) modifying by homologous recombination in a plant at least one chromosomal copy of the gene corresponding to an isolated nucleic acid molecule of the presently disclosed subject matter; (g) modifying by homologous recombination in a plant at least one chromosomal copy of the regulatory elements of a gene

corresponding to an isolated nucleic acid molecule of the presently disclosed subject matter; and (h) expressing in said plant an isolated nucleic acid molecule of the presently disclosed subject matter or a portion thereof in the "sense" and "antisense" orientation.

5 Embodiments of the presently disclosed subject matter also relate to a method for increasing the expression of an isolated nucleic acid molecule of the presently disclosed subject matter in a plant, the method comprising (a) inserting into the plant an expression cassette comprising an isolated
10 nucleic acid molecule of the presently disclosed subject matter; and (b) growing the plant comprising the expression cassette under suitable growth conditions, wherein the expression of the isolated nucleic acid molecule of the presently disclosed subject matter is increased.

 Embodiments of the presently disclosed subject matter also relate to a plant modified by a method that comprises introducing into the plant a
15 nucleic acid where the nucleic acid is expressible in the plant in an amount effective to effect the modification. The modification can be, for example, nutritional enhancement, increased nutrient uptake efficiency, enhanced production of endogenous compounds, and production of heterologous compounds. In some embodiments, the modified plant has increased or
20 decreased resistance to a herbicide, an abiotic stress, or a pathogen. In some embodiments, the modified plant has enhanced or diminished requirement for light, water, nitrogen, or trace elements. In some embodiments, the modified plant is enriched for an essential amino acid as a proportion of a polypeptide fraction of the plant. The polypeptide fraction
25 can be, for example, total seed polypeptide, soluble polypeptide, insoluble polypeptide, water-extractable polypeptide, and lipid-associated polypeptide. The modification can include, but is not limited to, overexpression, underexpression, antisense modulation, sense suppression, inducible expression, inducible repression, or inducible modulation of a gene.

30 The presently disclosed subject matter further relates to a seed from a modified plant or an isolated product of a modified plant, wherein the product includes, but is not limited to, an enzyme, a nutritional polypeptide, a structural polypeptide, an amino acid, a lipid, a fatty acid, a polysaccharide,

a sugar, an alcohol, an alkaloid, a carotenoid, a propanoid, a steroid, a pigment, a vitamin, or a plant hormone.

5 The presently disclosed subject matter will be further disclosed by reference to the following detailed examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

Examples

10 The following Examples have been included to illustrate representative and exemplary modes of the presently disclosed subject matter. Certain aspects of the following Examples are described in terms of techniques and procedures found or contemplated by the present inventors to work well in the practice of the presently disclosed subject matter. These Examples are exemplified through the use of standard laboratory practices of
15 the inventors. In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the spirit and scope of the presently disclosed subject matter.

20 Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are disclosed in Sambrook & Russell, 2001; Silhavy *et al.*, 1984; Ausubel *et al.*, 1994; Reiter *et al.*, 1992; and Schultz *et al.*, 1998.

25 Example 1

Plant Materials and Stress Treatment

A recombinant inbred population was developed from the cross of B35, an inbred sorghum line with a stay-green, drought tolerant phenotype, and Tx7000, an elite, high yielding sorghum accession with a non-stay-green, post-flowering drought susceptible phenotype (Xu *et al.*, 2000). The
30 two parental lines, two stay-green (stg) recombinant inbred lines (RILs; STG40 and STG62), and two non-stg RILs (NSG41 and NSG95) were used. Seeds of these lines were kindly provided by Dr. Darrell T. Rosenow of the Texas Agricultural Experiment Station in Lubbock, Texas. Plants were

grown in the greenhouse in a randomized complete block design with three replications. Two sorghum plants were grown in five-gallon plastic pots filled with Ball Growing Mix (Ball Seed Co., Chicago, Illinois, United States of America), with three pots per treatment, a single pot serving as a replicate. Plants were watered every alternate day and fertilized (MIRACLE-GRO®) every two weeks. Final irrigation was administered 3 days after anthesis to impose gradual water deficit treatment and to simulate natural post-flowering drought conditions.

Leaf samples were collected at 7, 14 and 21 days after withholding irrigation. Relative water content (RWC) was monitored for each sampling date. For the fourth treatment, RWC of the plants was allowed to drop to ~65% and then watering was resumed. Leaf sampling for the fourth treatment (7RW) was done 7 days after resuming irrigation. Corresponding fully irrigated plants were sampled at 7, 14, 21 and 7RW and the leaf tissues pooled to serve as fully irrigated control. Average greenhouse day conditions were 32°C, 43% humidity, 13-hour day length, 825 mE m⁻² sec⁻¹ light intensity. Average greenhouse night temperature was 27°C.

Example 2

Expression Profiling

Sample preparation and hybridization procedures were conducted as previously described in Zhu *et al.*, 2003. Total RNA was isolated from 300µl volume of ground, liquid nitrogen freeze-dried, leaf tissue using the RNAwiz reagent (Ambion, Austin, Texas, United States of America). Double strand cDNAs were synthesized from total RNA using oligo dT₍₂₄₎ primer containing 5' T7 RNA polymerase promoter sequence SUPERSCRIPT™ II (Invitrogen Corp., Carlsbad, California, United States of America). Probe preparation, hybridizations and normalizations, were conducted according to protocols optimized for sensitivity and reproducible comparisons (Zhu & Wang 2000, Harmer *et al.* 2000). Biotinylated complementary RNAs were transcribed *in vitro* from synthesized cDNA by T7 RNA polymerase (ENZO Biochem, New York, New York, United States of America). Hybridizations with labeled cRNAs were conducted with a rice GENECHIP® microarray (Affymetrix,

Santa Clara, California, United States of America). The GENECHIP® microarray contained probe sets for over 20,000 rice genes designed from the *O. sativa* spp. *japonica* genome sequence (Goff *et al.*, 2002). Expression data were normalized globally prior to data analysis. Genes with accurately detectable transcript levels were defined by probe sets showing averaged expression levels equal to or greater than 50 and those with lesser signals were adjusted to 50. Change in expression was calculated as fold-change difference from well-watered plants to corresponding dehydration stress treatments. While a two-fold change in gene expression cut-off was used by Zhu *et al.*, 2002 a 1.5-fold or greater change cut-off was employed to compensate for expected lower signal intensities due to cross-species hybridization (Chismar *et al.*, 2002).

Each transcript was assigned to functional categories based on the MIPS classification scheme (Schoof *et al.*, 2002). The scheme includes hierarchical structures of protein functions, enzymatic activities, and pathways. In order to classify a sequence according to the scheme, the sequence was compared to currently classified protein sequences using FASTX and FASTX-i. Top hits from FASTA searches were parsed based on a 200 cut-off z-score. The sequence was then assigned with the same hierarchical classification as the top hits.

Adaptive clustering of expression profiles of B35 and Tx7000 was performed using an adaptive, quality-based algorithm to cluster gene expression data and find groups of genes that have a similar expression profile (see Smet *et al.*, 2002). The software uses an adaptive, quality-based clustering algorithm to cluster gene expression data to find groups of genes that have a similar expression profile. User defined parameters were set to MIN_NR_GENES = 2 and S = 0.95 respectively. MIN_NR_GENES define the minimum number of genes that can belong to a gene cluster and S defines the probability of a gene belonging to a specific cluster.

Example 3

Expression Profile in Response to Drought

The transcription profile of two divergent sorghum lines and their four recombinant inbred (RIL) progenies was monitored in response to gradual

dehydration stress using a rice GENECHIP® array (Zhu *et al.*, 2003). Transcription profile was measured at four time points after imposing dehydration. Considerable differences in the overall gene expression profile were observed between stg and non-stg lines (Table 1). Almost two-fold more genes were modulated in B35, the stg parent, in response to dehydration than in the Tx7000, the non-stg parent across the four time points measured. An average of 1167 transcripts across the four sampling times was induced in B35 against 586 in Tx7000. Correspondingly, an average of 1176 transcripts was repressed in B35 and 525 in Tx7000. Among the stg parent and its two stg derived RIL progenies, STG40 and STG62, an average of 2115 transcripts showed a greater than 1.5-fold change in expression level. An average of 1470 transcripts exhibited a greater than 50% change in expression level in the non-stg parent and non-stg RILs across the four treatments. Similar number of transcripts were induced and repressed in the parent and in the two non-stg lines.

Table 1

General Trends in Expression Profile: Number of Transcripts that Exhibited 50% Change in Expression Level Relative to Well-irrigated Control at Corresponding Days After Initiation of Water Deficit Stress Treatments

Lines*	Induced				Repressed			
	7	14	21	7RW ^a	7	14	21	7RW ^a
B35	459	204	385	119	344	78	591	163
Tx7000	109	197	152	128	111	232	78	104
STG40	362	338	245	245	284	131	236	250
STG62	629	451	501	514	783	553	722	773
NSG41	569	176	222	139	585	170	153	285
NSG95	632	615	620	131	311	360	386	86

STG lines ^b									
(in total)	1204	911	1036	808		1262	727	1382	1130
NSG lines ^c									
(in total)	1156	932	843	372		883	700	563	434
Common to									
all STG	29	10	5	4		13	0	8	2
Common to									
all NSG	7	2	18	0		1	2	3	3

* STG= stay-green; NSG= non-stay-green

^a Relative water contents of plants were allowed to drop to ~65% and then re-watered. Sampling was done 7 days after re-watering.

^b Includes the STG parent and the two recombinant, STG lines.

5 ^c Includes the NSG parent and the two recombinant, NSG lines.

Differentially expressed transcripts in the parents under each sampling time point were classified according to MAtdB functional categories (Table 2; Schoof *et al.*, 2002). Many of the identified transcripts were assigned to multiple categories and considered synonymous with “unclear classification” to simplify data analysis. Altogether, these two categories make up more than half of the drought-responsive, modulated transcripts identified in the parents. For transcripts assigned to specific categories, transcriptional response was greatest for “metabolism” category followed by “cell rescue, defense, death and ageing” across the four time points. The categories “cell rescue, defense, death and ageing”, “cellular communication” and “cellular organization” showed bi-modal response to increased dehydration, with the most number of transcripts observed at 7 days and then at 21 days, respectively for both induced and repressed transcripts. The “metabolism” category showed peak response at 7 and 21 days only for induced transcripts.

Table 2

Functional Categorization^a of Transcripts Showing at least 1.5-fold Change
in Expression in Response to Drought Stress^b

Functional Category	Induced				Repressed			
	7	14	21	7RW ^c	7	14	21	7RW ^c
CELL GROWTH,	1	2	0	1	1	0	0	0
DIVISION	0	0	1	1	1	1	0	0
CELL RESCUE,	8	6	15	3	5	0	13	4
DEFENSE	3	8	1	0	5	6	1	3
Ageing	0	0	3	0	0	0	0	0
	0	0	0	0	0	0	0	0
DNA Repair	1	1	1	1	0	0	0	0
	0	0	0	0	0	0	0	0
Detoxification	2	1	3	1	1	0	2	0
	0	1	0	0	1	3	1	0
Defense/								
Biotic Stress	3	1	1	1	1	0	9	0
	2	0	0	0	1	0	0	3
Abiotic Stress	2	3	6	0	3	0	1	0
	1	7	1	0	3	3	0	0
Unknown	0	0	1	0	0	0	1	0
	0	0	0	0	0	0	0	0
CELLULAR	2	2	4	1	2	1	1	0
BIOGENESIS	1	2	0	0	0	0	0	0
CELLULAR	13	4	12	2	4	1	10	0
COMMUNICATION	0	4	2	1	1	2	1	0
CELLULAR	10	4	8	1	2	0	2	0
ORGANIZATION	0	2	5	0	0	0	0	0
CELLULAR	2	1	2	0	0	0	2	0
TRANSPORT	0	1	0	0	0	0	0	0
DEVELOPMENT	0	1	2	0	2	0	1	0
	0	0	1	0	0	0	0	0

ENERGY	0	2	2	2	6	0	11	2
	0	0	0	1	0	4	3	2
METABOLISM	17	8	22	3	12	2	1	5
	4	6	4	0	5	5	1	4
PROTEIN	2	1	3	1	2	0	3	3
DESTINATION	0	1	2	0	3	3	1	1
PROTEIN	15	1	1	3	2	0	7	0
SYNTHESIS	1	0	0	0	5	3	0	3
TRANSCRIPTION	6	1	5	1	4	0	5	1
	1	2	1	1	0	2	1	2
TRANSPORT	4	4	4	0	4	2	8	0
FACILITATION	1	1	2	0	1	8	1	1
TRANSPOSABLE	2	2	1	1	1	2	5	1
ELEMENTS	1	1	1	2	1	1	0	0
MOTILITY	0	0	0	0	0	0	1	0
	0	0	0	0	1	0	0	0
CLASSIFICATION	173	103	156	54	143	35	244	82
UNKNOWN	49	86	30	76	34	92	44	35
UNCLASSIFIED	122	41	87	29	81	14	147	36
PROTEINS	33	41	70	25	23	44	10	17
CLASSIFICATION	9	6	6	2	10	1	12	2
NOT CLEAR	0	4	5	0	2	2	6	2
MULTIPLE	73	15	55	15	63	20	118	25
CATEGORIES	15	38	29	21	29	59	9	34
TOTAL	459	204	385	119	344	78	591	168
	109	197	152	128	111	232	78	104

^a according to MIPS classification scheme (Schoof *et al.* 2002)

^b Numbers in each first line are for B35 while numbers in bold are corresponding values for Tx7000.

^c Relative water contents of plants were allowed to drop to ~65% and then re-watered. Sampling was done 7 days after re-watering.

Example 4Parental Response to Increased Dehydration

The stg QTL profile and phenotypic response of the two parents to dehydration at post-flowering were different (Table 3). The relative water content of Tx7000 was affected more slowly by increasing dehydration than B35. In contrast, the RWC of B35 recovered quicker after re-watering. However, these trends are not reflected by their correspondingly derived RILs. Thus, no clear trend correlation was observed between RWC and the stay-green trait. However, considerable differences in transcriptional response to increased dehydration were observed between the parents as almost two-fold more genes were modulated in B35 than in the Tx7000 (Table 1). Transcriptional response as indicated by the corresponding number of transcripts affected by increasing water deficit also varied between the parents (Figure 1). B35 had peak transcription induction at seven days after withholding irrigation while Tx7000 showed peak induction at 14 days, suggesting a more rapid induction response in the stg parent. Transcript down-regulation was greatest at 21 days for B35 and at 14 days for Tx7000. Interestingly, 107 transcripts down regulated in Tx7000 at 14 days were also found repressed at 21 days in B35, strongly indicating a slower transcript down-regulation in B35.

Table 3Phenotypic and QTL Profile of Sorghum Lines

25	Line	QTL Profile ^a				RWC at sampling ^b					Phenotype
		1	2	3	4	C ^c	7	14	21	7RW ^d	
	B35	+	+	+	+	94.0	91.3	86.0	62.5	93.8	stg, drought-tolerant parent
30	Tx7000	-	-	-	-	94.4	97.0	88.2	75.6	85.6	non-stg, susceptible parent

	SG40	+	+	+	H	92.6	95.7	92.9	92.8	88.9	stg, drought-tolerant RIL
	SG62	+	+	+	+	94.5	94.0	93.5	87.9	87.6	stg, drought-tolerant RIL
5	NSG41	+	-	+	-	92.9	92.3	88.6	78.2	91.7	non-stg, susceptible RIL
	NSG95	-	-	-	-	92.3	88.1	84.5	62.5	93.9	non-stg, susceptible RIL
10											RIL
	^a Genotype for each of the QTL loci; + = B35 allele, - = Tx7000 allele, H = heterozygous										
	^b Mean relative water content of three replicate samples										
	^c Control, fully irrigated tissues										
15	^d Irrigation was withdrawn until RWC is ~60% and then watering is resumed. Sampling is done 7 days after rewatering.										

Example 5

Transcriptional Response of the RILs

20 Two stay-green lines with defined tolerant response to post-flowering drought stress and two non-stay-green lines susceptible to post-flowering drought were selected from the RIL population derived from a cross between B35 and Tx7000. A greater number of drought-modulated transcripts were found in the two derived RILs than in the stay-green parent. More

25 differentially regulated transcripts were also observed in the two non-stay-green RIL than in Tx7000 (Table 1). Similarly as in the parents, fewer modulated transcripts were observed in the non-stg lines compared to the two stg lines. Forty-three up-regulated genes were found common in all the stg lines and 25 in all the non-stg lines. Twenty transcripts were commonly

30 down regulated in all the stg lines and 9 in the non-stg lines (Tables 4 and 5). There was negligible overlap between the stg and non-stg transcripts except two up-regulated transcripts (HNSb 38, a homeobox gene and HNSb 17, a phospholipid transfer protein). Annotation of the 43 induced transcripts

suggests various potential roles related to stress response. Among the 43 transcripts with expected stress-related functions are heat shock protein (HNSb 8) and a cation transport protein (OS018250.1_S_AT). Transcripts with known stress-related functions include HNSb 40 and HNSb 43 (detoxification), HNSb 31 (cellular reorganization), HNSb 45 (transcription regulation). A number of stress-related transcripts with unknown functions were also observed (HNSb 30, HNSb 36, HNSb 48). In addition, a number of transcripts commonly induced across all the stg lines are genes specific to photosystem II reaction center. Repressed functions common to all stg lines include protein degradation (HNSb 34) and signal transduction (HNSb 4, HNSb 29).

Table 4

Expression Values of Transcripts Common in All Stay-green Lines^a

15	Probe Set	Lines			Putative Function, Description
		B35	SG40	SG62	
	Induced at 7 days				
	HNSb 3	1.57	1.62	1.52	Unknown <i>S. propinquum</i> cDNA
	HNSb 5	2.44	1.96	2.21	Ribosomal protein (<i>O. sativa</i>)
	HNSb 6	1.78	9.00	18.39	Photosystem II reaction center protein (<i>O. sativa</i>)
	HNSb 10	2.90	2.87	7.66	Probable CLPP-like protease (<i>Z. mays</i>)
	HNSb 11	1.72	2.18	6.28	Cytochrome c biosynthesis protein (<i>D. carota</i>)
	HNSb 12	2.88	3.03	8.90	Photosystem I P700 chlorophyll a apoprotein (<i>O. sativa</i>)
	HNSb 14	2.15	2.20	1.85	24-methylene lophenol C24(1)methyltransferase (<i>O. sativa</i>)
	HNSb 16	4.84	3.00	1.69	Thiozole biosynthetic enzyme 1-1 (<i>Z. mays</i>)
	HNSb 18	2.01	2.40	4.63	Ribosomal protein (<i>A. thaliana</i>)

