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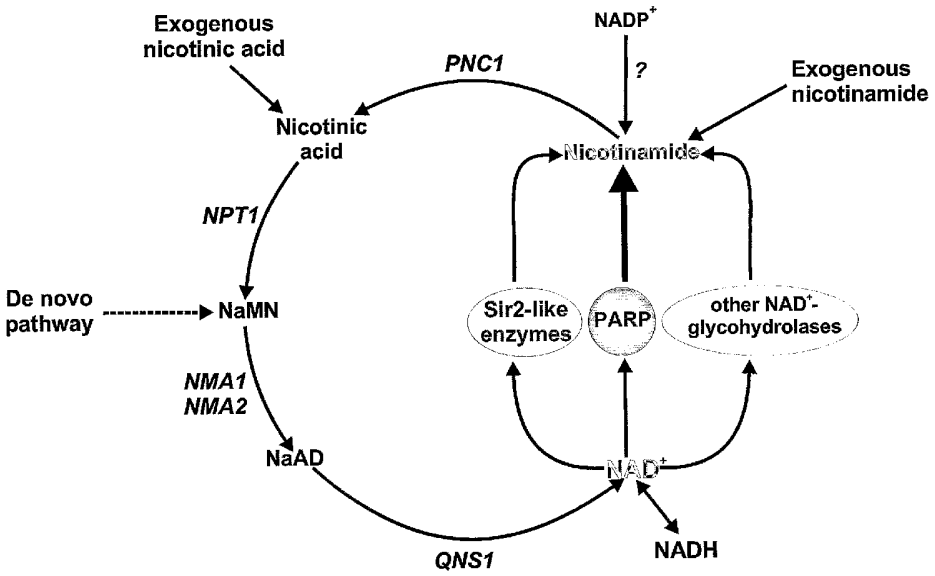
(54) Title: STRESS RESISTANT PLANTS



NaMN: nicotinic acid mononucleotide
NaAD: desamino-NAD+

PNC1: nicotinamidase
NPT1: nicotinate phosphoribosyl transferase
NMA: NAD+ pyrophosphorylase
QNS1: NAD+ synthetase

(57) Abstract: Stress tolerance in plants and plant cells is achieved by using nucleotide sequences encoding enzymes involved in the NAD salvage synthesis pathway and/or the NAD *de novo* synthesis pathway e.g. for overexpression in plants.

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STRESS RESISTANT PLANTS

Methods are provided for increasing the stress resistance in plants and plant cells whereby enzymes involved in the NAD salvage synthesis pathway and/or the NAD *de novo* synthesis pathway are expressed in plants.

Background art

Tolerance of plants to adverse growing conditions, including drought, high light intensities, high temperatures, nutrient limitations, saline growing conditions and the like, is a very desired property for crop plants, in view of the never-ending quest to ultimately increase the actual yield of these plants.

Various ways of achieving that goal of improving what is commonly known as the stress resistance or stress tolerance of plants have been described. Since different abiotic stress conditions frequently result in the generation of harmfull reactive oxygen species ("ROS") such as superoxides or hydrogen peroxides, initial attempts to improve stress resistance in plants focused on prevention of the generation of the ROS or the removal thereof. Examples of these approaches are overexpression of ROS scavenging enzymes such as catalases, peroxidases, superoxide dismutases etc. or even increasing the amount of ROS scavenging molecules such as ascorbic acid, glutathione etc. These approaches and other attempts to engineer stress tolerant plants are reviewed e.g. in Wang et al. 2003, Planta 218:1-14.

Stress tolerance in plant cells and plants can also be achieved by reducing the activity or the level of the endogenous poly-ADP-ribose polymerases (ParP) or poly(ADP-ribose) glycohydrolases (ParG) as described in WO00/04173 and PCT/EP2004/003995, respectively. It is thought that in this way, fatal NAD and ATP depletion in plant cells subject to stress conditions, resulting in traumatic cell death, can be avoided or sufficiently postponed for the stressed cells to survive and acclimate to the stress conditions.

Uchimiya et al. (2002) et al. describe the isolation of a rice gene denoted YK1, as well as use of a chimeric YK1 gene to increase the tolerance of transgenic rice plants harboring that gene to rice blast and several abiotic stresses such as

NaCl, UV-C, submergence, and hydrogen peroxide. (Uchimiya et al., 2002, Molecular breeding 9: 25-31).