HNSb 21	1.89	1.54	3.39	Ribosomal protein (<i>A. thaliana</i>)
HNSb 23	2.32	1.74	1.63	Unknown <i>H. vulgare</i> cDNA
HNSb 24	1.67	2.36	3.56	ATP synthase (<i>O. sativa</i>)
HNSb 31	2.07	1.56	2.64	Histone H1 protein (<i>Z. mays</i>)
HNSb 36	2.25	2.53	1.56	Unknown <i>T. aestivum</i> drought stressed cDNA
HNSb 37	1.58	1.62	2.11	ATHSAR1 EST (<i>A. thaliana</i>)
HNSb 40	2.61	4.08	4.26	NADH plastoquinone oxidoreductase (<i>O. sativa</i>)
HNSb 43	2.39	2.10	2.46	Geranylgeranyl hydrogenase (<i>G. max</i>)
HNSb 44	3.08	2.93	2.32	Cysteine protease component (<i>A. thaliana</i>)
HNSb 45	2.99	2.72	1.94	Initiation factor 5A
HNSb 48	1.53	2.04	1.80	Unknown cold stress <i>S. cereale</i> cDNA
HNSb 51	1.68	1.54	1.53	Pistil extensin like protein (<i>N. alata</i>)
HNSb 52	2.57	1.63	2.39	Unknown <i>S. bicolor</i> cDNA
HNSb 55	1.55	1.73	1.56	Cation transport protein
HNSb 59	1.70	1.55	3.57	Purple acid phosphatase precursor (<i>A. thaliana</i>)
induced at 14 days				
HNSb 8	2.93	8.94	2.59	Heat shock protein (<i>O. sativa</i>)
HNSb 15	1.62	1.55	2.64	Phenylalanine ammonia-lyase (<i>H. vulgare</i>)
HNSb 22	1.58	1.66	2.25	Ribosomal protein (<i>O. sativa</i>)
HNSb 38	1.54	1.63	2.07	Homeobox leucine zipper protein
HNSb 40	2.13	2.04	2.08	NADH plastoquinone oxidoreductase (<i>O. sativa</i>)
HNSb 49	1.58	1.61	1.96	Unknown <i>S. propinquum</i> cDNA
HNSb 56	1.53	1.86	1.64	Cytochrome P40 (<i>A. thaliana</i>)

induced at 21 days

HNSb 9	2.13	2.09	1.94	Lipid transfer protein (<i>O. sativa</i>)
HNSb 13	1.55	1.53	1.77	Ribosomal protein (<i>A. thaliana</i>)
HNSb 14	1.64	1.71	1.74	24-methylene lophenol C24(1)methyltransferase (<i>O. sativa</i>)
HNSb 17	4.90	2.48	1.71	Phospholipid transfer protein (<i>O. sativa</i>)

induced at 7 days after rewatering

HNSb 39	1.54	1.52	1.96	Glucose:flavonoid 3-o- glucosyltransferase -like protein (<i>Z. mays</i>)
HNSb 46	2.01	2.90	3.39	Enolase (2-phospho-D-glycerate hydrolyase) (<i>Z. mays</i>)
HNSb 53	1.54	1.76	1.60	Unknown <i>H. vulgare</i> cDNA

repressed at 7 days

HNSb 4	-1.54	-1.90	-1.65	Protein kinase (<i>A. thaliana</i>)
HNSb 19	-6.42	-3.51	-2.52	Photosystem I reaction protein (<i>H. vulgare</i>)
HNSb 25	-1.53	-1.85	-1.70	Similar to metallothionein
HNSb 27	-3.83	-4.26	-2.02	Lipoxygenase (<i>Zea mays</i>)
HNSb 28	-1.91	-1.90	-2.00	Photosystem I subunit (<i>H. vulgare</i>)
HNSb 30	-3.14	-1.94	-1.68	Unknown drought stressed <i>T. aestivum</i> cDNA
HNSb 34	-1.92	-2.18	-2.55	Ubiquitin conjugating enzyme (<i>A. thaliana</i>)
HNSb 47	-1.92	-2.48	-1.61	Amino acid transport protein AAP4 (<i>A. thaliana</i>)
HNSb 50	-1.84	-1.66	-1.67	<i>S. cereale</i> omega secalin gene
HNSb 54	-1.56	-1.53	-1.55	Similar to human prolyl 4- hydroxylase subunit
HNSb 60	-1.99	-1.65	-1.53	Unknown <i>L. luteus</i> cDNA

repressed at 21 days

HNSb 2	-1.54	-1.54	-1.81	Unknown <i>H. vulgare</i> cDNA
HNSb 7	-6.19	-2.20	-1.67	ATP synthase and tRNA-Met (<i>A. crassa</i>)
HNSb 26	-1.85	-1.64	-1.64	Unknown <i>G. max</i> cDNA
HNSb 29	-2.25	-1.70	-2.21	Calmodulin (<i>A. thaliana</i>)
HNSb 34	-2.63	-2.55	-2.25	Ubiquitin conjugating enzyme (<i>A. thaliana</i>)
HNSb 57	-2.41	-2.88	-1.71	Cinnamyl-alcohol dehydrogenase (<i>A. thaliana</i>)

repressed at 7 days after rewatering

HNSb 33	-1.61	-2.00	-1.59	Proteinase inhibitor (<i>Hordeum vulgare</i>)
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^a Probes in bold are also found in the non-stay-green lines.

Table 5

Expression Values of Transcripts Common in All Non-Stay-green Lines
And Also Found in Stay-green Lines

5

Probe Set	Lines			Putative Function, Description
	Tx7000	NSG41	NSG95	
induced at 14 days				
HNSb 17	1.54	1.54	1.75	phospholipid transfer protein (<i>O. sativa</i>)
induced at 21 days				
HNSb 17	1.62	3.63	4.83	phospholipid transfer protein (<i>O. sativa</i>)
HNSb 38	1.75	1.79	5.35	homeobox gene 13 protein (<i>A. thaliana</i>)

Example 6Gene Clusters with Common Expression Patterns

10

Microarray analysis can generate a vast amount of data which help elucidate global gene expression which can infer cellular behavior. Clustering of genes with similar expression profile is one way of analyzing massive amount of data. The parental expression profile was analyzed with an adaptive, quality-based software (Adapt_Cluster; Smet *et al.*, 2002) to

identify genes that share similar expression patterns. After global normalization of the expression data and removing probes showing unaltered levels of hybridization, a final set of 9056 probes was analyzed.

A total of 2487 probes were assigned into 16 gene clusters at the set parameters. The number of clustered probes account for 11% of the total number of probes in the rice GENECHIP® microarray (Affymetrix, Santa Clara, California, United States of America; see Zhu *et al.*, 2003). Cluster 1 has the greatest number of genes (583 probes) followed by cluster 3 that contains 267 genes. Cluster 1 contains genes which are continuously induced in the non-stay-green parent but continuously down-regulated in the stay-green parent across the duration of the imposed dehydration stress. Cluster 3 shows the reverse pattern: continuously induced in B35 and continuously repressed in Tx7000 during water deficit. Clusters 6 and 16 represent drought-responsive genes expressed only in B35 (Table 6). Cluster 6 genes were induced only in the early stage of dehydration while cluster 9 shows two expression peaks at 7 and 21 days respectively. Cluster 8 represents genes showing faster response to dehydration in B35 (7 days) but induced slower in Tx7000 (14 days).

20

Table 6

Gene Clusters Showing Similar Expression Patterns
Across the Drought Treatments

Expression Pattern ^a	Probes	Top MIPS Category ^a
induced in B35 at 7 days after drought (transient and early response in B35) (see Figure 2A)	154	Cell Rescue, Defense

induced in B35 at 7-21 days after drought; induced in Tx7000 at 14-21 days after drought (sustained response to dehydration) (see Figure 2B)	112	Metabolism
induced in Tx7000 at 14 days after drought (sustained response to dehydration) (see Figure 2C)	117	Cell Rescue, Defense, Cellular Organization
induced in B35 at 7-21 days after drought; induced in Tx7000 at 21 days after drought; (sustained response to dehydration) (see Figure 2D)	100	Cell Rescue, Defense
induced in B35 at 7 and 21 days after drought (bi-modal expression) (see Figure 2E)	53	Metabolism

^a See Figures 2A-2E for Graphical Representations of the Expression Patterns.

^b MIPS functional category according to Schoof *et al.*, 2002. The actual top MIPS category for each cluster is always the unclassified proteins followed by unclear classifications or multiple category classification.

5

Discussion of Examples 1-6

By pair-wise comparison of samples, gene expression profiling can indicate possible association between a phenotype and changes in gene expression. The association between genes and the resulting traits as revealed by transcription profiling could be used to develop plants with increased yields or with more resistance to diseases and tolerance to abiotic stresses (Zhu *et al.*, 2003). However, the variety of organisms for which

10

transcription profiling can be carried out is limited by the lack of a genomic sequence data. One solution to this limitation is to use microarrays designed for one species to profile RNA samples from a closely related species. The Affymetrix U95A human gene array is effective at measuring the transcript profiles of chimpanzee, orangutan, and rhesus macaque as is the *Mus musculus* gene array able to measure the transcript profile of *M. caroli* and *M. spretus* (Chismar *et al.*, 2002; Enard *et al.*, 2002). Similarly, the presently disclosed subject matter measured the transcript profile of dehydration stressed sorghum using a rice GENECHIP® microarray. The major cereal crops are highly related (Kellogg, 1998) and their transcriptomes likely detectable by the rice GENECHIP®. A large number of the rice probes detect genes expressed in maize and barley (Zhu *et al.*, 2001a). The present disclosure also shows that conservation of gene sequences between sorghum and rice was sufficient to generate good quality data. Even though the overall gene sequences of sorghum and rice and very similar, it was expected that not all of the 25-mer oligonucleotides that make up a probe set for a single gene will be alike. Therefore, a 1.5-fold change threshold was used rather than 2.0.

The number of modulated transcripts with at least a 1.5 fold change in expression level in response to gradual water deficit ranges from a low of 5% of all the probes surveyed found in the susceptible parent to a high of 21% in a stay-green RIL. These values are within range of previously published results in transcript profiling. Ozturk *et al.*, 2002 found that 15% of transcripts profiled were differentially expressed in dehydration stressed barley. Approximately 5% of the genome surveyed showed differential regulation in a water stressed rice plants (Hazen *et al.*, 2003a) and 12% of the *Arabidopsis* genome showed a change in transcription in response to hyperosmotic stress (Krebs *et al.*, 2002). These studies however used a 2-fold change to measure changes in gene expression.

The parents used in the presently disclosed subject matter showed widely divergent transcriptional response to dehydration. While the parents show equal modulation of both induced and repressed transcripts, approximately 10% of the probes surveyed were found to be modulated by

drought in the stay-green, drought tolerant parent across all the four time points used and only half of that amount was found in the susceptible parent. Hence, the magnitude of transcriptional response from B35 was higher than in Tx7000. B35 is more responsive transcriptionally to increasing
5 dehydration, indicating a higher response capacity to stress in the resistant line.

The onset of transcriptional response between the parents also differed. B35 shows a more rapid induction response and a slower transcript down-regulation compared to Tx7000. Kawasaki *et al.*, 2001 found that
10 sensitivity to salinity of two rice accessions was dependent on the speed of transcriptional changes over time. Similarly, the present disclosure indicates that in the drought resistant parent B35, transcriptional induction in response to drought was faster. However, general transcript down-regulation was found slower than in the susceptible parent. Taken together, these indicate
15 a longer window of active and functional cellular processes in the resistant lines under drought conditions.

The wide array of functional changes that occur in response to drought had been widely reviewed (Bray, 2002, Ramanjulu & Bartels 2002). Down-regulation of genes involved in photosynthesis or chloroplast
20 organization has been reported (Parry *et al.*, 2002; Lawlor & Cornic 2002). In a similar transcription profiling experiment involving drought stressed rice plants, Hazen *et al.*, 2003a found no genes with photosynthetic or chloroplast organization role up regulated. In contrast, many
photosynthesis-related transcripts were found induced in the dehydration
25 stressed, stay-green lines and none of these photosynthesis-related transcripts are induced in the non-stay-green lines. The transcripts are particularly specific to the photosystem reaction centers. These observations indicate that in the stay-green sorghum lines, photosynthetic processes remain active and functional even under water stress.

30 Transcripts related to signaling found repressed in stay-green lines and the corresponding absence of such repressed functions in the non-stg lines seem to indicate that a particular set of signaling cascade is down-regulated in stay-green sorghum. Down-regulated transcripts such as

protease inhibitors and protein ubiquitination found in the stay-green lines indicate less protein degradation and turnover and probably less damage to proteins. The longer window of time for active and functional cellular processes found in the resistant lines under drought conditions, the
5 increased number of transcripts involved in detoxification and antioxidant mechanisms and a corresponding decrease of transcripts related to protein degradation and turn-over, and up regulation of photosynthesis-related transcripts, all support the idea that stay-green sorghum lines resists
10 premature leaf senescence by maintaining greater functional photosynthetic leaf area even under soil moisture stress. The markedly contrasting response of rice and sorghum to dehydration especially with respect to photosynthetic functions could indicate that each utilizes a different drought response mechanism.

Annotation of many of the genes induced earlier in stay-green lines
15 indicate roles in detoxification and antioxidant mechanisms, energy metabolism, and ion homeostasis. Over all, these are responses contributing to initial cellular and metabolic adjustments in response to stress. Later induced functions such as modification of cell structure and cytoskeleton, and cellular membrane trafficking suggest final adjustments
20 indicating return to normal metabolism. Many of the genes induced in the stay-green lines are expectedly modulated by drought. Cluster analysis of the gene expression profiles identified genes that are specifically induced only in the resistant parent or are induced faster. Further analysis of
25 upstream genomic sequences of this gene cluster or other relevant gene clusters could lead to identification of common promoter elements which serve as "transcriptional switches" in the intricate coordination of stimuli sensing and stress response.

Drought-modulated changes in gene expression were also found
almost equal between the induced and down-regulated transcripts.
30 However, a great majority of the down regulated genes remain unclassified and their functions largely unknown

Example 7

Isolation of Orthologs

The nucleic acid sequences disclosed herein represent orthologs in rice of sorghum genes that are differentially expressed in response to changes in water availability. The sequences of these genes are known (see odd numbered SEQ ID NOs: 1-105), and thus identify reagents that are used to isolate the corresponding sorghum orthologs. This is accomplished using standard molecular biology techniques as exemplified in this example.

RNA is isolated as described in Example 2, and double-stranded cDNA is prepared. The cDNA is purified and cloned into a cDNA vector (for example, the SUPERScript™ Double-Stranded cDNA Synthesis Kit from Invitrogen) to produce a cDNA library. The cDNA library is then screened using probes corresponding to odd numbered SEQ ID NOs: 1-105 under the conditions used to screening the GENECHIP® array. Specifically, the library is transferred to a solid support (for example, nitrocellulose or nylon) and hybridized to detectably-labeled probes corresponding to odd numbered SEQ ID NOs: 1-105 in a hybridization buffer consisting of 0.1 mg/mL sonicated and denatured herring sperm DNA, 100 mM 2-N-morpholino-ethane-sulphonic acid (MES), 1 M NaCl, 20 mM EDTA, 0.01% Tween 20. The hybridization is performed 45 °C for 16 hours on a rotisserie at 60 r.p.m.

After hybridization, the solid support is washed with a first wash buffer consisting of 6X SSPE (0.9 M NaCl, 0.06 M NaH₂PO₄, 0.006 M EDTA), 0.01% Tween 20, and 0.005% Antifoam at 25 °C for 10 minutes, followed by a second wash in 100 mM MES, 0.1 M NaCl, 0.01% Tween 20 at 50°C for 20 minutes. After this wash, positive clones are identified by a procedure appropriate to the detectable label (for example, autoradiography or imaging with a PHOSPHORIMAGER™ device when a radioactive label is used). cDNA inserts are then isolated from the identified clones and sequenced to obtain the nucleic acid and derived amino acid sequences of the sorghum orthologs.

Example 8

Expression Vectors and Transformation of Plants

Binary destination vectors for plant transformation comprise or optionally consist of a binary backbone and a T-DNA portion. The binary backbone contains the sequences necessary for selection and growth in *Escherichia coli* DH-5 α (Invitrogen Corp., Carlsbad, California, United States of America) and *Agrobacterium tumefaciens* LBA4404, including the bacterial spectinomycin antibiotic resistance *aadA* gene from *E. coli* transposon Tn7, origins of replication for *E. coli* (ColE1) and *A. tumefaciens* (VS1), and the *A. tumefaciens* *virG* gene. The T-DNA portion is flanked by the right and left border sequences and includes the POSITECH™ (Syngenta Corp., Wilmington, Delaware, United States of America) plant selectable marker and a gene expression cassette that varies depending on the application. The POSITECH™ plant selectable marker in this instance consists of a rice actin (*ACT1*) promoter driving expression of the phosphomannose isomerase (*PMI*) gene, followed by the cauliflower mosaic virus transcriptional terminator, and confers resistance to mannose.

The gene expression cassette portion of the binary destination vectors varies depending on the application. In general, the cassette comprises and optionally consists of a promoter designed to express the gene in certain tissues of the plant, followed by cloning sites (in some cases interrupted by a segment of spacer DNA), and finally by the *A. tumefaciens* *nos* 3' end transcriptional terminator. The promoters used are designed to express the gene of interest in specific target tissues (e.g. endosperm: rice RS-4, wheat glutelin, maize ADPgpp or γ -zein, or barley α -thionin; e.g. embryo: maize globulin or oleosin; e.g. aleurone: barley α -amylase; e.g. root: maize MSR1 and MRS3; e.g. green tissue: maize PEPC) or constitutively (e.g. maize UBI plus intron), depending on the gene of interest. The cloning site contains either unique restriction enzyme sites (for conventional cloning) and/or a GATEWAY™ recombination-based cloning cassette (Invitrogen Corp., Carlsbad, California, United States of America), in either the forward or reverse orientation. In gene expression cassettes designed for double-stranded interfering RNA (dsRNA) production, the

cloning site is divided by a spacer region (e.g. first intron of the rice SH1 gene). The spacer permits the cloning of two gene fragments: one in the forward and one in the reverse orientation. Antisense (reverse orientation expression) is another technology available for silencing genes of interest.

5 Transformation of the nucleic acid molecules of the presently disclosed subject matter into plants is performed using methods disclosed herein above.

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10 The references listed below as well as all references cited in the specification are incorporated herein by reference to the extent that they supplement, explain, provide a background for or teach methodology, techniques, and/or compositions employed herein.

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It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

CLAIMS

What is claimed is:

1. An isolated polypeptide selected from the group consisting of:
 - 5 (a) a polypeptide comprising an amino acid sequence encoded by a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl, to a nucleic acid comprising a nucleotide sequence of one of odd numbered SEQ ID NOs: 1-105; and
 - 10 (b) a functional fragment of the polypeptide of (a).
2. The polypeptide of claim 1, wherein the polypeptide is involved in abiotic stress tolerance.
3. The polypeptide of claim 2, wherein the abiotic stress tolerance is drought resistance.
- 15 4. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - (a) a nucleotide sequence encoding the polypeptide of claim 1, or a fragment, domain, or feature thereof;
 - (b) a sequence fully complementary to (a); and
 - 20 (c) a nucleotide sequence that is the full reverse complement of (a).
5. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - 25 (a) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl, to a nucleic acid comprising a nucleotide sequence of one of odd numbered SEQ ID NOs: 1-105;
 - (b) a nucleotide sequence fully complementary to (a); and
 - 30 (c) a nucleotide sequence which is the full reverse complement of (a).
6. An expression cassette comprising a promoter operatively linked to a nucleic acid molecule of one of claims 4 and 5.

7. A recombinant vector comprising the expression cassette of claim 6.
8. A cell comprising the expression cassette of claim 6.
9. A transgenic plant comprising the expression cassette of claim 6.
10. The transgenic plant of claim 9, wherein the plant is a monocot.
11. The transgenic plant of claim 10, wherein the monocot is selected from the group consisting of rice, maize, wheat, barley, oats, rye, millet, sorghum, triticale, secale, einkorn, spelt, emmer, teff, milo, flax, gramma grass, *Tripsacum*, and *teosinte*.
12. The transgenic plant of claim 9, wherein the plant is selected from the group consisting of rice, wheat, barley, rye, corn, potato, canola, soybean, sunflower, carrot, sweet potato, sugarbeet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, squash, pumpkin, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.
13. The transgenic plant of claim 12, wherein the plant is sorghum.
14. The transgenic plant of claim 9, wherein the expression cassette is expressed in a tissue selected from the group consisting of the epidermis, root, vascular tissue, meristem, cambium, cortex, pith, leaf, flower, and combinations thereof.
15. The transgenic plant of claim 9, wherein the plant has altered abiotic stress tolerance.
16. The transgenic plant of claim 15, wherein the altered abiotic stress tolerance is enhanced drought resistance.
17. Progeny and seed from the transgenic plant of claim 9.
18. A transgenic plant comprising the nucleic acid molecule of claim 4.
19. Progeny and seed from the transgenic plant of claim 18.

20. A method for altering an abiotic stress tolerance of a plant, the method comprising expressing in the plant an expression cassette comprising a nucleic acid molecule encoding a polypeptide of claim 1.

5 21. A shuffled nucleic acid comprising a plurality of nucleotide sequence fragments, wherein at least one of the fragments corresponds to a region of a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl, to a nucleic acid comprising a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, and wherein at least two
10 of the plurality of sequence fragments are in an order, from 5' to 3', which is not an order in which the plurality of fragments naturally occur in a nucleic acid.

22. A method for producing a polypeptide of claim 1, comprising the steps of:

- 15 (a) growing cells comprising an expression cassette under suitable growth conditions, the expression cassette comprising a nucleic acid molecule encoding a polypeptide of claim 1; and
(b) isolating the polypeptide from the cells.

20 23. A method for decreasing expression of an isolated nucleic acid molecule of claim 4 in a plant, the method selected from the group consisting of:

- (a) expressing in said plant a nucleic acid molecule claim 4 or a portion thereof in "sense" orientation;
(b) expressing in said plant a nucleic acid molecule of claim 4 or a
25 portion thereof in "antisense" orientation;
(c) expressing in said plant a ribozyme capable of specifically cleaving a messenger RNA transcript encoded by an endogenous gene corresponding to an isolated nucleic acid molecule of claim 4;
30 (d) expressing in a plant an aptamer specifically directed to a polypeptide encoded by an isolated nucleic acid molecule of claim 4;

- 5 (e) expressing in a plant a mutated or a truncated form of an isolated nucleic acid molecule of claim 4;
- (f) modifying by homologous recombination in a plant at least one chromosomal copy of the gene corresponding to an isolated nucleic acid molecule of claim 4;
- (g) modifying by homologous recombination in a plant at least one chromosomal copy of the regulatory elements of a gene corresponding to an isolated nucleic acid molecule of claim 4; and
- 10 (h) expressing in said plant an isolated nucleic acid molecule of claim 4 or a portion thereof in the "sense" and "antisense" orientation.

24. A method for increasing the expression of an isolated nucleic acid molecule of claim 4 in a plant, the method comprising:

- 15 (a) inserting into the plant an expression cassette comprising an isolated nucleic acid molecule of claim 4; and
- (b) growing the plant comprising the expression cassette under suitable growth conditions, wherein the expression of the isolated nucleic acid molecule of claim 4 is increased.

20 25. A method for enhancing the drought resistance of a plant, the method comprising:

- (a) inserting into the plant an expression cassette comprising at least one isolated nucleic acid molecule of claim 4; and
- 25 (b) growing the plant comprising the expression cassette under suitable growth conditions, wherein the drought resistance of the plant is enhanced.

26. An antibody that specifically binds to the polypeptide of claim 1.

30 27. A method for altering the expression of a polypeptide of claim 1 in a plant, the method comprising expressing an expression cassette encoding a polypeptide of claim 1 in the plant.

28. The method for claim 27, wherein the polypeptide is expressed in a predetermined location or tissue of a plant.

29. The method for claim 28, wherein the location or tissue is selected from the group consisting of epidermis, root, vascular tissue, meristem, cambium, cortex, pith, leaf, flower, seed, and combinations thereof.

5 30. An isolated product from a plant comprising an expression cassette comprising a promoter sequence operatively linked to a nucleic acid, the nucleic acid comprising a nucleotide sequence selected from the group consisting of:

- 10 (a) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl, to a nucleic acid comprising a nucleotide sequence listed in odd numbered sequences of SEQ ID NOs: 1-105, or a fragment, domain, or feature thereof;
- (b) a nucleotide sequence fully complementary to (a); and
- 15 (c) a nucleotide sequence that is the full reverse complement of (a).

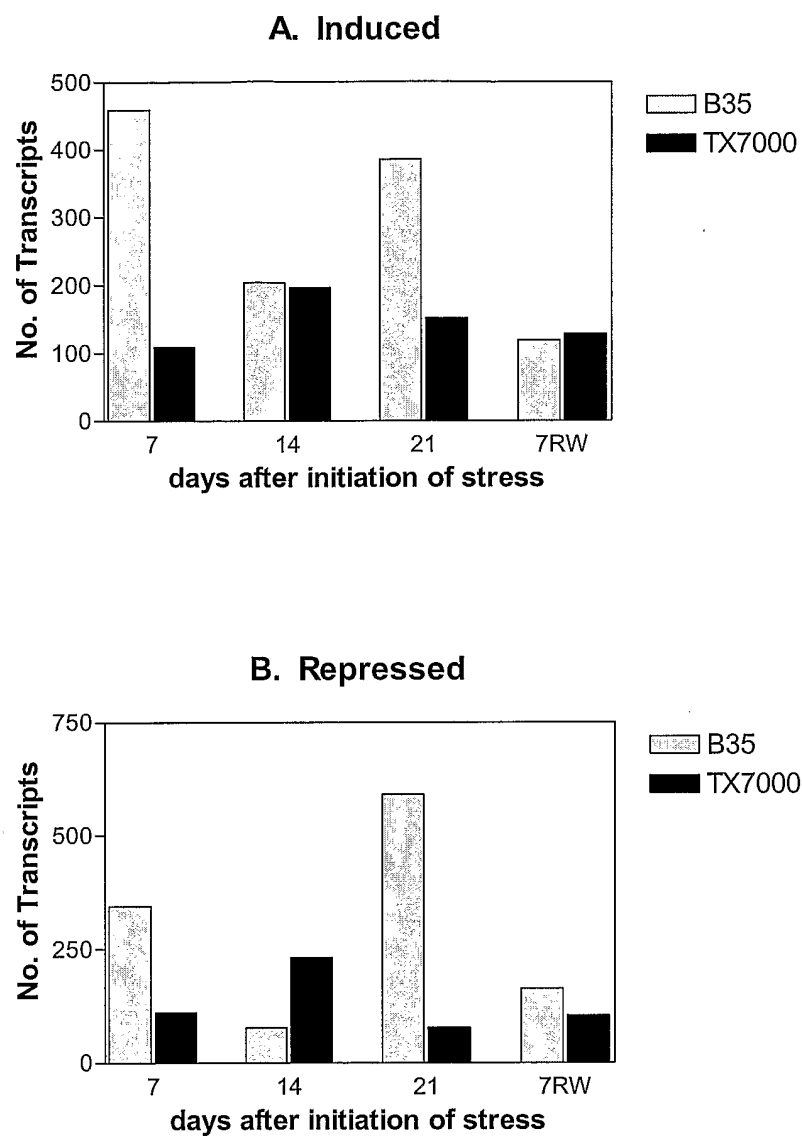
31. A method for producing a recombinant polypeptide, the method comprising:

- 20 (a) growing recombinant cells comprising a nucleic acid construct under suitable growth conditions, the construct comprising an expression vector and a nucleic acid comprising a nucleotide sequence selected from the group consisting of:
- 25 (i) a nucleotide sequence that hybridizes under conditions of hybridization of 45°C in 1 M NaCl, followed by a final washing step at 50°C in 0.1 M NaCl, to a nucleic acid comprising a nucleotide sequence listed in odd numbered nucleotide sequences of SEQ ID NOs: 1-105; and
- (ii) a subsequence of (i); and
- 30 (b) isolating from the recombinant cells the recombinant polypeptide expressed therein.

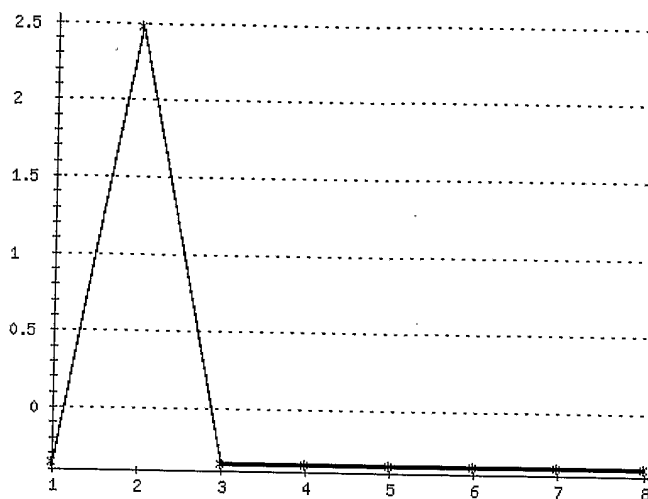
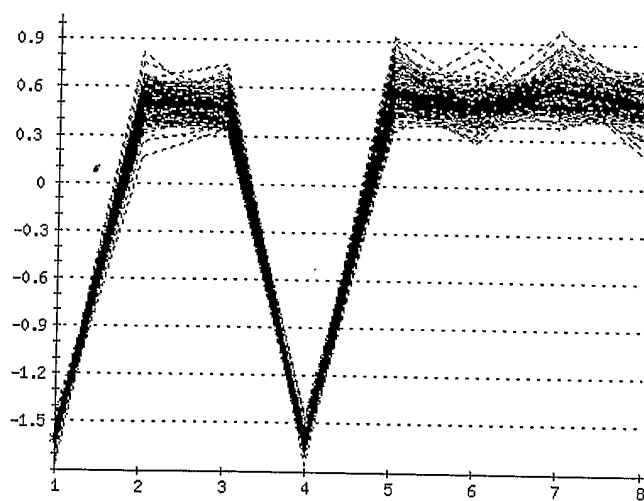
32. The method for claim 31, wherein the expression vector comprises one or more elements selected from the group consisting of a

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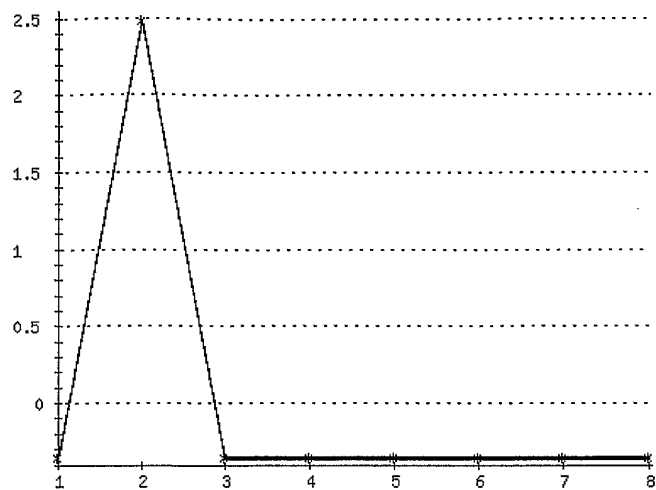
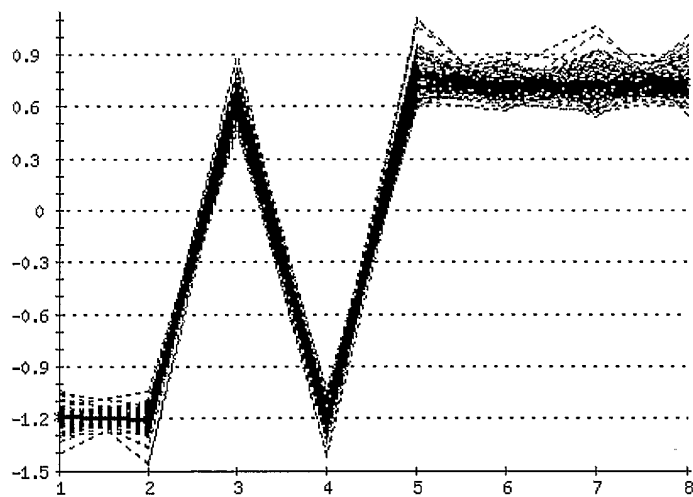
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**Figure 1**

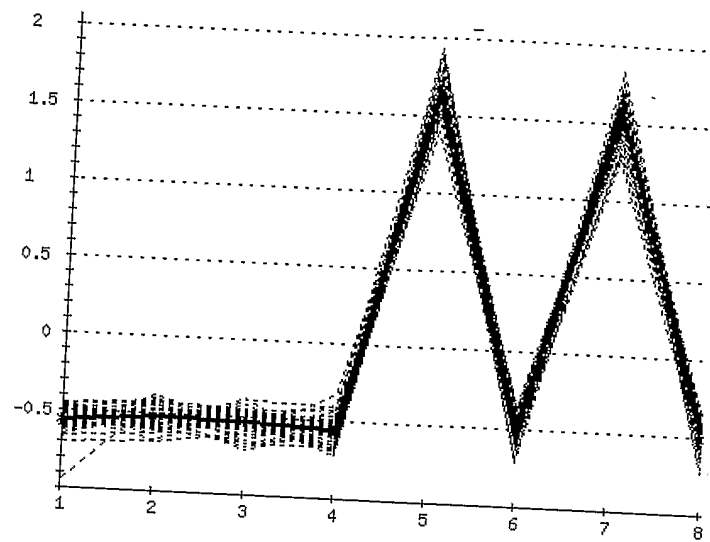
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**Figure 2A****Figure 2B**

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**Figure 2C****Figure 2D**

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**Figure 2E**

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 The Texas Tech University System
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Gln Pro Trp Pro Arg Pro Arg Cys Gly Ala Gly Ala Gly Gly Arg Thr
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45 Ser Gly Ser Gly Asp His His Arg Arg Arg Pro Arg Trp Leu Pro Arg
 195 200 205

50 Ser Pro Glu Arg Gly Pro Ala Gly Glu Arg Arg Ala Pro Pro Ala Pro
 210 215 220

Ser Leu Arg Leu His Cys Phe Ala Gly Gly Ser Lys Ala Glu Glu Gly
 225 230 235 240

55 Gly Gly Glu Glu Gly Val Ala Gly Ala Arg Asn Thr Arg Glu Asn Pro
 245 250 255

Asp Val Ser Leu Gln Met Gly Arg Val Ala Val Ala Arg Pro Thr Ser
 260 265 270

60 Ala Ser Pro Thr Arg Asn Leu Ala Gly His Leu His Pro Val Ala Leu
 275 280 285

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Pro Asn Gly Phe Val Trp Trp Val Leu Leu Thr Arg Gln Pro Thr His
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   Met His Ala Ala Gly Ser Ile Ser Val Thr Asp Gly Ala Leu Asp Arg
   1                5                10                15

   tcg agg tcg gca ggc ggt cgg cgt caa gcg ggc cgc gtg cat acg tta      96
20 Ser Arg Ser Ala Gly Gly Arg Arg Gln Ala Gly Arg Val His Thr Leu
   20                25                30

   cgc gca cac acc gcc cac gcc cac gtc cac gca cgc aca cgt ccg tcc      144
25 Arg Ala His Thr Ala His Ala His Val His Ala Arg Thr Arg Pro Ser
   35                40                45

   ggt tgc tct ccg cgc cca gct ggt cga tcg ccc agc tgc cac tcc aca      192
   Gly Cys Ser Pro Arg Pro Ala Gly Arg Ser Pro Ser Cys His Ser Thr
   50                55                60

30 ctc cac aga ctc gag act cca gcc ccg ccg ccg cca gaa atc aag      240
   Leu His Arg Leu Glu Thr Pro Ala Pro Pro Pro Pro Pro Glu Ile Lys
   65                70                75                80

35 cca gca aac gtt gac gtt ttc cca aaa gtc ttc gga gac ctc ttt ccg      288
   Pro Ala Asn Val Asp Val Phe Pro Lys Val Phe Gly Asp Leu Phe Pro
   85                90                95

   atg aaa aat ctt ttt tgt gta att cca aat gtg aag caa gtc tcc gta      336
40 Met Lys Asn Leu Phe Cys Val Ile Pro Asn Val Lys Gln Val Ser Val
   100                105                110

   tct tat tgg tga      348
   Ser Tyr Trp
   115

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   <211> 115
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50 <400>  8

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55 Ser Arg Ser Ala Gly Gly Arg Arg Gln Ala Gly Arg Val His Thr Leu
   20                25                30

   Arg Ala His Thr Ala His Ala His Val His Ala Arg Thr Arg Pro Ser
60 35                40                45

   Gly Cys Ser Pro Arg Pro Ala Gly Arg Ser Pro Ser Cys His Ser Thr
   50                55                60

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Leu His Arg Leu Glu Thr Pro Ala Pro Pro Pro Pro Glu Ile Lys
65          70          75          80

5  Pro Ala Asn Val Asp Val Phe Pro Lys Val Phe Gly Asp Leu Phe Pro
    85          90          95

Met Lys Asn Leu Phe Cys Val Ile Pro Asn Val Lys Gln Val Ser Val
    100        105        110

10 Ser Tyr Trp
    115

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15 <212> DNA
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25 1          5          10          15

tcg tct tgg tct agg tct aga act atc ttc aac ggt ttg tgt agc cat      96
Ser Ser Trp Ser Arg Ser Arg Thr Ile Phe Asn Gly Leu Cys Ser His
    20          25          30

30 aaa ttc tat tta atg att tgt gtt gac ttt gta tac tat tcc att gta      144
Lys Phe Tyr Leu Met Ile Cys Val Asp Phe Val Tyr Tyr Ser Ile Val
    35          40          45

35 gtt gta aat aca cga tct ttt cgt aga tcc att gta atc ttg aaa ttc      192
Val Val Asn Thr Arg Ser Phe Arg Arg Ser Ile Val Ile Leu Lys Phe
    50          55          60

40 tat aaa aat gga aac aac aac ttt agt cac cat ctc cat atc tgg ttt      240
Tyr Lys Asn Gly Asn Asn Asn Phe Ser His His Leu His Ile Trp Phe
    65          70          75          80

act tgt aag ctt tac tgg gta tgc ctt ata tac cgc gtt tgg gca acc      288
Thr Cys Lys Leu Tyr Trp Val Cys Leu Ile Tyr Arg Val Trp Ala Thr
45          85          90          95

ctc tca aca att aag aga tcc att cga aga aca gag gac taa      330
Leu Ser Thr Ile Lys Arg Ser Ile Arg Arg Thr Glu Asp
    100        105

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55 <400> 10

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60 Ser Ser Trp Ser Arg Ser Arg Thr Ile Phe Asn Gly Leu Cys Ser His
    20          25          30

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	Lys Phe Tyr Leu Met Ile Cys Val Asp Phe Val Tyr Tyr Ser Ile Val	
	35 40 45	
5	Val Val Asn Thr Arg Ser Phe Arg Arg Ser Ile Val Ile Leu Lys Phe	
	50 55 60	
	Tyr Lys Asn Gly Asn Asn Asn Phe Ser His His Leu His Ile Trp Phe	
	65 70 75 80	
10	Thr Cys Lys Leu Tyr Trp Val Cys Leu Ile Tyr Arg Val Trp Ala Thr	
	85 90 95	
	Leu Ser Thr Ile Lys Arg Ser Ile Arg Arg Thr Glu Asp	
	100 105	
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	1 5 10 15	
30	tgt gaa gtg aaa gaa atc att tta tct act aat agt ggc caa att ggc	96
	Cys Glu Val Lys Glu Ile Ile Leu Ser Thr Asn Ser Gly Gln Ile Gly	
	20 25 30	
	gta tta cca aac cac gcc ccc att aac aca gct gta gat atg ggt ccc	144
35	Val Leu Pro Asn His Ala Pro Ile Asn Thr Ala Val Asp Met Gly Pro	
	35 40 45	
	ttg aga ata cgc ctc ctc aac gat caa tgg tta acg gcg gtt ctg tgg	192
40	Leu Arg Ile Arg Leu Leu Asn Asp Gln Trp Leu Thr Ala Val Leu Trp	
	50 55 60	
	agc ggt ttt gcc aga ata gtt aat aat gag atc atc att tta gga aat	240
	Ser Gly Phe Ala Arg Ile Val Asn Asn Glu Ile Ile Ile Leu Gly Asn	
	65 70 75 80	
45	gat gcg gaa ctg ggt agt gac att gat ccg gaa gaa gct caa cag gca	288
	Asp Ala Glu Leu Gly Ser Asp Ile Asp Pro Glu Glu Ala Gln Gln Ala	
	85 90 95	
50	ctt gaa ata gcc gaa gct aac gtg agt aga gct gag ggt acg aaa gaa	336
	Leu Glu Ile Ala Glu Ala Asn Val Ser Arg Ala Glu Gly Thr Lys Glu	
	100 105 110	
	ttg gtt gaa gcg aag gta gct ctc aga cga gct agg ata cga gtc gag	384
55	Leu Val Glu Ala Lys Val Ala Leu Arg Arg Ala Arg Ile Arg Val Glu	
	115 120 125	
	gct gtt aat tgg att ccc cca tct aat tga	414
	Ala Val Asn Trp Ile Pro Pro Ser Asn	
60	130 135	

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 10 Cys Glu Val Lys Glu Ile Ile Leu Ser Thr Asn Ser Gly Gln Ile Gly
 20 25 30
 Val Leu Pro Asn His Ala Pro Ile Asn Thr Ala Val Asp Met Gly Pro
 35 40 45
 15 Leu Arg Ile Arg Leu Leu Asn Asp Gln Trp Leu Thr Ala Val Leu Trp
 50 55 60
 Ser Gly Phe Ala Arg Ile Val Asn Asn Glu Ile Ile Ile Leu Gly Asn
 20 65 70 75 80
 Asp Ala Glu Leu Gly Ser Asp Ile Asp Pro Glu Glu Ala Gln Gln Ala
 85 90 95
 25 Leu Glu Ile Ala Glu Ala Asn Val Ser Arg Ala Glu Gly Thr Lys Glu
 100 105 110
 Leu Val Glu Ala Lys Val Ala Leu Arg Arg Ala Arg Ile Arg Val Glu
 115 120 125
 30 Ala Val Asn Trp Ile Pro Pro Ser Asn
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 45 Met Ser Leu Ile Arg Arg Gly Asn Ala Phe Asp Pro Phe Ser Leu Asp
 1 5 10 15
 ctc tgg gat ccc gtc gac ggc ttc ccc ttc ggc tcc ggc ggc agc agc 96
 50 Leu Trp Asp Pro Val Asp Gly Phe Pro Phe Gly Ser Gly Gly Ser Ser
 20 25 30
 agc agc agc ggc agc ctc ttc cct cgg gcc aac tcc gac gcg gcg gcc 144
 Ser Ser Ser Gly Ser Leu Phe Pro Arg Ala Asn Ser Asp Ala Ala Ala
 35 40 45
 55 ttc gcc ggc gcg cgc atc gac tgg aag gag acg ccc gag gtg cac gtg 192
 Phe Ala Gly Ala Arg Ile Asp Trp Lys Glu Thr Pro Glu Val His Val
 50 55 60
 60 ttc aag gcg gac gtg ccg ggg ctg aag aag gag gag gtc aag gtg gag 240
 Phe Lys Ala Asp Val Pro Gly Leu Lys Lys Glu Glu Val Lys Val Glu
 65 70 75 80

gtg gac gac ggc aac atc ctg cag atc agc ggc gag cgc agc agg gag 288
 Val Asp Asp Gly Asn Ile Leu Gln Ile Ser Gly Glu Arg Ser Arg Glu
 85 90 95