Uchimiya et al. further published a poster abstract describing that overexpression of a NAD dependent reductase gene (YK1) in rice cells also promoted the level of NAD(P)(H) through up-regulating NAD synthetase activities, and concluded that this modification in turn generated a pool of redox substances needed for ROS stress resistance (Uchimiya et al. 2003 Keystone symposium on Plant biology: Functions and control of cell death, Snowbird Utah April 10-15, 2003).

NAD synthetase from yeast has been well characterized and is the last enzyme in both the NAD *de novo* synthesis pathway and the NAD salvage pathway (see Figure 1). In the *de novo* pathway, quinolate is the precursor for NAD synthesis and is generated as a product of tryptophan degradation. In the salvage pathway, nicotinamide (which is a degradation product of NAD, generated through the action of various enzymes such as PARP, NAD-dependent deacetylases or other NAD glycohydrolases) is the precursor molecule. In a first step, nicotinamide is deamidated to nicotinic cid by a nicotinamidase. The nicotinic acid is transferred to 5-phosphoribosyl-1-pyrophosphate by the enzyme nicotinate phosphoribosyl transferase to yield nicotinic acid mononucleotide. This compound is shared between the *de novo* and the salvage pathway. Hence, further conversion of this compound by NAD+ pyrophosphorylase and NAD synthetase is achieved as in the *de novo* pathway.

In yeast, overexpression of PNC1 (encoding nicotinamidase) has been correlated with life span extension by calorie restriction and low-intensity stress (Anderson et al., 2003 Nature 423: p181-185; Gallo et al., 2004, Molecular and Cellular Biology 24: 1301-1312).

Little is known about the respective enzymes of the NAD biosynthesis pathways in plants. Hunt et al., 2004 describe the use of the available genomic information from Arabidopsis to identify the plant homologues of these enzymes (Hunt et al. , 2004, New Phytologist163(1): 31-44). The identified DNA sequences have the following Accession numbers: for nicotinamidase: At5g23220; At5g23230 and At3g16190; for nicotinate phosphoribosyltransferase: At4g36940, At2g23420, for

nicotinic acid mononucleotide adenyltransferase: At5g55810 and for NAD synthetase: At1g55090 (all nucleotide sequences are incorporated herein by reference).

Alternative methods for increasing stress tolerance in plants are still required and the embodiments described hereinafter, including the claims, provide such methods and means.

Summary of the invention

In one embodiment of the invention, a method is provided for obtaining a plant with increased stress resistance comprising introducing a chimeric gene into a cells of a plant to obtain transgenic cells whereby the chimeric gene comprises the following operably linked DNA fragments:

i. A plant-expressible promoter;
ii. A DNA region coding for a plant-functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase;
iii. A 3'end region involved in transcription termination and polyadenylation,

followed by regenerating the transgenic cells to obtain a population of transgenic plants; and selecting a plant from the population of transgenic plants which exhibits increased stress resistance or selecting a plant which exhibits a reduced level of reactive oxygen species or maintains a high level of NADH under stress conditions when compared to a similar non-transgenic plant. The DNA region may code for a protein comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID

No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway such as the nucleotide sequences of SEQ ID No.:1, SEQ ID No.:3, SEQ ID No.:5; SEQ ID No.:7, SEQ ID No.:9, SEQ ID No.:11; SEQ ID No.:13; SEQ ID No.:15, SEQ ID No.:17, SEQ ID No.:19, SEQ ID No.: 21 or SEQ ID No.:23.

In another embodiment, the invention relates to the chimeric genes as described herein, plant cells comprising these chimeric genes, and plants consisting essentially of plant cells comprising these chimeric genes, and seeds of such plants. These plants and plant cells may be characterized in that they have a lower level of reactive oxygen species under stress conditions than a similar plant not comprising such a chimeric gene.

In yet another embodiment, the invention relates to the use of the described chimeric genes to increase the stress resistance of a plant or to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions.

The invention further provides the use of a DNA sequence encoding a plant functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase, such as a a DNA sequence encoding a protein comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway, including a DNA sequence

comprising an nucleotide sequence selected from the nucleotide sequence of SEQ ID No.:1, SEQ ID No.:3, SEQ ID No.:5; SEQ ID No.:7, SEQ ID No.:9, SEQ ID No.:11; SEQ ID No.:13; SEQ ID No.:15, SEQ ID No.:17, SEQ ID No.:19, SEQ ID No.:21 or SEQ ID No.:23, to increase the stress resistance of a plant or to decrease the level of reactive oxygen species or maintain the level of NADH in a plant or a plant cell under stress conditions.

Brief description of the Figures

Figure 1 is a schematic representation of the NAD salvage pathway and the de novo NAD synthesis pathway as known in baker's yeast (*Saccharomyces cerevisea*)

Figures 2 to 11 are schematic representations of the various T-DNA vectors comprising DNA regions encoding enzymes from the NAD salvage pathway or the NAD *de novo* synthesis pathway under control of plant-expressible control elements. Abbreviations used are: RB: right T-DNA border; 3'35S: transcription termination and polyadenylation signal from CaMV 35S transcript; Cab22L:untranslated leader sequence of the Cab22L transcript; P35S2: CaMV 35S promoter; 3'g7: transcription termination and polyadenylation signal from Agrobacterium tumefaciens T-DNA gene 7; bar: phosphinotricin acetyltransferase coding region; pSSUAra promoter of the Rubisco small subunit transcript from Arabidopsis; LB; left T-DNA border; Sm/Sp: Spectinomycin and streptomycin resistance gene; pVS1ori; origin of VS1 suitable for replication in Agrobacterium; ColE1: origin of replication; NLS: nuclear localization signal; PNC1: DNA region coding for nicotinamidase from *Saccharomyces cereviseae*; npt1: the nicotinate phosphoribosyltransferase from *Saccharomyces cereviseae*; nma1: nicotinic acid mononucleotide adenyl transferase 1 from *Saccharomyces cereviseae*; nma2: nicotinic acid mononucleotide adenyl transferase 2 from *Saccharomyces cereviseae*; qns1: NAD synthetase (QNS1) from *Saccharomyces cerevisea.*

Detailed description

The current invention is based on the finding that DNA sequences encoding plant-functional enzymes from the NAD salvage pathway in yeasts could be used to obtain transgenic plants which were more resistant to stress, particularly abiotic stress, than plants not comprising these DNA sequences. The transgenic plants also exhibited a significantly reduced level of reactive oxygen species ("ROS") and maintained a high level of NADH, when put under stress conditions, compared to control plants

Thus in one embodiment of the invention, a method is provided to obtain a plant with increased stress resistance, whereby the method comprises the steps of

- introducing a stress resistant chimeric gene as herein described into cells of a plant to obtain cells comprising the stress resistant chimeric gene;
- regenerating these cells comprising the stress resistant chimeric gene to obtain a population of plants comprising the stress resistant chimeric gene; and
- selecting a plant from the population of these plants which exhibits increased stress resistance and/or decreased ROS level under stress conditions and/or maintains a high level of NADH, when compared to a similar non-transgenic plant.

The stress resistant chimeric gene thereby comprises a plant-expressible promoter operably linked to a DNA region coding for a plant-functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase and a 3'end region involved in transcription termination and polyadenylation.

As used herein, "a plant-functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway" is an enzyme which when introduced into plants, linked to appropriate control elements such as plant expressible promoter and terminator region, can be transcribed and translated to yield a enzyme of the NAD salvage synthesis pathway functional in plant cells. Included are the enzymes (and encoding genes) from the NAD salvage synthesis, which are obtained from a plant source, but also the enzymes obtained from yeast (Saccharomyces cereviseae) or from other yeasts or fungi. It is thought that the latter proteins may be even more suitable for the methods according to the invention, since these are less likely to be subject to the enzymatic feedback regulation etc. to which similar plant-derived enzymes may be subject.

Enzymes involved in the NAD salvage synthesis pathway comprise the following

- Nicotinamidase (EC 3.5.1.19) catalyzing the hydrolysis of the amide group of nicotinamide, thereby releasing nicotinate and NH3. The enzyme is also known as nicotinamide deaminase, nicotinamide amidase, YNDase or nicotinamide amidohydrolase
- Nicotinate phophoribosyltransferase (EC 2.4.2.11) also known as niacin ribonucleotidase, nicotinic acid mononucleotide glycohydrolase; nicotinic acid mononucleotide pyrophosphorylase; nicotinic acid phosphoribosyltransferase catalyzing the following reaction