5 cag gag gag aag tcg gac aag tgg cac cgc gtg gag cgc agc agc ggc 336
 Gln Glu Glu Lys Ser Asp Lys Trp His Arg Val Glu Arg Ser Ser Gly
 100 105 110

10 aag ttc ctc cgc agg ttc cgc ctc ccc gag aac acc aag ccg gag cag 384
 Lys Phe Leu Arg Arg Phe Arg Leu Pro Glu Asn Thr Lys Pro Glu Gln
 115 120 125

15 atc aag gcg tcc atg gag aat ggc gtg ctc acc gtc acc gtg ccc aag 432
 Ile Lys Ala Ser Met Glu Asn Gly Val Leu Thr Val Thr Val Pro Lys
 130 135 140

20 gaa gag ccc aag aag ccc gac gtc aag tcc atc cag atc tcc ggc tag 480
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 145 150 155

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25 <400> 14

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 20 25 30

35 Ser Ser Ser Gly Ser Leu Phe Pro Arg Ala Asn Ser Asp Ala Ala Ala
 35 40 45
 Phe Ala Gly Ala Arg Ile Asp Trp Lys Glu Thr Pro Glu Val His Val
 50 55 60

40 Phe Lys Ala Asp Val Pro Gly Leu Lys Lys Glu Glu Val Lys Val Glu
 65 70 75 80
 Val Asp Asp Gly Asn Ile Leu Gln Ile Ser Gly Glu Arg Ser Arg Glu
 85 90 95

45 Gln Glu Glu Lys Ser Asp Lys Trp His Arg Val Glu Arg Ser Ser Gly
 100 105 110

50 Lys Phe Leu Arg Arg Phe Arg Leu Pro Glu Asn Thr Lys Pro Glu Gln
 115 120 125
 Ile Lys Ala Ser Met Glu Asn Gly Val Leu Thr Val Thr Val Pro Lys
 130 135 140

55 Glu Glu Pro Lys Lys Pro Asp Val Lys Ser Ile Gln Ile Ser Gly
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10 ctc gcc gcc ccg cac gcc gcc gtg gcc atc acc tgc ggc cag gtc aac 96
Leu Ala Ala Pro His Ala Ala Val Ala Ile Thr Cys Gly Gln Val Asn
20 25 30

15 tcc gcc gtc ggg ccc tgc ctg acc tac gcc cgc ggc ggc gcc ggg ccg 144
Ser Ala Val Gly Pro Cys Leu Thr Tyr Ala Arg Gly Gly Ala Gly Pro
35 40 45

20 tgc ggc gcc tgc tgc agc ggc gtg agg agc ctc aag gcg gca gca agc 192
Ser Ala Ala Cys Cys Ser Gly Val Arg Ser Leu Lys Ala Ala Ala Ser
50 55 60

25 acg acc gca gac cgg cgc acc gcc tgc aac tgc ctc aag aac gcg gcc 240
Thr Thr Ala Asp Arg Arg Thr Ala Cys Asn Cys Leu Lys Asn Ala Ala
65 70 75 80

25 cgc ggc atc aag ggg ctc aac gcc ggc aac gcc gcc agc atc ccc tcc 288
Arg Gly Ile Lys Gly Leu Asn Ala Gly Asn Ala Ala Ser Ile Pro Ser
85 90 95

30 aag tgc ggc gtc agc gtc ccc tac acc atc agc gct tcc atc gac tgc 336
Lys Cys Gly Val Ser Val Pro Tyr Thr Ile Ser Ala Ser Ile Asp Cys
100 105 110

35 tcc agg gtg agc tga 351
Ser Arg Val Ser
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20 25 30

50 Ser Ala Val Gly Pro Cys Leu Thr Tyr Ala Arg Gly Gly Ala Gly Pro
35 40 45

Ser Ala Ala Cys Cys Ser Gly Val Arg Ser Leu Lys Ala Ala Ala Ser -
50 55 60

55 Thr Thr Ala Asp Arg Arg Thr Ala Cys Asn Cys Leu Lys Asn Ala Ala
65 70 75 80

Arg Gly Ile Lys Gly Leu Asn Ala Gly Asn Ala Ala Ser Ile Pro Ser
85 90 95

60 Lys Cys Gly Val Ser Val Pro Tyr Thr Ile Ser Ala Ser Ile Asp Cys
100 105 110

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Ser Arg Val Ser
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   1 5 10 15

   gaa gcg act tgg gtt gac tta tac aat gtt atg tat cga gaa agg aca 96
   Glu Ala Thr Trp Val Asp Leu Tyr Asn Val Met Tyr Arg Glu Arg Thr
   20 20 25 30

   ctt ttt tta ggt caa gag att cgt tgc gag gtc acg aat cat att aca 144
   Leu Phe Leu Gly Gln Glu Ile Arg Cys Glu Val Thr Asn His Ile Thr
   25 35 40 45

   ggt ctc atg gta tat gtc agt ata gaa gat gga att agc gat att ttt 192
   Gly Leu Met Val Tyr Val Ser Ile Glu Asp Gly Ile Ser Asp Ile Phe
   50 55 60

   ttg ttt ata aac tcc ccc ggc ggg tgg tta atc tca gga atg gcg att 240
   Leu Phe Ile Asn Ser Pro Gly Gly Trp Leu Ile Ser Gly Met Ala Ile
   65 70 75 80

   ttt gat acg atg caa acg gtg aca cca gat ata tat aca ata tgc ctc 288
   Phe Asp Thr Met Gln Thr Val Thr Pro Asp Ile Tyr Thr Ile Cys Leu
   85 90 95

   gga ata gcc gcg tcc atg gcg tcc ttc att ctg ctt gga gga gaa ccc 336
   Gly Ile Ala Ala Ser Met Ala Ser Phe Ile Leu Leu Gly Gly Glu Pro
   100 105 110

   acc aag cgt ata gca ttc ccc cac gcg agg att atg ctt cac caa cct 384
   Thr Lys Arg Ile Ala Phe Pro His Ala Arg Ile Met Leu His Gln Pro
   115 120 125

45 gct agt gct tat tat cgg gca aga aca cca gaa ttt tta cta gaa gtg 432
   Ala Ser Ala Tyr Tyr Arg Ala Arg Thr Pro Glu Phe Leu Leu Glu Val
   130 135 140

50 gaa gag tta cac aaa gtt cgc gaa atg atc aca agg gtt tat gca cta 480
   Glu Glu Leu His Lys Val Arg Glu Met Ile Thr Arg Val Tyr Ala Leu
   145 150 155 160

   aga aca ggc aag cct ttt tgg gtt gta tcc gaa gac atg gaa agg gat 528
   Arg Thr Gly Lys Pro Phe Trp Val Val Ser Glu Asp Met Glu Arg Asp
   165 170 175

   gtt ttt atg tca gca gac gaa gcc aaa gct tat gga ctt gtc gat att 576
   Val Phe Met Ser Ala Asp Glu Ala Lys Ala Tyr Gly Leu Val Asp Ile
   180 185 190

60 gta ggg gat gaa atg ctt gac gag cac tgc gat act gat cca gtg tgg 624
   Val Gly Asp Glu Met Leu Asp Glu His Cys Asp Thr Asp Pro Val Trp
   195 200 205

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 20 25 30
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 20 35 40 45
 Gly Leu Met Val Tyr Val Ser Ile Glu Asp Gly Ile Ser Asp Ile Phe
 50 55 60
 25
 Leu Phe Ile Asn Ser Pro Gly Gly Trp Leu Ile Ser Gly Met Ala Ile
 65 70 75 80
 Phe Asp Thr Met Gln Thr Val Thr Pro Asp Ile Tyr Thr Ile Cys Leu
 85 90 95
 30
 Gly Ile Ala Ala Ser Met Ala Ser Phe Ile Leu Leu Gly Gly Glu Pro
 100 105 110
 Thr Lys Arg Ile Ala Phe Pro His Ala Arg Ile Met Leu His Gln Pro
 115 120 125
 35
 Ala Ser Ala Tyr Tyr Arg Ala Arg Thr Pro Glu Phe Leu Leu Glu Val
 130 135 140
 40
 Glu Glu Leu His Lys Val Arg Glu Met Ile Thr Arg Val Tyr Ala Leu
 145 150 155 160
 Arg Thr Gly Lys Pro Phe Trp Val Val Ser Glu Asp Met Glu Arg Asp
 165 170 175
 45
 Val Phe Met Ser Ala Asp Glu Ala Lys Ala Tyr Gly Leu Val Asp Ile
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 Val Gly Asp Glu Met Leu Asp Glu His Cys Asp Thr Asp Pro Val Trp
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5	gtt gca ttc act tac aac aag aaa caa cca cca gcg ttt ggt gca gca	96
	Val Ala Phe Thr Tyr Asn Lys Lys Gln Pro Pro Ala Phe Gly Ala Ala	
	20 25 30	
10	cct gca ttt tgg tgc att ctt ctt cct ttc ctt ggt ctt tcg ttc cgt	144
	Pro Ala Phe Trp Cys Ile Leu Leu Pro Phe Leu Gly Leu Ser Phe Arg	
	35 40 45	
15	cat att cca aat aac tta tct aat tac aac gta tta acc ggt aat gca	192
	His Ile Pro Asn Asn Leu Ser Asn Tyr Asn Val Leu Thr Gly Asn Ala	
	50 55 60	
20	tct ttt ttt tat caa atc tca ggg gca tgg tct aat cat gag ggt agt	240
	Ser Phe Phe Tyr Gln Ile Ser Gly Ala Trp Ser Asn His Glu Gly Ser	
	65 70 75 80	
25	att tta tca tgg tgt tgg atc cca agt ttt tat gga ttc ctt ttt tgt	288
	Ile Leu Ser Trp Cys Trp Ile Pro Ser Phe Tyr Gly Phe Leu Phe Cys	
	85 90 95	
30	tac cgg ggt cga ccc caa agc cat aat gtc tca aaa cga aga ggc tat	336
	Tyr Arg Gly Arg Pro Gln Ser His Asn Val Ser Lys Arg Arg Gly Tyr	
	100 105 110	
35	aga gaa act ttt att ttt tcc ttt gtc tcg aac ttc gtg aag aac tcc	384
	Arg Glu Thr Phe Ile Phe Ser Phe Val Ser Asn Phe Val Lys Asn Ser	
	115 120 125	
40	att cta tct ctc caa caa aaa agt ggg gct gcg ccc cag ttg tac acc	432
	Ile Leu Ser Leu Gln Gln Lys Ser Gly Ala Ala Pro Gln Leu Tyr Thr	
	130 135 140	
45	ccc ttc gtt cgg aga acc ttt gtt gat tct gaa ctt cgt tcg caa agt	480
	Pro Phe Val Arg Arg Thr Phe Val Asp Ser Glu Leu Arg Ser Gln Ser	
	145 150 155 160	
50	aag cgt cct ttt aac ggg cca gcc ctt ttt aat gcg ccg ctt gac cct	528
	Lys Arg Pro Phe Asn Gly Pro Ala Leu Phe Asn Ala Pro Leu Asp Pro	
	165 170 175	
55	gtt ttg aaa atg agc ttt gct ctt ctg ggc gct ggg cgc tcc cgt ggt	576
	Val Leu Lys Met Ser Phe Ala Leu Leu Gly Ala Gly Arg Ser Arg Gly	
	180 185 190	
60	tcg cga gaa gga aaa agg act aat ctt ttg tta cat ctg gca cga gat	624
	Ser Arg Glu Gly Lys Arg Thr Asn Leu Leu Leu His Leu Ala Arg Asp	
	195 200 205	
65	gaa aaa gag aga gct tcg tct atc gat gaa cag cag att gac gga gct	672
	Glu Lys Glu Arg Ala Ser Ser Ile Asp Glu Gln Gln Ile Asp Gly Ala	
	210 215 220	
70	ctt gcc att gct ttg ttt ttc tct tct ttc cta tca gcg agt tcc gat	720
	Leu Ala Ile Ala Leu Phe Phe Ser Ser Phe Leu Ser Ala Ser Ser Asp	
	225 230 235 240	
75	cct ttt gtt cga aat ttc ttc gtt cgt acc gaa ccg ctt gca gaa tca	768
	Pro Phe Val Arg Asn Phe Phe Val Arg Thr Glu Pro Leu Ala Glu Ser	
	245 250 255	

	aat cct gtt cca caa gat cct ata tca gct ata cat cct cct tgc att	816
	Asn Pro Val Pro Gln Asp Pro Ile Ser Ala Ile His Pro Pro Cys Ile	
	260 265 270	
5	tat gcc gga gac gtc gcc agt gct atg ggc ttt ggg tta tgt aga tca	864
	Tyr Ala Gly Asp Val Ala Ser Ala Met Gly Phe Gly Leu Cys Arg Ser	
	275 280 285	
10	aaa atg atg aat ggg att gtg gca ctc cac tcg ccg cca atg cgg aag	912
	Lys Met Met Asn Gly Ile Val Ala Leu His Ser Pro Pro Met Arg Lys	
	290 295 300	
15	gat gcc gcc gaa aag aat gga acg ctg ctt cgc tct gct gga tgc gtc	960
	Asp Ala Ala Glu Lys Asn Gly Thr Leu Leu Arg Ser Ala Gly Cys Val	
	305 310 315 320	
20	gga tcc cat ata aga agc tcg ctc ttt acc cga tca ttc aaa cat ttt	1008
	Gly Ser His Ile Arg Ser Ser Leu Phe Thr Arg Ser Phe Lys His Phe	
	325 330 335	
25	gtg ggc ggc gcg cct gct cta ttg ttg cgt agc aat aga agc ctg ctc	1056
	Val Gly Gly Ala Pro Ala Leu Leu Arg Ser Asn Arg Ser Leu Leu	
	340 345 350	
30	atg ctg ctt cgg cgg cgc ttt ttc gcc ttc tct tcg ctc tgg aca gga	1104
	Met Leu Leu Arg Arg Arg Phe Phe Ala Phe Ser Ser Leu Trp Thr Gly	
	355 360 365	
35	gca cta atg gac acg ggg agg gag cag gcg aag cgt gtc gtt cgt aat	1152
	Ala Leu Met Asp Thr Gly Arg Glu Gln Ala Lys Arg Val Val Arg Asn	
	370 375 380	
40	gga aag aaa gac acc act act tcg cct ctt tgt tgg acc gcc ggc gcg	1200
	Gly Lys Lys Asp Thr Thr Thr Ser Pro Leu Cys Trp Thr Ala Gly Ala	
	385 390 395 400	
45	aac aca gtg gtc tct gac cag gac cag gaa cca att cga att tgg atc	1248
	Asn Thr Val Val Ser Asp Gln Asp Gln Glu Pro Ile Arg Ile Trp Ile	
	405 410 415	
50	ttg aca tgt cgg ttg ttt tta acc gta ggc atc tcg cca gga agt tgg	1296
	Leu Thr Cys Arg Leu Phe Leu Thr Val Gly Ile Ser Pro Gly Ser Trp	
	420 425 430	
55	tgg gct cat cat gaa tta ggt cgg ggt ggc tgg tgg ttt cgg gat ccc	1344
	Trp Ala His His Glu Leu Gly Arg Gly Gly Trp Trp Phe Arg Asp Pro	
	435 440 445	
60	gta gaa aat gcg tct ttt atg cct cgg gta tta gcc aca gct cgt att	1392
	Val Glu Asn Ala Ser Phe Met Pro Arg Val Leu Ala Thr Ala Arg Ile	
	450 455 460	
65	cat tca gta att cta ccc ctt ctt cat tct tgg acc tcg ctc ctg aat	1440
	His Ser Val Ile Leu Pro Leu Leu His Ser Trp Thr Ser Leu Leu Asn	
	465 470 475 480	
70	att ttg act ctt cca tgc tgt gtc tca gga acc ttt tca ata cgg tcc	1488
	Ile Leu Thr Leu Pro Cys Cys Val Ser Gly Thr Phe Ser Ile Arg Ser	
	485 490 495	
75	gga ttg cta gct ccc gtt cat agt tct gct aca gac gat aca cga gga	1536
	Gly Leu Leu Ala Pro Val His Ser Ser Ala Thr Asp Asp Thr Arg Gly	
	500 505 510	

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    aga ttt tta tgg cgg ttc ttc ctt cta att aca ggc ata tct atg act      1584
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Arg Met Asp Phe Val Pro Asp Arg Ser Ala Leu Glu Val Asp Asp Ile 95
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55 Arg Val Asp Lys Glu Thr Leu Asp Met Leu Thr Ser Leu Gly Met Ala 110
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   Leu Val Tyr Trp Phe Val Trp Val Met Gly Ala Ala Glu Val Lys Gly
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25	ggg cag ctg ttc tcc gcc atg gtg gtg cgc aag ccg gcg cac ctg ttc	432
	Gly Gln Leu Phe Ser Ala Met Val Val Arg Lys Pro Ala His Leu Phe	
	130 135 140	
30	ctc gac gag ctc gcc gtc gcg tac gac gag cag gag gac tac gtc gtc	480
	Leu Asp Glu Leu Gly Val Ala Tyr Asp Glu Gln Glu Asp Tyr Val Val	
	145 150 155 160	
35	atc aag cac gcc gcg ctc ttc acc tcc acc gtc atg agc cgc ctc ctg	528
	Ile Lys His Ala Ala Leu Phe Thr Ser Thr Val Met Ser Arg Leu Leu	
	165 170 175	
40	gcg cgc ccc aac gtg aag ctg ttc aac gcc gtc gcc gtc gag gac ctc	576
	Ala Arg Pro Asn Val Lys Leu Phe Asn Ala Val Ala Val Glu Asp Leu	
	180 185 190	
45	atc gtc aag gag gcc gcg gtc gcc gcc gtg gtc acc aac tgg gcg ctg	624
	Ile Val Lys Glu Gly Arg Val Gly Gly Val Val Thr Asn Trp Ala Leu	
	195 200 205	
50	gtg tcg atg aac cac gac acg cag tcg tgc atg gac ccc aac gtg atg	672
	Val Ser Met Asn His Asp Thr Gln Ser Cys Met Asp Pro Asn Val Met	
	210 215 220	
55	gag tcc agg gtg gtg gtg agc tcc tgc gcc cac gac ggg ccg ttc gcc	720
	Glu Ser Arg Val Val Val Ser Ser Cys Gly His Asp Gly Pro Phe Gly	
	225 230 235 240	
60	gcc acg gcc gtc aag cgg ctg cag gac atc gcc atg atc gac gcc gtg	768
	Ala Thr Gly Val Lys Arg Leu Gln Asp Ile Gly Met Ile Asp Ala Val	
	245 250 255	
65	ccc gcc atg cgc gcc ctc gac atg aac acc gcc gag gac gag atc gtc	816
	Pro Gly Met Arg Ala Leu Asp Met Asn Thr Ala Glu Asp Glu Ile Val	
	260 265 270	
70	cgc ctc acc cgc gag gtc gtc ccc gcc atg atc gtc acc gcc atg gag	864
	Arg Leu Thr Arg Glu Val Val Pro Gly Met Ile Val Thr Gly Met Glu	
	275 280 285	
75	gtc gcc gag atc gac gcc gcc ccg aga atg gcc ccg acg ttc gga gcc	912
	Val Ala Glu Ile Asp Gly Ala Pro Arg Met Gly Pro Thr Phe Gly Ala	
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      Met Met Ile Ser Gly Gln Lys Ala Ala His Leu Ala Leu Lys Ala Leu
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5      ggc cgg ccg aac gcc atc gac ggc acg atc aag aag gcg gcg gcg gcg      1008
      Gly Arg Pro Asn Ala Ile Asp Gly Thr Ile Lys Lys Ala Ala Ala Ala
                        325                      330                      335

10     gcg gcg cac ccg gag ctg atc ctg gcg tcg aag gac gac ggc gag atc      1056
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30     Ala Pro Arg Thr Gly Gly Ala Ile Cys Asn Ser Ile Ser Ser Ser Ser
      35                      40                      45

      Ser Thr Pro Pro Tyr Asp Leu Asn Ala Ile Arg Phe Ser Pro Ile Lys
      50                      55                      60

35     Glu Ser Ile Val Ser Arg Glu Met Thr Arg Arg Tyr Met Thr Asp Met
      65                      70                      75                      80

      Ile Thr Tyr Ala Asp Thr Asp Val Val Val Val Gly Ala Gly Ser Ala
      85                      90                      95

      Gly Leu Ser Cys Ala Tyr Glu Leu Ser Lys Asp Pro Ser Val Ser Val
      100                      105                      110

45     Ala Val Ile Glu Gln Ser Val Ser Pro Gly Gly Gly Ala Trp Leu Gly
      115                      120                      125

      Gly Gln Leu Phe Ser Ala Met Val Val Arg Lys Pro Ala His Leu Phe
      130                      135                      140

50     Leu Asp Glu Leu Gly Val Ala Tyr Asp Glu Gln Glu Asp Tyr Val Val
      145                      150                      155                      160

      Ile Lys His Ala Ala Leu Phe Thr Ser Thr Val Met Ser Arg Leu Leu
      165                      170                      175

55     Ala Arg Pro Asn Val Lys Leu Phe Asn Ala Val Ala Val Glu Asp Leu
      180                      185                      190

60     Ile Val Lys Glu Gly Arg Val Gly Gly Val Val Thr Asn Trp Ala Leu
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      Val Ser Met Asn His Asp Thr Gln Ser Cys Met Asp Pro Asn Val Met
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	275 280 285	
	Val Ala Glu Ile Asp Gly Ala Pro Arg Met Gly Pro Thr Phe Gly Ala	
	290 295 300	
15	Met Met Ile Ser Gly Gln Lys Ala Ala His Leu Ala Leu Lys Ala Leu	
	305 310 315 320	
	Gly Arg Pro Asn Ala Ile Asp Gly Thr Ile Lys Lys Ala Ala Ala Ala	
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	ctg gcg ggc cca cac acc acc atg gcc gcc atc agc tgc ggc cag gtc	96
	Leu Ala Gly Pro His Thr Thr Met Ala Ala Ile Ser Cys Gly Gln Val	
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45	aac tcc gcc gtg tcg ccc tgc ctc agc tac gcc cgc ggc ggc tcc ggc	144
	Asn Ser Ala Val Ser Pro Cys Leu Ser Tyr Ala Arg Gly Gly Ser Gly	
	35 40 45	
50	ccg tcg gcg gcc tgc tgc agc ggc gtc agg agc ctc aac tcc gcc gcc	192
	Pro Ser Ala Ala Cys Cys Ser Gly Val Arg Ser Leu Asn Ser Ala Ala	
	50 55 60	
	acc acc acc gcc gac cgc cgc acc gcc tgc aac tgc ctc aag aac gtg	240
55	Thr Thr Thr Ala Asp Arg Arg Thr Ala Cys Asn Cys Leu Lys Asn Val	
	65 70 75 80	
	gcc ggc agc atc agc ggc ctc aac gcc ggc aat gcc gcc agc atc ccc	288
	Ala Gly Ser Ile Ser Gly Leu Asn Ala Gly Asn Ala Ala Ser Ile Pro	
	85 90 95	
60	tcc aag tgc ggc gtc agc atc ccc tac acc atc agc ccc tcc atc gac	336
	Ser Lys Cys Gly Val Ser Ile Pro Tyr Thr Ile Ser Pro Ser Ile Asp	
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 35 40 45
 20 Pro Ser Ala Ala Cys Cys Ser Gly Val Arg Ser Leu Asn Ser Ala Ala
 50 55 60
 25 Thr Thr Thr Ala Asp Arg Arg Thr Ala Cys Asn Cys Leu Lys Asn Val
 65 70 75 80
 Ala Gly Ser Ile Ser Gly Leu Asn Ala Gly Asn Ala Ala Ser Ile Pro
 85 90 95
 30 Ser Lys Cys Gly Val Ser Ile Pro Tyr Thr Ile Ser Pro Ser Ile Asp
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 Pro Leu Ser Ala Arg Gly Arg Ser Ser Ser Val Ser Phe Pro Val Pro
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 55 aag aaa ggg ggt att ggt cat ggt ggc ctg cgg atc gag tgc att cgt
 Lys Lys Gly Gly Ile Gly His Gly Gly Leu Arg Ile Glu Cys Ile Arg
 35 40 45 144
 att ggt gga gtg gag atc ccg aac cac aag agg gta gag tac tca ctg
 Ile Gly Gly Val Glu Ile Pro Asn His Lys Arg Val Glu Tyr Ser Leu
 50 55 60 192
 60 cag tac atc cat ggc att ggg cgg tca cgc tcc cgt cag atc ctc ttg
 Gln Tyr Ile His Gly Ile Gly Arg Ser Arg Ser Arg Gln Ile Leu Leu
 65 70 75 80 240

gac ctc aat ttt gac aac aag gtc aca aag gac ctc tcc gag gag gaa 288
 Asp Leu Asn Phe Asp Asn Lys Val Thr Lys Asp Leu Ser Glu Glu Glu
 85 90 95

5

gtc atc act ctc cgt aag gag aaa cgg ttt aat cgt gtg gcg att gag 336
 Val Ile Thr Leu Arg Lys Glu Lys Arg Phe Asn Arg Val Ala Ile Glu
 100 105 110

10

agg ttg aag gag att agg tgt tac agg ggc atc cgc cac aag cta ggg 384
 Arg Leu Lys Glu Ile Arg Cys Tyr Arg Gly Ile Arg His Lys Leu Gly
 115 120 125

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ctg cca gtt cgt gga cag agg acg aag aac aat tgc agg acg ctg aag 432
 Leu Pro Val Arg Gly Gln Arg Thr Lys Asn Asn Cys Arg Thr Leu Lys
 130 135 140

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gga agg agg gct tct gtt gcg aag aag aag tgc cct gct tcc cag gag 480
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 Glu

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Lys Lys Gly Gly Ile Gly His Gly Gly Leu Arg Ile Glu Cys Ile Arg
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Ile Gly Gly Val Glu Ile Pro Asn His Lys Arg Val Glu Tyr Ser Leu
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Gln Tyr Ile His Gly Ile Gly Arg Ser Arg Ser Arg Gln Ile Leu Leu
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Asp Leu Asn Phe Asp Asn Lys Val Thr Lys Asp Leu Ser Glu Glu Glu
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Val Ile Thr Leu Arg Lys Glu Lys Arg Phe Asn Arg Val Ala Ile Glu
 100 105 110

55

Arg Leu Lys Glu Ile Arg Cys Tyr Arg Gly Ile Arg His Lys Leu Gly
 115 120 125

Leu Pro Val Arg Gly Gln Arg Thr Lys Asn Asn Cys Arg Thr Leu Lys
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Gly Arg Arg Ala Ser Val Ala Lys Lys Lys Ser Pro Ala Ser Gln Glu
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    ctc cgg acc tac tcc tct cct agg tcc atg gtg acg ctg cca tcg ttg      96
15  Leu Arg Thr Tyr Ser Ser Pro Arg Ser Met Val Thr Leu Pro Ser Leu
    20          25          30

    agg atg agc aag aag agg tcc cag gcc atc cgg tgc gac tac atc ggc      144
20  Arg Met Ser Lys Lys Arg Ser Gln Gly Ile Arg Cys Asp Tyr Ile Gly
    35          40          45

    tca gcc acc aac gtt atc atg gtg acg acg acg acg ctg atg ctg ttc      192
    Ser Ala Thr Asn Val Ile Met Val Thr Thr Thr Thr Leu Met Leu Phe
    50          55          60

25  gcg ggg agg ttc ggg ctg gcg ccg tcg gcg aac agg aag tcg acg gcg      240
    Ala Gly Arg Phe Gly Leu Ala Pro Ser Ala Asn Arg Lys Ser Thr Ala
    65          70          75          80

30  ggg ctg aag ctg gag gcg cgc gac tcc gcc ctg cag acc gcc gac ccc      288
    Gly Leu Lys Leu Glu Ala Arg Asp Ser Gly Leu Gln Thr Gly Asp Pro
    85          90          95

35  gcc ggg ttc acg ctc gcc gac acg ctg gcc tgc gcc gcc gtc gcc cac      336
    Ala Gly Phe Thr Leu Ala Asp Thr Leu Ala Cys Gly Ala Val Gly His
    100          105          110

    atc atg gcc gtc gcc gtc gtc ctc gcc ctc aag aac atc gcc gtc ctg      384
40  Ile Met Gly Val Gly Val Val Leu Gly Leu Lys Asn Ile Gly Val Leu
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    Asp Gln Ile Ile Gly
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    Arg Met Ser Lys Lys Arg Ser Gln Gly Ile Arg Cys Asp Tyr Ile Gly
60  35          40          45

    Ser Ala Thr Asn Val Ile Met Val Thr Thr Thr Thr Leu Met Leu Phe
    50          55          60

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5 Gly Leu Lys Leu Glu Ala Arg Asp Ser Gly Leu Gln Thr Gly Asp Pro
85 90 95

Ala Gly Phe Thr Leu Ala Asp Thr Leu Ala Cys Gly Ala Val Gly His
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Asp Gln Ile Ile Gly
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Arg Gly Arg Arg Arg Arg Leu Pro Ser Pro Pro Pro Ser Gln Ile Trp
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Ala Trp Gly Gly Gly Gly Gly Gly Gly Asp Asp Ala Arg Gly Ala Ala Gly
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Gly Gly Gly Ser Pro Gly Pro Leu Pro Pro Arg Ser Gly Arg Arg Arg
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Glu Gly Gly Gly Arg Trp Arg Gly Arg Arg Arg Gly Ala Ala Ala
100 105 110

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Thr Thr Thr Ala Pro Leu Pro Ser Pro Leu Arg Ser Gly Arg Gly Gly
115 120 125

60 ggg cga ggc gtg gcg gcg gcg gcg agc cgc ttt tgg ccg ctt ggg aaa 432
Gly Arg Gly Val Ala Ala Ala Ala Ser Arg Phe Trp Pro Leu Gly Lys
130 135 140

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145 150 155 160

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10	gtg cct aga tct agt cgc atg gcg gcg caa gtg gtt gcg ttg gta ggc	576
	Val Pro Arg Ser Ser Arg Met Ala Ala Gln Val Val Ala Leu Val Gly	
	180 185 190	
15	ttc tcg gca ctg agg agt gga cag agc agt gcc cag att agg ttg caa	624
	Phe Ser Ala Leu Arg Ser Gly Gln Ser Ser Ala Gln Ile Arg Leu Gln	
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	Ser Gly Asn Phe Ser Ser Ile Ser Asp Cys Val Thr Val Arg Val Met	
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	Thr Ile Val Ala Val Leu Phe Gly Met Val Val Ala Met Thr Leu Gly	
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	Ala Val Ala Ala Lys Ser Ser Val Tyr Arg Pro Lys Ser Asn His Val	
	275 280 285	
45	gat tgg gag gtg gct cta gct agt acg aca atg aca agc ttg gtg gct	912
	Asp Trp Glu Val Ala Leu Ala Ser Thr Thr Met Thr Ser Leu Val Ala	
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	Val Leu Val Ala Thr Gly Glu Glu Gly Ser Asn Leu Lys Lys Ala Lys	
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	Cys Gln His Asn Ala Ala Asp His Glu Arg Gln Arg Ser Leu Trp Ala	
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60	cga gtc tgc agg ctt gca gcg gaa ggc ctc gtt aca gcg tcg aaa tgg	1056
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65	gcc agg cca ggc cgg gcc gga aca cgt ggg cct agc cca ctc aaa cag	1104
	Ala Arg Pro Gly Arg Ala Gly Thr Arg Gly Pro Ser Pro Leu Lys Gln	
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70	ccc ttg acg cgc acc acc aac cac tat ata agc ttc cga gag ctc ttc	1152
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	370 375 380	
75	ccc caa acc cta acc gcc gcc gcc gcc gtc tcc tcc tcc tca tcc aag	1200
	Pro Gln Thr Leu Thr Ala Ala Ala Ala Val Ser Ser Ser Ser Ser Lys	
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80	cag ctc gcg cct ccg acc cct cgc ctc gag ctc cag ctc tcc cca acc	1248
	Gln Leu Ala Pro Pro Thr Arg Leu Glu Leu Gln Leu Ser Pro Thr	
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5	gga gcg ccg aag aag agg acg ttc cgc aag tac agc tac cgc ggc gtg	1344
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10	gac ctc gac gcg ctc ctc gac atg tcc acc gac gac ctc gtc cag ctc	1392
	Asp Leu Asp Ala Leu Leu Asp Met Ser Thr Asp Asp Leu Val Gln Leu	
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35	ttt gtg gat att ttt gct aga atg tgg ttc ttg ctg aaa aag gat gca	1632
	Phe Val Asp Ile Phe Ala Arg Met Trp Phe Leu Leu Lys Lys Asp Ala	
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40	cct gct ggt gag aag cca gag cct gtg agg acc cac ctc agg aac atg	1680
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55	ctc gca gag ttc tcc atc tcg tac aag cca gtc aag cac ggt agg cct	1824
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	Glu	Gly	Gly	Gly	Arg	Trp	Arg	Gly	Arg	Arg	Arg	Gly	Ala	Ala	Ala	Ala
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	Thr	Thr	Thr	Ala	Pro	Leu	Pro	Ser	Pro	Leu	Arg	Ser	Gly	Arg	Gly	Gly
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	Ile	Ser	Phe	Leu	Val	Val	Arg	Pro	Pro	Phe	Lys	Pro	Val	Val	Val	Arg
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25	Pro	Tyr	Asn	Val	Glu	Pro	Glu	Val	Val	Val	Val	Glu	Phe	Trp	Val	Phe
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 405 410 415
 10 Pro Ser Thr Met Ala Asp Val Glu Val Glu Ala Glu Val Ala Ala Ala
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 Gly Ala Pro Lys Lys Arg Thr Phe Arg Lys Tyr Ser Tyr Arg Gly Val
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 15 Asp Leu Asp Ala Leu Leu Asp Met Ser Thr Asp Asp Leu Val Gln Leu
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 Phe Pro Ala Arg Ala Arg Arg Arg Phe Gln Arg Gly Leu Lys Arg Lys
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 Pro Met Ala Leu Ile Lys Lys Leu Arg Lys Ala Gly Ser Asn Phe Glu
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 Val Ser Tyr Val Gly Val Met Tyr Lys Val Tyr Ile Arg Thr Val Ala
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 30 Phe Val Asp Ile Phe Ala Arg Met Trp Phe Leu Leu Lys Lys Asp Ala
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 Pro Ala Gly Glu Lys Pro Glu Pro Val Arg Thr His Leu Arg Asn Met
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 Ile Ile Val Pro Glu Met Ile Gly Ser Ile Val Gly Val Tyr Asn Gly
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 40 Lys Thr Phe Asn Gln Val Glu Ile Lys Pro Glu Met Ile Gly His Tyr
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 Lys Ser Gly Leu Arg Lys Leu Ala Arg Lys Val Pro Thr Asp Arg Leu
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5 ctt aag ttc gaa cgg gtt ttc aaa gca caa aaa cgc atc cac atg tcc 144
 Leu Lys Phe Glu Arg Val Phe Lys Ala Gln Lys Arg Ile His Met Ser
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10 gtt ttc aaa gta caa aga gtt ctg gat gag att cgt tgg cgt tac tac 192
 Val Phe Lys Val Gln Arg Val Leu Asp Glu Ile Arg Trp Arg Tyr Tyr
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15 gaa gaa act gtt atg ata ttg aac ctg atg cct tat cga gca tct tat 240
 Glu Glu Thr Val Met Ile Leu Asn Leu Met Pro Tyr Arg Ala Ser Tyr
 65 70 75 80

20 ccc atc tta aag ttg gtt tat tcg gca gca gca aat gct act cat tat 288
 Pro Ile Leu Lys Leu Val Tyr Ser Ala Ala Ala Asn Ala Thr His Tyr
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agg gat ttc gac aaa gca aat tta ttc atc act aaa gcc gaa gtc agt 336
 Arg Asp Phe Asp Lys Ala Asn Leu Phe Ile Thr Lys Ala Glu Val Ser
 100 105 110

25 agg agt act att atg aat aaa ttc aga cct cgg gct cga gga cgt agt 384
 Arg Ser Thr Ile Met Asn Lys Phe Arg Pro Arg Ala Arg Gly Arg Ser
 115 120 125

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 Ser Pro Ile Lys Lys Thr Met Cys His Ile Thr Ile Val Leu Asn Ile
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 35 40 45
 Val Phe Lys Val Gln Arg Val Leu Asp Glu Ile Arg Trp Arg Tyr Tyr
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55 Glu Glu Thr Val Met Ile Leu Asn Leu Met Pro Tyr Arg Ala Ser Tyr
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 Pro Ile Leu Lys Leu Val Tyr Ser Ala Ala Ala Asn Ala Thr His Tyr
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Arg Asp Phe Asp Lys Ala Asn Leu Phe Ile Thr Lys Ala Glu Val Ser
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	Arg Ser Thr Ile Met Asn Lys Phe Arg Pro Arg Ala Arg Gly Arg Ser	
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	Ala Ala Lys Pro His Leu Ser Cys Ala Gln Ser Pro Arg Pro Pro Ser	
	20 25 30	
30	gcc cag ccg ccg ccg gag cag caa ccg ccg acg ccg acg ccg acg cag	144
	Ala Gln Pro Pro Pro Glu Gln Gln Pro Pro Thr Pro Thr Pro Thr Gln	
	35 40 45	
35	cag cag tcc atg caa gcc cag ccg cag gcg agg ccg agg cgc gcg ccg	192
	Gln Gln Ser Met Gln Ala Gln Pro Gln Ala Arg Pro Arg Arg Ala Pro	
	50 55 60	
40	gcg gcg gcg gcg tcc ggc gcg gac tcg acg gac tgg gtg gcg acg tcg	240
	Ala Ala Ala Ala Ser Gly Ala Asp Ser Thr Asp Trp Val Ala Thr Ser	
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45	ctg acg ccg ccg ttc ggc atc ggc gcc ggg ctc gcg tgg gtc ggg ttc	288
	Leu Thr Arg Arg Phe Gly Ile Gly Ala Gly Leu Ala Trp Val Gly Phe	
	85 90 95	
50	ctc gcc ttc ggc gtc gtc tcc gag cag ctc aag acc cgc ttc gag gtc	336
	Leu Ala Phe Gly Val Val Ser Glu Gln Leu Lys Thr Arg Phe Glu Val	
	100 105 110	
55	gcg cag cag ctg gcc aac acc aag gac gtg gag caa gag cag gag gtc	384
	Ala Gln Gln Leu Ala Asn Thr Lys Asp Val Glu Gln Glu Glu Val	
	115 120 125	
60	gtc ctt ccc aac gga ata ccg tac tac gag atg cga gta ggc ggc ggc	432
	Val Leu Pro Asn Gly Ile Arg Tyr Tyr Glu Met Arg Val Gly Gly Gly	
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	Asp Val Pro Arg Pro Gly Asp Leu Val Val Ile Asp Leu Lys Gly Arg	
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	Val Thr Gly Gly Glu Ala Phe Val Asp Thr Phe Gly Asp Gly Lys Arg	
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 Glu Gly Val Glu Tyr Val Leu Arg Ser Met Arg Ala Gly Gly Lys Arg
 195 200 205

10 cgg gtg gtc gtc ccg ccg gcg ctc ggc ttc ggc gac gac ggc gcc gac 672
 Arg Val Val Val Pro Pro Ala Leu Gly Phe Gly Asp Asp Gly Ala Asp
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 Phe Gly Asp Ala Ala Ala Gln Val Pro Pro Gly Ala Thr Leu Glu Tyr
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Gln Gln Ser Met Gln Ala Gln Pro Gln Ala Arg Pro Arg Arg Ala Pro
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45 Leu Ala Phe Gly Val Val Ser Glu Gln Leu Lys Thr Arg Phe Glu Val
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50 Ala Gln Gln Leu Ala Asn Thr Lys Asp Val Glu Gln Glu Gln Glu Val
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Val Leu Pro Asn Gly Ile Arg Tyr Tyr Glu Met Arg Val Gly Gly Gly
 130 135 140