  Nicotinate-D-ribonucleotide + diphosphate = nicotinate + 5-phospho-α-D-ribose 1-diphosphate
- Nicotinate-nucleotide adenylyltransferase, (EC 2.7.7.18) also known as deamido-NAD+ pyrophosphorylase; nicotinate mononucleotide adenylyltransferase; deamindonicotinamide adenine dinucleotide pyrophsophorylase; NaMT-ATase; nicotinic acid mononucleotide adenylyltransferase catalyzing the following reaction

  ATP+nicotinate ribonucleotide = diphosphate + deamido-NAD^+

- NAD-synthase (EC 6.3.1.5) also known as NAD synthetase; NAD^+synthase; nicotinamide adenine dinucleotide synthetase; diphosphopyridine nucleotide synthetase, catalyzing the following reaction

  Deamido-NAD^++ATP+NH3 = AMP+ diphosphate + NAD^+

In one embodiment of the invention, the coding regions encoding the different enzymes of the NAD salvage pathway comprise a nucleotide sequence encoding proteins with the amino acid sequences as set forth in SEQ ID Nos 2, 4, 6, 8 or 10, such as the nucleotide sequences of SEQ ID Nos 1, 3, 5, 7 or 9.

However, it will be clear that variants of these nucleotide sequences, including insertions, deletions and substitutions thereof may be also be used to the same effect. Equally, homologues to the mentioned nucleotide sequences from species different from *Saccharomyces cerevisea* can be used. These include but are not limited to nucleotide sequences from plants, and nucleotide sequences encoding proteins with the same amino acid sequences, as well as variants of such nucleotide sequences. Examples of the latter are nucleotide sequences encoding a protein with an amino acid sequence as set forth in SEQ ID Nos 12, 14, 16, 18, 20, 22 or 24 such as the nucleotide sequences of SEQ ID Nos 11, 13, 15, 17, 19, 21 or 23.

Variants of the described nucleotide sequence will have a sequence identity which is preferably at least about 80%, or 85 or 90% or 95% with identified nucleotide sequences encoding enzymes from the NAD salvage pathway, such as the ones identified in the sequence listing. Preferably, these variants will encode functional proteins with the same enzymatic activity as the enzymes from the NAD salvage pathway. For the purpose of this invention, the "sequence identity" of two related nucleotide or amino acid sequences, expressed as a percentage, refers to the number of positions in the two optimally aligned sequences which have identical residues (x100) divided by the number of positions compared. A gap, i.e. a position in an alignment where a residue is

present in one sequence but not in the other, is regarded as a position with non-identical residues. The alignment of the two sequences is performed by the Needleman and Wunsch algorithm (Needleman and Wunsch 1970). The computer-assisted sequence alignment above, can be conveniently performed using standard software program such as GAP which is part of the Wisconsin Package Version 10.1 (Genetics Computer Group, Madision, Wisconsin, USA) using the default scoring matrix with a gap creation penalty of 50 and a gap extension penalty of 3.

Nucleotide sequences homologous to the nucleotide sequences encoding an enzyme from the NAD salvage pathway in yeast, or encoding a homologous enzyme from an organism different than yeast may be identified by in silico analysis of genomic data, as described by Hunt et al. (vide supra).

Homologous nucleotide sequence may also be identified and isolated by hybridization under stringent conditions using as probes identified nucleotide sequences encoding enzymes from the NAD salvage pathway, such as the ones identified in the sequence listing.

"Stringent hybridization conditions" as used herein means that hybridization will generally occur if there is at least 95% and preferably at least 97% sequence identity between the probe and the target sequence. Examples of stringent hybridization conditions are overnight incubation in a solution comprising 50% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared carrier DNA such as salmon sperm DNA, followed by washing the hybridization support in 0.1 x SSC at approximately 65 °C, preferably twice for about 10 minutes. Other hybridization and wash conditions are well known and are exemplified in Sambrook et al, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly chapter 11.

Such variant sequences may also be obtained by DNA amplification using oligonucleotides specific for genes encoding enzymes from the NAD salvage pathway as primers, such as but not limited to oligonucleotides comprising about 20 to about 50 consecutive nucleotides selected from the nucleotide sequences of SEQ ID Nos 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 or their complement.

The methods of the invention can be used to obtain plants tolerant to different kinds of stress-inducing conditions, particularly abiotic stress conditions including submergence, high light conditions, high UV radiation levels, increased hydrogen peroxide levels, drought conditions, high or low temperatures, increased salinity conditions. The methods of the invention can also be used to reduce the level of ROS in the cells of plants growing under adverse conditions, particularly abiotic stress conditions including submergence, high light conditions, high UV radiation levels, increased hydrogen peroxide levels, drought conditions, high or low temperatures, increased salinity conditions etc. The level of ROS or the level of NADH can be determined using the methods known in the art, including those described in Example 3.