55 Asp Val Pro Arg Pro Gly Asp Leu Val Val Ile Asp Leu Lys Gly Arg
 145 150 155 160

Val Thr Gly Gly Glu Ala Phe Val Asp Thr Phe Gly Asp Gly Lys Arg
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	Ile Glu Gln Tyr Asn Arg Lys Val Gly Ile Glu Asn Ile Gly Arg Val	
	20 25 30	
30	gtt caa gtg ggg gat ggg att gct cgt att ata ggt ctt ggt gaa ata	144
	Val Gln Val Gly Asp Gly Ile Ala Arg Ile Ile Gly Leu Gly Glu Ile	
	35 40 45	
35	atg tca ggc gaa tta gtc gaa ttt gca gaa ggg act agg ggt att gct	192
	Met Ser Gly Glu Leu Val Glu Phe Ala Glu Gly Thr Arg Gly Ile Ala	
	50 55 60	
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	Leu Asn Leu Glu Ser Lys Asn Val Gly Ile Val Leu Met Gly Asp Gly	
	65 70 75 80	
	ttg atg ata caa gag ggc agt ttt gta aaa gca aca gga aga att gct	288
	Leu Met Ile Gln Glu Gly Ser Phe Val Lys Ala Thr Gly Arg Ile Ala	
	85 90 95	
45	cag ata ccc gtg agc gag gct tac ttg ggt cgt gtt ata aat gct ctg	336
	Gln Ile Pro Val Ser Glu Ala Tyr Leu Gly Arg Val Ile Asn Ala Leu	
	100 105 110	
50	gct aaa cct att gat ggg aga ggc gaa att gta gct tcg gaa tct cgc	384
	Ala Lys Pro Ile Asp Gly Arg Gly Glu Ile Val Ala Ser Glu Ser Arg	
	115 120 125	
55	tta att gaa tct cct gct ccg ggt ata att tcc agg cgt tct gta tat	432
	Leu Ile Glu Ser Pro Ala Pro Gly Ile Ile Ser Arg Arg Ser Val Tyr	
	130 135 140	
60	gaa ccc ctt caa acg ggg ctt att gct atc gat tcg atg att cct att	480
	Glu Pro Leu Gln Thr Gly Leu Ile Ala Ile Asp Ser Met Ile Pro Ile	
	145 150 155 160	

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	Gly Arg Gly Gln Arg Glu Leu Ile Ile Gly Asp Arg Gln Thr Gly Lys	
	165 170 175	
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	Thr Ala Val Ala Thr Asp Thr Ile Leu Asn Gln Lys Gly Gln Asp Val	
	180 185 190	
10	ata tgt gtt tat gta gct atc ggt caa aga gca tcc tcc gta gct caa	624
	Ile Cys Val Tyr Val Ala Ile Gly Gln Arg Ala Ser Ser Val Ala Gln	
	195 200 205	
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	Val Val Thr Thr Phe His Glu Glu Gly Ala Met Glu Tyr Thr Ile Val	
	210 215 220	
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	Val Ala Glu Met Ala Asp Ser Pro Ala Thr Leu Gln Tyr Leu Ala Pro	
	225 230 235 240	
	tat acg gga gca gcc ctg gct gag tat ttt atg tac cgc gaa cgg cat	768
	Tyr Thr Gly Ala Ala Leu Ala Glu Tyr Phe Met Tyr Arg Glu Arg His	
	245 250 255	
25	act tta ata att tat gat gat ctc tcc aaa cag gca caa gct tat cgc	816
	Thr Leu Ile Ile Tyr Asp Asp Leu Ser Lys Gln Ala Gln Ala Tyr Arg	
	260 265 270	
30	caa atg tcc ctt cta tta aga aga ccc ccc ggc cgc gaa gct tac cca	864
	Gln Met Ser Leu Leu Leu Arg Arg Pro Pro Gly Arg Glu Ala Tyr Pro	
	275 280 285	
35	ggg gat gtt ttt tat ttg cat tca cgc ctt tta gaa aga gcc gct aaa	912
	Gly Asp Val Phe Tyr Leu His Ser Arg Leu Leu Glu Arg Ala Ala Lys	
	290 295 300	
40	tta aat tct ctt tta ggg gaa gga agt atg act gct tta cca ata gtt	960
	Leu Asn Ser Leu Leu Gly Glu Gly Ser Met Thr Ala Leu Pro Ile Val	
	305 310 315 320	
	gag act caa tct gga gac gtt tcc gcc tat att cct act aat gta atc	1008
	Glu Thr Gln Ser Gly Asp Val Ser Ala Tyr Ile Pro Thr Asn Val Ile	
	325 330 335	
45	tcc att aca gat gga caa ata ttc tta tcc gca gat cta ttc aat gcc	1056
	Ser Ile Thr Asp Gly Gln Ile Phe Leu Ser Ala Asp Leu Phe Asn Ala	
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	Gly Ile Arg Pro Ala Ile Asn Val Gly Ile Ser Val Ser Arg Val Gly	
	355 360 365	
55	tcc gcg gct caa att aaa gcc atg aaa caa gta gct ggc aaa tca aaa	1152
	Ser Ala Ala Gln Ile Lys Ala Met Lys Gln Val Ala Gly Lys Ser Lys	
	370 375 380	
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	Leu Glu Leu Ala Gln Phe Ala Glu Leu Gln Ala Phe Ala Gln Phe Ala	
	385 390 395 400	
	tct gct ctc gat aaa aca agt cag aat caa ttg gca agg ggc cga cga	1248
	Ser Ala Leu Asp Lys Thr Ser Gln Asn Gln Leu Ala Arg Gly Arg Arg	
	405 410 415	

	tta cga gaa ttg ctt aaa caa tcc caa gca aat cct ctt cca gtg gaa Leu Arg Glu Leu Leu Lys Gln Ser Gln Ala Asn Pro Leu Pro Val Glu	1296
	420 425 430	
5	gag cag ata gct act att tat atc gga aca aga gga tat ctt gat tcc Glu Gln Ile Ala Thr Ile Tyr Ile Gly Thr Arg Gly Tyr Leu Asp Ser	1344
	435 440 445	
10	tta gaa att gga cag gta aag aaa ttt ctg gat gag tta cgt aaa cac Leu Glu Ile Gly Gln Val Lys Lys Phe Leu Asp Glu Leu Arg Lys His	1392
	450 455 460	
15	cta aaa gat act aaa cct caa ttc caa gaa att ata tct tct agc aag Leu Lys Asp Thr Lys Pro Gln Phe Gln Glu Ile Ile Ser Ser Ser Lys	1440
	465 470 475 480	
20	aca ttc acc gag gaa gcg gaa atc ctt ttg aag gaa gct att cag gaa Thr Phe Thr Glu Glu Ala Glu Ile Leu Leu Lys Glu Ala Ile Gln Glu	1488
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	Leu Met Ile Gln Glu Gly Ser Phe Val Lys Ala Thr Gly Arg Ile Ala 85 90 95	
50	Gln Ile Pro Val Ser Glu Ala Tyr Leu Gly Arg Val Ile Asn Ala Leu 100 105 110	
55	Ala Lys Pro Ile Asp Gly Arg Gly Glu Ile Val Ala Ser Glu Ser Arg 115 120 125	
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60	Glu Pro Leu Gln Thr Gly Leu Ile Ala Ile Asp Ser Met Ile Pro Ile 145 150 155 160	
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			355					360					365				
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				420					425					430			
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		450					455					460					
	Leu	Lys	Asp	Thr	Lys	Pro	Gln	Phe	Gln	Glu	Ile	Ile	Ser	Ser	Ser	Lys	
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 Ala Pro Gly Lys Leu Val Thr Pro Glu Gln Phe Gln Arg Ala Arg Ala
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cta ccg cgc cag ctg gcg gcg gtg gcg gcg tcc ccg cgg caa ccg cgc 192
 Leu Pro Arg Gln Leu Ala Ala Val Ala Ala Ser Pro Arg Gln Pro Arg
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ccg ctc gcc atg cag cag cag cag cag cag atg tcg cag tcg atg atc 240
 Pro Leu Ala Met Gln Gln Gln Gln Gln Met Ser Gln Ser Met Ile
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ttc ggg cgg ccg tcc gcg ccg gcg ccg acg acg ctg cag ggg ttc ggg 288
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ttc ggg cgc ggc gca ggc cct ggt gcg gcg gcg gtg gcg gcg cgt tcg 336
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 165 170 175

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 180 185 190

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 195 200 205

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	Asp Asp Asp His Ala Glu Ala Ala Arg Asn Gly Val Arg Arg Trp Arg	
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10	agc ggg agc ggg cag cag cag ccg tgc acg atc ctc gcg ccg ccg ccg	768
	Ser Gly Ser Gly Gln Gln Gln Pro Ser Thr Ile Leu Ala Pro Pro Pro	
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	Ala Ala Ala Gly Val Ala Pro Pro Pro Ser Leu Arg Gly Met Pro Met	
	275 280 285	
25	gcg tct ggg ccg cgt ccc gct agg cag ccg cag cgc aag cgg aag cag	912
	Ala Ser Gly Pro Arg Pro Ala Arg Gln Pro Gln Arg Lys Arg Lys Gln	
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30	cgg acc ctc gcg ccg ccg cag cag aag aat cag ggc atc ggc gtg tcc	960
	Arg Thr Leu Ala Pro Pro Gln Gln Lys Asn Gln Gly Ile Gly Val Ser	
	305 310 315 320	
35	ggg ggc cgc ggc tgg agc tgg agc tgc gcc cgt gtc ttc tcc gcc gag	1008
	Gly Gly Arg Gly Trp Ser Trp Ser Cys Ala Arg Val Phe Ser Ala Glu	
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	Arg Ser Glu Gly Val Gln Pro Gly His His Asp Ala Val Arg Leu Arg	
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	Arg Gly Arg His Gln Gln Val Arg Val Leu Pro Leu Arg Arg Ser Ile	
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	Ser Leu Leu Leu Leu Leu Leu Leu Leu Leu Leu Leu Pro Ser Pro Pro	
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	Asn Val Tyr Ala Glu Tyr Glu Glu Asp Glu Ile Tyr Val Glu Asp Gln	
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	Val Ala Leu Gln Lys Gln Gln Gln Gln Gln Glu Glu Ala Ala Leu Gln	
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	Glu Gln Gln Gln Gln Gln Gln Met Met Ala Ala Leu Gln Gln Gln Gln	
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	Pro Leu Ala Met Gln Gln Gln Gln Gln Met Ser Gln Ser Met Ile	
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	Ala Glu Gly Asp Gly Asp Gly Gly Ala Ala Ser Arg His Ala Ala Val	115	120	125
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	Arg Trp Thr Ser Gly Gly Gly Ala Phe Ala Ala Arg Asp Asp Asp Gly	145	150	155
15	Cys Gly Ala Ala Ser Arg Arg Ala Ala Glu Ala Glu Ala Ala Tyr Pro	165	170	175
	Arg Ala Ala Ala Ala Ala Ala Ala Glu Ser Gly Leu Gln Arg Gly Arg	180	185	190
	Gly Trp Gly Asp Gly Gly Ala Phe Ala Ala Gly Asp Gly Gly Gly Asn	195	200	205
25	Ala Ser Arg His Ala Ala Ala Ala Val His Asp Pro Arg Ala Ala	210	215	220
	Asp Asp Asp His Ala Glu Ala Ala Arg Asn Gly Val Arg Arg Trp Arg	225	230	235
30	Ser Gly Ser Gly Gln Gln Gln Pro Ser Thr Ile Leu Ala Pro Pro Pro	245	250	255
	Pro Thr Pro Thr Arg Gln Lys Gln Gln Gly Ile Val Phe Gly Gly Gly	260	265	270
	Ala Ala Ala Gly Val Ala Pro Pro Pro Ser Leu Arg Gly Met Pro Met	275	280	285
40	Ala Ser Gly Pro Arg Pro Ala Arg Gln Pro Gln Arg Lys Arg Lys Gln	290	295	300
	Arg Thr Leu Ala Pro Pro Gln Gln Lys Asn Gln Gly Ile Gly Val Ser	305	310	315
45	Gly Gly Arg Gly Trp Ser Trp Ser Cys Ala Arg Val Phe Ser Ala Glu	325	330	335
	Arg Asp Val Gly Gly Gly Gly Asp Gly Val Gly Gly Tyr Gly Gly Gly	340	345	350
	Arg Ser Glu Gly Val Gln Pro Gly His His Asp Ala Val Arg Leu Arg	355	360	365
55	Arg Gly Arg His Gln Gln Val Arg Val Leu Pro Leu Arg Arg Ser Ile	370	375	380
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	Trp Arg Pro Gly Asp Glu Gly His Gly Pro Ala Leu Gly Leu Leu Gln	
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	Gly Asp Val Tyr Met Gly Gln Met Gly Arg Phe Glu Arg Gly His Ile	
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	Arg Ala Gly Arg Met Glu Pro Asp Pro Val Arg Leu Ala Pro Cys Ile	
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Gly Leu Arg Gly Val Gly Gly Val Leu Pro Glu His Leu Pro Gly Gly
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	Pro Ala Glu Phe Pro Tyr Met Leu Leu Tyr Pro Asn Thr Ser Asp Val	
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35	Asp	Asp	Gly	Val	Leu	Arg	Gly	Asp	Ala	Glu	Leu	Gln	Ala	Trp	Trp	Ala	660	665		670	
40	Glu	Val	Arg	Glu	Val	Gly	His	Gly	Asp	Leu	Lys	Gly	Ala	Ala	Trp	Trp	675	680		685	
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	Gly	Ile	Ser	Leu	Ile	Glu	Val	Leu	Ser	Lys	His	Ser	Ser	Asp	Glu	Val	770	775		780	
60	Tyr	Leu	Gly	Gln	Arg	Asp	Thr	Pro	Ala	Trp	Thr	Ser	Asp	Ala	Arg	Ala	785	790	795		800

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 Ala Gly Gly Asn Gly Gly Ser Gly Gly Gly Gly Ala Ser Arg Val Ser
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 Phe Ala Ala Asn Arg Val Gly Arg Arg Met Val Val Val Arg Ala Glu
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 40 gag gag gcc gcg gcg ccg ccg ccg ccg ccg ccg ccc gcg gcg gag gag 192
 Glu Glu Ala Ala Ala Pro Pro Pro Pro Pro Pro Ala Ala Glu Glu
 50 55 60
 aag ccg gcg gag gcc gag gcg gcc gtg gcg acc aag gag ccc gcc gcc 240
 Lys Pro Ala Glu Ala Glu Ala Ala Val Ala Thr Lys Glu Pro Ala Ala
 65 70 75 80
 45 gcc aag ccg cct ccc att ggc ccc aag aga ggc acc aag gtg aag atc 288
 Ala Lys Pro Pro Ile Gly Pro Lys Arg Gly Thr Lys Val Lys Ile
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 50 ctg agg agg gag tcc tac tgg tac aac ggc act ggc tcc gtc gtc acc 336
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 Val Asp Gln Asp Pro Asn Thr Arg Tyr Pro Val Val Val Arg Phe Ala
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 60 aag gtg aac tac gcc ggc gtg tcg acc aac aac tac gcc ctg gac gag 432
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 Ala Lys Pro Pro Pro Ile Gly Pro Lys Arg Gly Thr Lys Val Lys Ile
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 Arg Leu Cys Met Lys Thr Thr Leu Gly Glu Glu Val Ser Asp Glu Glu
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 Ala Gly Gln Leu Val Ala Ser Val Asp Ala Asp Gly Asp Gly Leu Leu
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	Glu Asp Glu Arg Arg Gly Thr Gly Leu Arg Glu Ala Phe Gly Met Tyr	
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10	gag atg gaa ggg gaa ggg tgc atc acg ccg acg agc ctc agg cgg atg	336
	Glu Met Glu Gly Glu Gly Cys Ile Thr Pro Thr Ser Leu Arg Arg Met	
	100 105 110	
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    His Thr Thr Thr Thr Gly Arg Leu Ser Cys Ala Cys Ala Pro Pro Thr
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15    agg gga aca agg cgg cgg cgg ctc gcg gtg gcg gtg cgg gtg cgg gtg      144
    Arg Gly Thr Arg Arg Arg Arg Leu Ala Val Ala Val Arg Val Arg Val
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20    cgg gtg cgg gcg tcg gcg tcg gag gcg atg gcg acg gag aag ctc ggc      192
    Arg Val Arg Ala Ser Ala Ser Glu Ala Met Ala Thr Glu Lys Leu Gly
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25    gtc cgt gtg gag cgc aac ccg ccc gag tcc cgc ctc tcc gag ctc ggc      240
    Val Arg Val Glu Arg Asn Pro Pro Glu Ser Arg Leu Ser Glu Leu Gly
    65          70          75          80

30    gtc cgc gag tgg ccc aag tgg ggg tgc gag aag agc aag ttc ccg tgg      288
    Val Arg Glu Trp Pro Lys Trp Gly Cys Glu Lys Ser Lys Phe Pro Trp
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35    acg tac tcg gcc aag gag acg tgc tac ctg ctg cag ggg aag gtg aag      336
    Thr Tyr Ser Ala Lys Glu Thr Cys Tyr Leu Leu Gln Gly Lys Val Lys
          100          105          110

    gtg ttc ccg gac ggc gcc gcc gag gat ttc gtg gag atc gcg gcg ggg      384
    Val Phe Pro Asp Gly Ala Gly Glu Asp Phe Val Glu Ile Ala Ala Gly
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40    gac ctg gtg gtg ttc ccc aag ggg atg agc tgc acc tgg gac gtc gcc      432
    Asp Leu Val Val Phe Pro Lys Gly Met Ser Cys Thr Trp Asp Val Ala
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60    Arg Gly Thr Arg Arg Arg Arg Leu Ala Val Ala Val Arg Val Arg Val
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	Ala	Pro	Lys	Lys	Gln	Lys	Ala	Lys	Pro	Ser	Ala	Ala	Ala	Gly	Gly	Ser	
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	Ile	Gln	Leu	Arg	Gly	Phe	Ala	Ser	Lys	Gly	Lys	Leu	Val	Lys	Val	Lys	
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 Ile Gln Leu Arg Gly Phe Ala Ser Lys Gly Lys Leu Val Lys Val Lys
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   Arg Lys Thr Ser Trp Pro Glu Val Val Gly Leu Ser Ala Glu Glu Ala
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 35 40 45
 His Thr His Thr His Ser Gly Asp Gly Ala Arg Arg Arg Gly His Leu
 50 55 60
 20 Pro Leu Gln Leu Pro Arg Arg Arg Ala Pro Leu Leu Arg Ala Gly Ala
 65 70 75 80
 25 Arg Gly Arg Leu Ala Arg Val His Leu Leu Leu Pro Leu His Arg Arg
 85 90 95
 Leu Leu Arg Ala Pro Ala Glu Asp Arg Arg Gly Gly Gly Pro Pro Pro
 100 105 110
 30 Gly Glu His Glu Val Arg Gly Gly Arg Arg Arg Gly Ala Ala Ala Arg
 115 120 125
 Arg Ala Ala Ala Ala Ala Asp Gly Gly Val His Gly Gly Arg Gly Gly
 130 135 140
 35 Arg Arg Gly Arg Gly Gly Arg Ala Arg Gly Gly Ala Arg Arg Gly Gly
 145 150 155 160
 40 Gly Cys Arg Gly Glu Leu Arg Gly Arg Gly Arg Val Arg Val Asp Gly
 165 170 175
 Gly Gly Arg Gly Gly Glu Gly Trp Gly Gln Val Gly Ala Gly Val Asp
 180 185 190
 45 Gly Arg Val Gln Arg Ala Arg Arg Ala Pro Pro His Arg Arg Ala Pro
 195 200 205
 Arg Arg Arg Arg Arg Arg Gly Tyr Ala His Phe Ser Pro Glu Leu Ala
 210 215 220
 50 Ala Ser Arg Ala Val Glu Leu Leu Thr Ser His Pro Gly Leu Glu Ser
 225 230 235 240
 Tyr Arg Ile Arg Asp Leu Pro Asp Gly Val Val Ser Gly Asp Leu Asn
 245 250 255
 Tyr Val Ile Asn Leu Leu Leu His Arg Met Ala Gln Arg Leu Pro Arg
 260 265 270
 60 Ala Ala Thr Ala Val Ala Leu Asn Thr Phe Pro Gly Leu Asp Pro Pro
 275 280 285
 Thr Val Thr Ala Ala Leu Thr Ala Val Leu Pro Thr Cys Leu Pro Leu
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5	Asn	Gly	Cys	Leu	Ala	Trp	Leu	Asp	Arg	His	Ala	Pro	Arg	Thr	Val	Ala	
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	Tyr	Val	Ser	Phe	Gly	Thr	Val	Ala	Ser	Pro	Arg	Pro	Asp	Glu	Leu	Arg	
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	Gly	Trp	Ala	Ser	Val	Met	Glu	Gly	Ala	Ser	Ser	Gly	Val	Pro	Met	Ala	
				420					425					430			
25	Cys	Arg	Pro	Phe	Phe	Gly	Asp	Gln	Arg	Thr	Asn	Ala	Arg	Ser	Val	Ser	
		435					440						445				
	His	Val	Trp	Gly	Phe	Gly	Thr	Ala	Phe	Asp	Gly	Ala	Met	Thr	Arg	Gly	
30		450					455					460					
	Gly	Val	Ala	Thr	Ala	Val	Ala	Ser	Leu	Val	Gly	Gly	Glu	Asp	Gly	Arg	
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35	Arg	Met	Arg	Ala	Arg	Ala	Gln	Glu	Leu	Gln	Ala	Lys	Val	Ala	Ser	Ala	
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55	Met	Ala	Pro	Ser	Leu	Val	Arg	Leu	Tyr	Glu	Gln	Met	Pro	Glu	Pro	Lys	
	1				5					10				15			
	tac	gtc	att	gct	atg	gga	gct	tgt	act	att	act	ggt	gga	atg	ttc	agt	96
	Tyr	Val	Ile	Ala	Met	Gly	Ala	Cys	Thr	Ile	Thr	Gly	Gly	Met	Phe	Ser	
60				20				25					30				
	acg	gat	tcc	tat	agt	act	gtt	cgg	gga	gtt	gat	aag	tta	att	cct	gtg	144
	Thr	Asp	Ser	Tyr	Ser	Thr	Val	Arg	Gly	Val	Asp	Lys	Leu	Ile	Pro	Val	
			35					40					45				

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	Asp Val Tyr Leu Pro Gly Cys Pro Pro Lys Pro Glu Ala Val Ile Asp	
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	gcc cta aca aaa ctt cgt aag aag ata tcg cga gaa ata gtt gag gat	240
	Ala Leu Thr Lys Leu Arg Lys Lys Ile Ser Arg Glu Ile Val Glu Asp	
	65 70 75 80	
10	cga act cta tct caa aag aaa aat cga tgt ttt act acc agc cac aag	288
	Arg Thr Leu Ser Gln Lys Lys Asn Arg Cys Phe Thr Thr Ser His Lys	
	85 90 95	
15	ctt tat gtt cgg cgc agt act aat act gga act tat gag caa gaa ttg	336
	Leu Tyr Val Arg Ser Thr Asn Thr Gly Thr Tyr Glu Gln Glu Leu	
	100 105 110	
20	ctc tat caa tca cca tct act tta gac ata tct tct gaa act ttt ttc	384
	Leu Tyr Gln Ser Pro Ser Thr Leu Asp Ile Ser Ser Glu Thr Phe Phe	
	115 120 125	
25	aaa tcc aaa aga gga gag atc aag ata atg cag cag ggt tgg tta tct	432
	Lys Ser Lys Arg Gly Glu Ile Lys Ile Met Gln Gln Gly Trp Leu Ser	
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	aat tgg cta gtc aaa cat gag gtg gtt cat aga tct ttg ggc ttc gat	480
	Asn Trp Leu Val Lys His Glu Val Val His Arg Ser Leu Gly Phe Asp	
	145 150 155 160	
30	cat cga gga ata gag act ttg caa ata aaa gca gag gat tgg gat tcc	528
	His Arg Gly Ile Glu Thr Leu Gln Ile Lys Ala Glu Asp Trp Asp Ser	
	165 170 175	
35	att gct gtc att tta tat gta tat ggt tac aat tat tta cgt tcc caa	576
	Ile Ala Val Ile Leu Tyr Val Tyr Gly Tyr Asn Tyr Leu Arg Ser Gln	
	180 185 190	
40	tgt gct tat gac gtt gca ccc ggt gga tct tta gct agc gtg tat cat	624
	Cys Ala Tyr Asp Val Ala Pro Gly Gly Ser Leu Ala Ser Val Tyr His	
	195 200 205	
45	ctt acg aga ata cag tat ggt ata gat aac cca gaa gaa gta tgc ata	672
	Leu Thr Arg Ile Gln Tyr Gly Ile Asp Asn Pro Glu Glu Val Cys Ile	
	210 215 220	
	aaa gtc ttt gcc caa aag gat aat cct aga atc cca tct gtc ttc tgg	720
	Lys Val Phe Ala Gln Lys Asp Asn Pro Arg Ile Pro Ser Val Phe Trp	
	225 230 235 240	
50	att tgg aga agt tcc gat ttt caa gaa cgc gaa tct ttt gat atg gtg	768
	Ile Trp Arg Ser Ser Asp Phe Gln Glu Arg Glu Ser Phe Asp Met Val	
	245 250 255	
55	gga atc tct tat gat aat cat cca cgc ctt aaa cgt atc cta atg cct	816
	Gly Ile Ser Tyr Asp Asn His Pro Arg Leu Lys Arg Ile Leu Met Pro	
	260 265 270	
60	gaa agt tgg ata ggc tgg ccc tta cgt aag gac tat ata acc ccc aat	864
	Glu Ser Trp Ile Gly Trp Pro Leu Arg Lys Asp Tyr Ile Thr Pro Asn	
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 35 40 45
 Asp Val Tyr Leu Pro Gly Cys Pro Pro Lys Pro Glu Ala Val Ile Asp
 50 55 60
 20 Ala Leu Thr Lys Leu Arg Lys Lys Ile Ser Arg Glu Ile Val Glu Asp
 65 70 75 80
 Arg Thr Leu Ser Gln Lys Lys Asn Arg Cys Phe Thr Thr Ser His Lys
 25 85 90 95
 Leu Tyr Val Arg Arg Ser Thr Asn Thr Gly Thr Tyr Glu Gln Glu Leu
 100 105 110
 30 Leu Tyr Gln Ser Pro Ser Thr Leu Asp Ile Ser Ser Glu Thr Phe Phe
 115 120 125
 Lys Ser Lys Arg Gly Glu Ile Lys Ile Met Gln Gln Gly Trp Leu Ser
 130 135 140
 35 Asn Trp Leu Val Lys His Glu Val Val His Arg Ser Leu Gly Phe Asp
 145 150 155 160
 His Arg Gly Ile Glu Thr Leu Gln Ile Lys Ala Glu Asp Trp Asp Ser
 40 165 170 175
 Ile Ala Val Ile Leu Tyr Val Tyr Gly Tyr Asn Tyr Leu Arg Ser Gln
 180 185 190
 45 Cys Ala Tyr Asp Val Ala Pro Gly Gly Ser Leu Ala Ser Val Tyr His
 195 200 205
 Leu Thr Arg Ile Gln Tyr Gly Ile Asp Asn Pro Glu Glu Val Cys Ile
 210 215 220
 50 Lys Val Phe Ala Gln Lys Asp Asn Pro Arg Ile Pro Ser Val Phe Trp
 225 230 235 240
 Ile Trp Arg Ser Ser Asp Phe Gln Glu Arg Glu Ser Phe Asp Met Val
 55 245 250 255
 Gly Ile Ser Tyr Asp Asn His Pro Arg Leu Lys Arg Ile Leu Met Pro
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 60 Glu Ser Trp Ile Gly Trp Pro Leu Arg Lys Asp Tyr Ile Thr Pro Asn
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 Phe Tyr Glu Ile Gln Asp Ala His
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15   atg ccg tcg tcg gtt cgt ggg ggc atg agc cgc ggc agg agg atg gcg      96
    Met Pro Ser Ser Val Arg Gly Gly Met Ser Arg Gly Arg Arg Met Ala
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20   agg ctg gtg acg cgc gcg gcg gcg tcg agc cgc aag ctc ccg agc ggg      144
    Arg Leu Val Thr Arg Ala Ala Ala Ser Ser Pro Lys Leu Pro Ser Gly
                    35                    40                    45

25   cgg cgg ctg cgg gtg gcg gtg gtc gga ggc gga ccc gcg ggc ggc gcg      192
    Arg Arg Leu Arg Val Ala Val Val Gly Gly Gly Pro Ala Gly Gly Ala
    50                    55                    60

30   gcg gcg gag gcg ctg gcc aag ggc ggc gtg gag acg gtg ctc atc gaa      240
    Ala Ala Glu Ala Leu Ala Lys Gly Gly Val Glu Thr Val Leu Ile Glu
    65                    70                    75                    80

    cgg aag ctg gac aac tgc aag ccc tgc ggc ggg gcc atc ccg ctg tgc      288
    Arg Lys Leu Asp Asn Cys Lys Pro Cys Gly Gly Ala Ile Pro Leu Cys
                    85                    90                    95

35   atg gtg tcg gag ttc gac ctg ccg ctg gac ctc gtc gac agg aag gtg      336
    Met Val Ser Glu Phe Asp Leu Pro Leu Asp Leu Val Asp Arg Lys Val
                    100                    105                    110

40   agg aag atg aag atg att tcg ccg tcc aac gtc gcc gtc gac atc ggc      384
    Arg Lys Met Lys Met Ile Ser Pro Ser Asn Val Ala Val Asp Ile Gly
                    115                    120                    125

45   ggc acg ctc gcg ccg cac gag tac atc ggc atg gtc cgc cgc gag gtg      432
    Gly Thr Leu Ala Pro His Glu Tyr Ile Gly Met Val Arg Arg Glu Val
    130                    135                    140

50   ctc gac gcc tac ctc cgc tcc cgc gcc gag ggg gcc ggc gcc gag gtc      480
    Leu Asp Ala Tyr Leu Arg Ser Arg Ala Glu Gly Ala Gly Ala Glu Val
    145                    150                    155                    160

    gtc aat ggt ctc ttc ctg agg tat gag gcg ccc aag gaa ccc aac ggg      528
    Val Asn Gly Leu Phe Leu Arg Tyr Glu Ala Pro Lys Glu Pro Asn Gly
                    165                    170                    175

55   tcg tac gtg gtg cac tac aac cac tac gac agc tcc aac ggc aag gcc      576
    Ser Tyr Val Val His Tyr Asn His Tyr Asp Ser Ser Asn Gly Lys Ala
                    180                    185                    190

60   ggc ggc gag aag cgg acg ttc gag gtg gac gcg atc gtg ggc gcg gac      624
    Gly Gly Glu Lys Arg Thr Phe Glu Val Asp Ala Ile Val Gly Ala Asp
    195                    200                    205

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	Tyr Ala Ile Ala Phe Gln Glu Arg Val Lys Ile Pro Asp Asp Lys Met	
	225 230 235 240	
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	Lys Tyr Tyr Glu Glu Arg Ala Glu Met Tyr Val Gly Asp Asp Val Ser	
	245 250 255	
15	ccg gac ttc tac ggc tgg gtg ttc ccc aag tgc gac cac gtc gcc gtc	816
	Pro Asp Phe Tyr Gly Trp Val Phe Pro Lys Cys Asp His Val Ala Val	
	260 265 270	
20	ggc acc ggc acc gtc acc cac aag ccc gac atc aag aag ttc cag gcc	864
	Gly Thr Gly Thr Val Thr His Lys Pro Asp Ile Lys Lys Phe Gln Ala	
	275 280 285	
25	gcc acc cgc ctt cgc gcc aag gac cgg atc gag ggc ggc aag atc atc	912
	Ala Thr Arg Leu Arg Ala Lys Asp Arg Ile Glu Gly Gly Lys Ile Ile	
	290 295 300	
30	cgc gtc gag gct cac ccc atc ccc gag cac ccg agg cct aag agg gtg	960
	Arg Val Glu Ala His Pro Ile Pro Glu His Pro Arg Pro Lys Arg Val	
	305 310 315 320	
35	gcg ggg agg gtg acg ctg gtg ggg gac gcg gcg ggg tac gtg aca aag	1008
	Ala Gly Arg Val Thr Leu Val Gly Asp Ala Ala Gly Tyr Val Thr Lys	
	325 330 335	
40	tgc tcc ggc gag ggg atc tac ttc gcg gcg aag agc ggg cgg atg tgc	1056
	Cys Ser Gly Glu Gly Ile Tyr Phe Ala Ala Lys Ser Gly Arg Met Cys	
	340 345 350	
45	gcg gag gcg atc gtg gcc ggg tcg gcg aac ggg acg cgg atg gtg gag	1104
	Ala Glu Ala Ile Val Ala Gly Ser Ala Asn Gly Thr Arg Met Val Glu	
	355 360 365	
50	gag agc gac ctg cgc agg tac ctg gcc gag ttc gac cgc ctc tac tgg	1152
	Glu Ser Asp Leu Arg Arg Tyr Leu Ala Glu Phe Asp Arg Leu Tyr Trp	
	370 375 380	
55	ccg acg tac aag gtg ctc gac gtg ctc cag aag gtg ttc tac cgc tcc	1200
	Pro Thr Tyr Lys Val Leu Asp Val Leu Gln Lys Val Phe Tyr Arg Ser	
	385 390 395 400	
60	aac gcc gcc cgc gag gcg ttc gtc gag atg tgc gcc gac gac tac gtc	1248
	Asn Ala Ala Arg Glu Ala Phe Val Glu Met Cys Ala Asp Asp Tyr Val	
	405 410 415	
65	cag agg atg acc ttc gac agc tac ctc tac aag cgc gtc gtg ccg ggc	1296
	Gln Arg Met Thr Phe Asp Ser Tyr Leu Tyr Lys Arg Val Val Pro Gly	
	420 425 430	
70	aac ccg ctc gac gac atc aag ctc gcc gtc aac acc atc ggc agc ctc	1344
	Asn Pro Leu Asp Asp Ile Lys Leu Ala Val Asn Thr Ile Gly Ser Leu	
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Arg Leu Val Thr Arg Ala Ala Ala Ser Ser Pro Lys Leu Pro Ser Gly
 35 40 45

Arg Arg Leu Arg Val Ala Val Val Gly Gly Gly Pro Ala Gly Gly Ala
 50 55 60

20 Ala Ala Glu Ala Leu Ala Lys Gly Gly Val Glu Thr Val Leu Ile Glu
 65 70 75 80

Arg Lys Leu Asp Asn Cys Lys Pro Cys Gly Gly Ala Ile Pro Leu Cys
 85 90 95

25 Met Val Ser Glu Phe Asp Leu Pro Leu Asp Leu Val Asp Arg Lys Val
 100 105 110

Arg Lys Met Lys Met Ile Ser Pro Ser Asn Val Ala Val Asp Ile Gly
 115 120 125

Gly Thr Leu Ala Pro His Glu Tyr Ile Gly Met Val Arg Arg Glu Val
 130 135 140

35 Leu Asp Ala Tyr Leu Arg Ser Arg Ala Glu Gly Ala Gly Ala Glu Val
 145 150 155 160

Val Asn Gly Leu Phe Leu Arg Tyr Glu Ala Pro Lys Glu Pro Asn Gly
 165 170 175

40 Ser Tyr Val Val His Tyr Asn His Tyr Asp Ser Ser Asn Gly Lys Ala
 180 185 190

Gly Gly Glu Lys Arg Thr Phe Glu Val Asp Ala Ile Val Gly Ala Asp
 195 200 205

Gly Ala Asn Ser Arg Val Ala Lys Asp Met Gly Ala Gly Asp Tyr Glu
 210 215 220

50 Tyr Ala Ile Ala Phe Gln Glu Arg Val Lys Ile Pro Asp Asp Lys Met
 225 230 235 240

Lys Tyr Tyr Glu Glu Arg Ala Glu Met Tyr Val Gly Asp Asp Val Ser
 245 250 255

55 Pro Asp Phe Tyr Gly Trp Val Phe Pro Lys Cys Asp His Val Ala Val
 260 265 270

Gly Thr Gly Thr Val Thr His Lys Pro Asp Ile Lys Lys Phe Gln Ala
 275 280 285

60 Ala Thr Arg Leu Arg Ala Lys Asp Arg Ile Glu Gly Gly Lys Ile Ile
 290 295 300

	Arg Val Glu Ala His Pro Ile Pro Glu His Pro Arg Pro Lys Arg Val	
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	325 330 335	
	Cys Ser Gly Glu Gly Ile Tyr Phe Ala Ala Lys Ser Gly Arg Met Cys	
	340 345 350	
10	Ala Glu Ala Ile Val Ala Gly Ser Ala Asn Gly Thr Arg Met Val Glu	
	355 360 365	
	Glu Ser Asp Leu Arg Arg Tyr Leu Ala Glu Phe Asp Arg Leu Tyr Trp	
	370 375 380	
15	Pro Thr Tyr Lys Val Leu Asp Val Leu Gln Lys Val Phe Tyr Arg Ser	
	385 390 395 400	
20	Asn Ala Ala Arg Glu Ala Phe Val Glu Met Cys Ala Asp Asp Tyr Val	
	405 410 415	
	Gln Arg Met Thr Phe Asp Ser Tyr Leu Tyr Lys Arg Val Val Pro Gly	
	420 425 430	
25	Asn Pro Leu Asp Asp Ile Lys Leu Ala Val Asn Thr Ile Gly Ser Leu	
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	Ser Leu Ala Ala Ala Asp Met Ser Ile Val Ser Tyr Gly Glu Arg Ser	
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50	gag gag gag gcg cgg cgg ctg tac gcg gag tgg aag gcc gag cac ggc	144
	Glu Glu Glu Ala Arg Arg Leu Tyr Ala Glu Trp Lys Ala Glu His Gly	
	35 40 45	
55	aag agc tac aac gcc gtc ggc gag gag gag cgg cgg tac gcg gcg ttc	192
	Lys Ser Tyr Asn Ala Val Gly Glu Glu Glu Arg Arg Tyr Ala Ala Phe	
	50 55 60	
60	agg gac aac ctc cgg tac atc gac gag cac aac gcc gcc gcc gac gcc	240
	Arg Asp Asn Leu Arg Tyr Ile Asp Glu His Asn Ala Ala Ala Asp Ala	
	65 70 75 80	
	ggg gtc cac tcc ttc cgc ctc ggc ctc aac cgc ttc gcc gac ctc acc	288
	Gly Val His Ser Phe Arg Leu Gly Leu Asn Arg Phe Ala Asp Leu Thr	
	85 90 95	

	aac gag gag tac cgg gac acc tac ctc ggc ctc agg aac aag ccg cgg	336
	Asn Glu Glu Tyr Arg Asp Thr Tyr Leu Gly Leu Arg Asn Lys Pro Arg	
	100 105 110	
5	agg gag agg aag gtc agc gac agg tac ctc gcc gcc gac aac gag gcg	384
	Arg Glu Arg Lys Val Ser Asp Arg Tyr Leu Ala Ala Asp Asn Glu Ala	
	115 120 125	
10	ctg ccg gag tcc gtt gac tgg agg acc aag ggg gcc gtg gct gag atc	432
	Leu Pro Glu Ser Val Asp Trp Arg Thr Lys Gly Ala Val Ala Glu Ile	
	130 135 140	
15	aag gac cag gga ggt tgc gga agt tgc tgg gct ttc tca gca ata gca	480
	Lys Asp Gln Gly Gly Cys Gly Ser Cys Trp Ala Phe Ser Ala Ile Ala	
	145 150 155 160	
20	gct gta gaa ggc atc aac cag att gtt act ggc gac ctg ata tct ttg	528
	Ala Val Glu Gly Ile Asn Gln Ile Val Thr Gly Asp Leu Ile Ser Leu	
	165 170 175	
25	tct gag cag gag cta gtt gac tgt gac act tca tat aac gag gga tgc	576
	Ser Glu Gln Glu Leu Val Asp Cys Asp Thr Ser Tyr Asn Glu Gly Cys	
	180 185 190	
30	aat gga ggt ctg atg gac tat gca ttt gat ttc atc atc aac aat ggt	624
	Asn Gly Gly Leu Met Asp Tyr Ala Phe Asp Phe Ile Ile Asn Asn Gly	
	195 200 205	
35	gga atc gac acc gag gat gac tac cct tac aaa ggc aag gac gaa ccg	672
	Gly Ile Asp Thr Glu Asp Asp Tyr Pro Tyr Lys Gly Lys Asp Glu Arg	
	210 215 220	
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	Cys Asp Val Asn Arg Lys Asn Ala Lys Val Val Thr Ile Asp Ser Tyr	
	225 230 235 240	
45	gaa gat gta aca cca aac agt gag acg agt ctg cag aag gcg gta gca	768
	Glu Asp Val Thr Pro Asn Ser Glu Thr Ser Leu Gln Lys Ala Val Ala	
	245 250 255	
50	aac cag ccc gtc agt gtt gca att gaa gcc ggt ggc aga gca ttc cag	816
	Asn Gln Pro Val Ser Val Ala Ile Glu Ala Gly Gly Arg Ala Phe Gln	
	260 265 270	
55	ctc tac tca tcg ggt atc ttc acg ggt aaa tgt gga aca gca cta gac	864
	Leu Tyr Ser Ser Gly Ile Phe Thr Gly Lys Cys Gly Thr Ala Leu Asp	
	275 280 285	
60	cac ggc gtc gct gct gtt ggg tac ggc aca gag aat ggc aag gac tac	912
	His Gly Val Ala Ala Val Gly Tyr Gly Thr Glu Asn Gly Lys Asp Tyr	
	290 295 300	
65	tgg atc gtg agg aac tcg tgg ggc aag agc tgg ggt gag tcc ggc tac	960
	Trp Ile Val Arg Asn Ser Trp Gly Lys Ser Trp Gly Glu Ser Gly Tyr	
	305 310 315 320	
70	gtc cgt atg gag cgc aac atc aag gcg agc agt ggc aag tgc ggc att	1008
	Val Arg Met Glu Arg Asn Ile Lys Ala Ser Ser Gly Lys Cys Gly Ile	
	325 330 335	
75	gcc gtt gag cca tcc tac cct ctg aag aag ggc gag aac ccc ccg aac	1056
	Ala Val Glu Pro Ser Tyr Pro Leu Lys Lys Gly Glu Asn Pro Pro Asn	
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ccc ggc cct act ccg ccg tcc cca acc ccg cct ccc acc gtc tgc gac 1104
 Pro Gly Pro Thr Pro Pro Ser Pro Thr Pro Pro Pro Thr Val Cys Asp
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5 aac tac tac act tgc ccc gat agc acc acc tgc tgc tgc atc tac gag 1152
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 370 375 380

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 385 390 395 400

15 acc tgc tgc gat gat cac tac agc tgc tgc cct cac gag tac ccc atc 1248
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 405 410 415

20 tgc aat gtt cag cag gga acc tgc ctc atg gct aag gac agt cca ctg 1296
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gca gtg aag gct ctg aag cgt acc ctg gca aag cca aac ttg tct ttc 1344
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 35 40 45

45 Lys Ser Tyr Asn Ala Val Gly Glu Glu Glu Arg Arg Tyr Ala Ala Phe
 50 55 60

Arg Asp Asn Leu Arg Tyr Ile Asp Glu His Asn Ala Ala Ala Asp Ala
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Gly Val His Ser Phe Arg Leu Gly Leu Asn Arg Phe Ala Asp Leu Thr
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Ala Ser Lys Thr Tyr Pro Gln Gln Ala Gly Thr Ile Arg Lys Asn Gly
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His Ile Val Ile Lys Asn Arg Pro Cys Lys Val Val Glu Val Ser Thr
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Ser Lys Thr Gly Lys His Gly His Ala Lys Cys His Phe Val Ala Ile
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gac atc ttc aat ggg aag aag ctt gaa gat att gtg ccc tcc tcc cac      240
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Asn Cys Asp Val Pro His Val Asn Arg Thr Asp Tyr Gln Leu Ile Asp
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att tct gaa gat gga ttt gtc agc ctc ctg act gaa agt gga ggc act      336
Ile Ser Glu Asp Gly Phe Val Ser Leu Leu Thr Glu Ser Gly Gly Thr
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aag gat gac ctg agg ctc cct agt gat gag gct ctg ctt act cag atc      384
Lys Asp Asp Leu Arg Leu Pro Ser Asp Glu Ala Leu Leu Thr Gln Ile
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gcc atg ggt gag gag cag atc tgc gct ctg aag gat att ggc ccc aag      480
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	Ser	Pro	Leu	Leu	Phe	Asn	Pro	Thr	Arg	Ser	Lys	Ile	Asn	Val	Glu	Gly	
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	Ala	Phe	Cys	Leu	Pro	Cys	Tyr	Asn	Arg	Lys	Lys	Ala	Ser	Asn	Arg	Ser	
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	ggc	gaa	gaa	gca	cca	tca	aag	gca	gga	att	ttc	gga	aat	atg	caa	aat	240
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	ctt	tat	gaa	act	gtg	aag	aag	gcc	cag	atg	gtt	gtc	caa	gtt	gag	gct	288
	Leu	Tyr	Glu	Thr	Val	Lys	Lys	Ala	Gln	Met	Val	Val	Gln	Val	Glu	Ala	
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	gtc	cgg	gtg	caa	aag	gag	ctt	gca	gcg	act	gag	atc	gat	ggc	tac	tgt	336
	Val	Arg	Val	Gln	Lys	Glu	Leu	Ala	Ala	Thr	Glu	Ile	Asp	Gly	Tyr	Cys	
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 Arg Val Glu Ile Thr Glu Ala Ala Met Glu Val Gly Ala Glu Lys Leu
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tct gag ctg gtg aac gac gcc tac aag gat gca cat cag agg agt gtc 480
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 145 150 155 160

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cag gca atg aag gag agg atg gct gat ctg gca cag agc tta gga atg 528
 Gln Ala Met Lys Glu Arg Met Ala Asp Leu Ala Gln Ser Leu Gly Met
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Ala Phe Cys Leu Pro Cys Tyr Asn Arg Lys Lys Ala Ser Asn Arg Ser
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Phe Arg Val Tyr Ser Leu Phe Gly Gly Lys Lys Asp Lys Asp Glu Asn
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Gly Glu Glu Ala Pro Ser Lys Ala Gly Ile Phe Gly Asn Met Gln Asn
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Leu Tyr Glu Thr Val Lys Lys Ala Gln Met Val Val Gln Val Glu Ala
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Val Arg Val Gln Lys Glu Leu Ala Ala Thr Glu Ile Asp Gly Tyr Cys
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Glu Gly Glu Leu Ile Lys Val Thr Leu Ser Gly Asn Gln Gln Pro Val
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Arg Val Glu Ile Thr Glu Ala Ala Met Glu Val Gly Ala Glu Lys Leu
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Ser Glu Leu Val Asn Asp Ala Tyr Lys Asp Ala His Gln Arg Ser Val
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    Leu Thr Val Arg Gly Pro Gln Leu Arg Leu Cys Asn Trp Ala Pro Arg
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20   ccc cgg tgg cgg ccg ccc ggc gcc ggc gcg gcg gac tcc tgt tgt ctc      144
    Pro Arg Trp Arg Pro Pro Gly Ala Gly Ala Ala Asp Ser Cys Cys Leu
          35          40          45

25   ctc ttc cgt gcg cgc gcg cgc cgc cgt ggc cac gcc cat gtg gcc agg      192
    Leu Phe Arg Ala Arg Ala Arg Arg Arg Gly His Gly His Val Ala Arg
    50          55          60

30   ttt gcg gcg tcc gct tcg ggg gcc ggc ggc gag gag gcc ggg gag ccg      240
    Phe Ala Ala Ser Ala Ser Gly Ala Gly Gly Glu Glu Ala Gly Glu Pro
    65          70          75          80

35   tcg gag gac gag gcg cag agg gag tgg gag gcg gag atg gcg cgg cgg      288
    Ser Glu Asp Glu Ala Gln Arg Glu Trp Glu Ala Glu Met Ala Arg Arg
          85          90          95

    ctg aag gag gcc gag gag atg gag gag ctc gag cgc acc gcc gag gag      336
    Leu Lys Glu Ala Glu Glu Met Glu Glu Leu Glu Arg Thr Ala Glu Glu
    100          105          110

40   ctg cag agc cag gcc gcc gcc gag gcc ccc gac gag tcc gag gag gag      384
    Leu Gln Ser Gln Ala Ala Ala Glu Ala Pro Asp Glu Ser Glu Glu Glu
    115          120          125

45   aag cgc gag cgc gtc cgc cgc gag ctc cag aag gtg gca aag gag caa      432
    Lys Arg Glu Arg Val Arg Arg Glu Leu Gln Lys Val Ala Lys Glu Gln
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50   gca gag agg cgg gcg acg gcg aag caa atg ttt gat ttg ggg caa agg      480
    Ala Glu Arg Arg Ala Thr Ala Lys Gln Met Phe Asp Leu Gly Gln Arg
    145          150          155          160

55   gcc tac gga aga gga atg tat ggc cgc tct att gaa ttc ttg gag gct      528
    Ala Tyr Gly Arg Gly Met Tyr Gly Arg Ser Ile Glu Phe Leu Glu Ala
          165          170          175

    gca ctc aca att att cgc cct tca tcg ctc ctt ggt ggg gag gta gca      576
    Ala Leu Thr Ile Ile Arg Pro Ser Ser Leu Leu Gly Gly Glu Val Ala
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 35 40 45
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 50 55 60
 20 Phe Ala Ala Ser Ala Ser Gly Ala Gly Gly Glu Glu Ala Gly Glu Pro
 65 70 75 80
 Ser Glu Asp Glu Ala Gln Arg Glu Trp Glu Ala Glu Met Ala Arg Arg
 85 90 95
 25 Leu Lys Glu Ala Glu Glu Met Glu Glu Leu Glu Arg Thr Ala Glu Glu
 100 105 110
 30 Leu Gln Ser Gln Ala Ala Ala Glu Ala Pro Asp Glu Ser Glu Glu Glu
 115 120 125
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 130 135 140
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 145 150 155 160
 Ala Tyr Gly Arg Gly Met Tyr Gly Arg Ser Ile Glu Phe Leu Glu Ala
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	gcc ggg ggt gga ggc gga ggt gga ggc ggg aag ggg agc tcg gtg tac	144
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5	ccg gcg ccc gtg gtg tac ccg cac cac tcc cgc cag atc tcc tgg aag	192
	Pro Ala Pro Val Val Tyr Pro His His Ser Arg Gln Ile Ser Trp Lys	
	50 55 60	
10	ccc agg gtg ttc ctc tac cag cat ttc ctc agc gac gac gag gcc aac	240
	Pro Arg Val Phe Leu Tyr Gln His Phe Leu Ser Asp Asp Glu Ala Asn	
	65 70 75 80	
15	cac ctc gtc tcc ctc gcg aga acg gag ctc aag agg tca gcg gtg gct	288
	His Leu Val Ser Leu Ala Arg Thr Glu Leu Lys Arg Ser Ala Val Ala	
	85 90 95	
20	gat aac ctg tcc ggc aag agc gag ctc agc gac gcc cgc acc agc tcc	336
	Asp Asn Leu Ser Gly Lys Ser Glu Leu Ser Asp Ala Arg Thr Ser Ser	
	100 105 110	
25	ggc acc ttc atc cgc aag agc cag gat cca att gtt gct ggt ata gag	384
	Gly Thr Phe Ile Arg Lys Ser Gln Asp Pro Ile Val Ala Gly Ile Glu	
	115 120 125	
30	gaa aaa att gct gcg tgg aca ttt ctt cct aaa gag aat ggt gag gat	432
	Glu Lys Ile Ala Ala Trp Thr Phe Leu Pro Lys Glu Asn Gly Glu Asp	
	130 135 140	
35	atc caa gtg tta agg tat aag cac ggc gag aag tat gaa cgg cac tat	480
	Ile Gln Val Leu Arg Tyr Lys His Gly Glu Lys Tyr Glu Arg His Tyr	
	145 150 155 160	
40	gat tac ttc agc gac aat gtt aac act ctc cgt ggt ggt cat cgc ata	528
	Asp Tyr Phe Ser Asp Asn Val Asn Thr Leu Arg Gly Gly His Arg Ile	
	165 170 175	
45	gct act gtt ctt atg tac tta aca gat gtt gca gaa ggt gga gaa aca	576
	Ala Thr Val Leu Met Tyr Leu Thr Asp Val Ala Glu Gly Gly Glu Thr	
	180 185 190	
50	gtt ttc cct cta gct gag gag ttt aca gaa agt ggc aca aac aat gaa	624
	Val Phe Pro Leu Ala Glu Glu Phe Thr Glu Ser Gly Thr Asn Asn Glu	
	195 200 205	
55	gat tca acc ttg tca gaa tgt gct aaa aaa ggt gtt gca gtg aaa cca	672
	Asp Ser Thr Leu Ser Glu Cys Ala Lys Lys Gly Val Ala Val Lys Pro	
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60	cga aaa ggg gat gca ctt ctg ttc ttc aac ctt agc cca gat gct tca	720
	Arg Lys Gly Asp Ala Leu Leu Phe Phe Asn Leu Ser Pro Asp Ala Ser	
	225 230 235 240	
65	aaa gat tca ttg agc ctc cac gct gga tgc cca gtc ata aag ggt gag	768
	Lys Asp Ser Leu Ser Leu His Ala Gly Cys Pro Val Ile Lys Gly Glu	
	245 250 255	
70	aaa tgg tct gct aca aag tgg att cgt gta gcc tca ttc gac aag gtt	816
	Lys Trp Ser Ala Thr Lys Trp Ile Arg Val Ala Ser Phe Asp Lys Val	
	260 265 270	
75	tat cac aca caa ggc aat tgc act gac gac aat gag agt tgt gaa aaa	864
	Tyr His Thr Gln Gly Asn Cys Thr Asp Asp Asn Glu Ser Cys Glu Lys	
	275 280 285	

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      Ala Gly Gly Gly Gly Gly Gly Gly Gly Gly Lys Gly Ser Ser Val Tyr
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25  Pro Ala Pro Val Val Tyr Pro His His Ser Arg Gln Ile Ser Trp Lys
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      Pro Arg Val Phe Leu Tyr Gln His Phe Leu Ser Asp Asp Glu Ala Asn
30  65                                70                                75                                80

      His Leu Val Ser Leu Ala Arg Thr Glu Leu Lys Arg Ser Ala Val Ala
      85                                90                                95

35  Asp Asn Leu Ser Gly Lys Ser Glu Leu Ser Asp Ala Arg Thr Ser Ser
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      Gly Thr Phe Ile Arg Lys Ser Gln Asp Pro Ile Val Ala Gly Ile Glu
      115                                120                                125

40  Glu Lys Ile Ala Ala Trp Thr Phe Leu Pro Lys Glu Asn Gly Glu Asp
      130                                135                                140

      Ile Gln Val Leu Arg Tyr Lys His Gly Glu Lys Tyr Glu Arg His Tyr
45  145                                150                                155                                160

      Asp Tyr Phe Ser Asp Asn Val Asn Thr Leu Arg Gly Gly His Arg Ile
      165                                170                                175

50  Ala Thr Val Leu Met Tyr Leu Thr Asp Val Ala Glu Gly Gly Glu Thr
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      Val Phe Pro Leu Ala Glu Glu Phe Thr Glu Ser Gly Thr Asn Asn Glu
      195                                200                                205

55  Asp Ser Thr Leu Ser Glu Cys Ala Lys Lys Gly Val Ala Val Lys Pro
      210                                215                                220

      Arg Lys Gly Asp Ala Leu Leu Phe Phe Asn Leu Ser Pro Asp Ala Ser
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      Lys Asp Ser Leu Ser Leu His Ala Gly Cys Pro Val Ile Lys Gly Glu
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	275 280 285	
	Trp Ala Ala Leu Gly Glu Cys Ile Lys Asn Pro Glu Tyr Met Ile Gly	
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	ttc gac ttc gat gag aag atc ctg ggc ttc gtc aag ggc tac aaa cgc	96
	Phe Asp Phe Asp Glu Lys Ile Leu Gly Phe Val Lys Gly Tyr Lys Arg	
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	Thr Phe Asn Leu Ala Cys Ile Asp His Arg Gly Thr Pro Glu His Pro	
	35 40 45	
35	gcg agg acc tgc aca ctt gag tcc gac gag gaa gcc ata tgc tgg ggg	192
	Ala Arg Thr Cys Thr Leu Glu Ser Asp Glu Glu Ala Ile Cys Trp Gly	
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40	att gca tac tgt gtc aaa ggg ggg ctt aaa aag gag caa gaa gca atg	240
	Ile Ala Tyr Cys Val Lys Gly Gly Leu Lys Lys Glu Gln Glu Ala Met	
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	aag tac ttg gaa aga aga gag tgt gag tat gac cag aag atc tct gtg	288
45	Lys Tyr Leu Glu Arg Arg Glu Cys Glu Tyr Asp Gln Lys Ile Ser Val	
	85 90 95	
	gat ttc tac aag gaa gga gat tct ttg aag cca gct gtg aca ggt gta	336
	Asp Phe Tyr Lys Glu Gly Asp Ser Leu Lys Pro Ala Val Thr Gly Val	
	100 105 110	
50	cta gtc ttt gta tcc act cct gat cca gta ggc aac aaa tac tat ctt	384
	Leu Val Phe Val Ser Thr Pro Asp Pro Val Gly Asn Lys Tyr Tyr Leu	
	115 120 125	
55	ggg cct gct cct ttg gag gac atg gca agg caa att gct aca gcc aat	432
	Gly Pro Ala Pro Leu Glu Asp Met Ala Arg Gln Ile Ala Thr Ala Asn	
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60	ggc ccc aat ggg aat aac agg gat tac ctg ttc tca atg gag aag gca	480
	Gly Pro Asn Gly Asn Asn Arg Asp Tyr Leu Phe Ser Met Glu Lys Ala	
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10 gat agc cca caa aaa tct cac gct ctc gta cac ctg tct gcg ctt cct 624
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Thr Phe Asn Leu Ala Cys Ile Asp His Arg Gly Thr Pro Glu His Pro
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 130 135 140

Gly Pro Asn Gly Asn Asn Arg Asp Tyr Leu Phe Ser Met Glu Lys Ala
 145 150 155 160

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 165 170 175

60 Glu Val Arg Lys Val Leu Ser Arg Pro Lys Glu Lys Ile Thr Gly Ser
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	225 230 235 240	
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	Ser Phe Pro Asn Ser Phe Pro Gly Thr Leu Tyr Tyr Lys Ala Ile Gln	
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	Ala Arg Arg Arg Leu His Gly Val Leu Ser Asp Ile Met Arg Glu Arg	
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	Arg Ala Arg Gly Glu Pro Gly Ser Asp Leu Leu Gly Cys Leu Met Gln	
	275 280 285	
25	tcg cgg gcg ggc gac gac ggc gcg ctc ctc acc gac gag cag gtc gcc	912
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	Tyr Ile His His Cys Leu Val Asp Gly Leu Glu Tyr Asn Thr Lys Tyr	115	120	125
10	Tyr Tyr Lys Ile Gly Ser Gly Asp Ser Ala Arg Glu Phe Trp Phe Glu	130	135	140
	Thr Pro Pro Ala Ile Asp Pro Asp Ala Ser Tyr Thr Phe Gly Ile Ile	145	150	155
15	Gly Asp Leu Gly Gln Thr Phe Asn Ser Leu Ser Thr Leu Gln His Tyr	165	170	175
	Glu Lys Ser Glu Gly Gln Thr Val Leu Phe Val Gly Asp Leu Ser Tyr	180	185	190
20	Ala Asp Arg Tyr Gln His Asn Asp Gly Val Arg Trp Asp Ser Trp Gly	195	200	205
	Arg Leu Val Glu Arg Ser Thr Ala Tyr Gln Pro Trp Ile Trp Ser Ala	210	215	220
	Gly Asn His Glu Ile Glu Tyr Arg Pro Asp Leu Gly Glu Thr Ser Thr	225	230	235
30	Phe Lys Pro Tyr Leu His Arg Cys His Thr Pro Tyr Leu Ala Ser Lys	245	250	255
	Ser Ser Ser Pro Met Trp Tyr Ala Ile Arg Arg Ala Ser Ala His Ile	260	265	270
35	Ile Val Leu Ser Ser Tyr Ser Pro Phe Val Lys Tyr Thr Pro Gln Trp	275	280	285
	Thr Trp Leu Lys Tyr Glu Leu Lys His Val Asp Arg Glu Lys Thr Pro	290	295	300
	Trp Leu Ile Val Leu Met His Ser Pro Met Tyr Asn Ser Asn Glu Ala	305	310	315
45	His Tyr Met Glu Gly Glu Ser Met Arg Ala Ala Phe Glu Lys Trp Phe	325	330	335
	Val Lys Tyr Lys Val Asp Leu Val Phe Ala Gly His Val His Ala Tyr	340	345	350
50	Glu Arg Ser Tyr Arg Ile Ser Asn Ile Asn Tyr Asn Ile Thr Ser Gly	355	360	365
	Asn Arg Tyr Pro Val Pro Asp Lys Ser Ala Pro Val Tyr Ile Thr Val	370	375	380
	Gly Asp Gly Gly Asn Gln Glu Gly Leu Ala Ser Arg Phe Ser Asp Pro	385	390	395
60	Gln Pro Asp Tyr Ser Ala Phe Arg Glu Ala Ser Tyr Gly His Ser Ile	405	410	415

Leu Gln Leu Lys Asn Arg Thr His Ala Ile Tyr Gln Trp Asn Arg Asn
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 5 Asp Asp Gly Lys His Val Pro Ala Asp Asn Val Val Phe His Asn Gln
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 Ile Gln Leu Val Leu Leu Val Leu Val Ile Gly Ser Gly Ile Leu Val
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 Val Pro Ala Ala Gly Gly Thr Gly Ser Ser Gly His Gly Arg Asp Arg
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 Ser Gly Met Gly Trp Thr Thr Leu His Gly Pro Leu Val Ser Ser Gly
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 Ser Gln Trp Leu Gly Cys Ser Ser Glu His Gly Asp Gly Gly Ala Arg
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 Arg Arg Glu Arg His Thr Gly Glu Arg Val Ala Ala Arg Gly Arg Gly
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 Val Asp Gly Val Arg Gly Gly Gly Gly Gln Ser Gln Arg Arg Gly Gly
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