Using the methods described herein, plants may be obtained wherein the level of ROS is equal to or lower than in control plants under non-stressed conditions, such as but not limited to low light. In these plants, under non-stressed conditions, the level of ROS may range from 50% to 100% of the level of control plants under low light conditions, more particularly from about 60% to about 85%. The level of the ROS in these plants under stress conditions is about 50% to 80% of the level of ROS in control plants under stress conditions, corresponding to about 60 to 80% of the level of ROS in control plants under non-stressed conditions. Similarly, the NADH level in these plants is equal to or higher than in control plants under non-stressed conditions, such as but not limited to low light. In these plants, under non-stressed conditions, the level of NADH may range from 100% to 160% of the level of NADH in control plants under low light

conditions, more particularly from about 120% to about 140%. The level of NADH in these plants under stress conditions is about 200 to 300% of the level of NADH in control plants under stress conditions, corresponding to about 100 to 160% of the level of ROS in control plants under non-stressed conditions.

Methods to obtain transgenic plants are not deemed critical for the current invention and any transformation method and regeneration suitable for a particular plant species can be used. Such methods are well known in the art and include *Agrobacterium*-mediated transformation, particle gun delivery, microinjection, electroporation of intact cells, polyethyleneglycol-mediated protoplast transformation, electroporation of protoplasts, liposome-mediated transformation, silicon-whiskers mediated transformation etc. The transformed cells obtained in this way may then be regenerated into mature fertile plants.

The obtained transformed plant can be used in a conventional breeding scheme to produce more transformed plants with the same characteristics or to introduce the chimeric gene according to the invention in other varieties of the same or related plant species, or in hybrid plants. Seeds obtained from the transformed plants contain the chimeric genes of the invention as a stable genomic insert and are also encompassed by the invention.

It will be clear that the different stress resistant chimeric genes described herein, with DNA regions encoding different enzymes from the NAD salvage pathway can be combined within one plant cell or plant, to further enhance the stress tolerance of the plants comprising the chimeric genes. Thus, in one embodiment of the invention, plant cells and plants are provided which comprise at least two stress resistant chimeric genes each comprising a different coding region.

The transgenic plant cells and plant lines according to the invention may further comprise chimeric genes which will reduce the expression of endogenous PARP and/or PARG genes as described in WO 00/04173 and PCT/EP2004/003995 .

These further chimeric genes may be introduced e.g. by crossing the transgenic plant lines of the current invention with transgenic plants containing PARP and/or PARG gene expression reducing chimeric genes. Transgenic plant cells or plant lines may also be obtained by introducing or transforming the chimeric genes of the invention into transgenic plant cells comprising the PARP or PARG gene expression reducing chimeric genes or vice versa.

For the purpose of the invention, the promoter is a plant-expressible promoter. As used herein, the term "plant-expressible promoter" means a DNA sequence which is capable of controlling (initiating) transcription in a plant cell. This includes any promoter of plant origin, but also any promoter of non-plant origin which is capable of directing transcription in a plant cell, i.e., certain promoters of viral or bacterial origin such as the CaMV35S (Harpster et al., 1988 *Mol. Gen. Genet.* **212**, 182-190), the subterranean clover virus promoter No 4 or No 7 (WO9606932), or T-DNA gene promoters but also tissue-specific or organ-specific promoters including but not limited to seed-specific promoters (e.g., WO89/03887), organ-primordia specific promoters (An et al., 1996, *The Plant Cell* **8**, 15-30), stem-specific promoters (Keller et al., 1988, *EMBO J.* **7**, 3625-3633), leaf specific promoters (Hudspeth et al., 1989, *Plant Mol Biol* **12**, 579-589), mesophyl-specific promoters (such as the light-inducible Rubisco promoters), root-specific promoters (Keller et al.,1989, *Genes Devel.* **3**, 1639-1646), tuber-specific promoters (Keil et al., 1989, *EMBO J.* **8**, 1323-1330), vascular tissue specific promoters (Peleman et al., 1989, *Gene* **84**, 359-369), stamen-selective promoters (WO 89/10396, WO 92/13956), dehiscence zone specific promoters (WO 97/13865) and the like.

The chimeric genes of the inventions may also be equipped with a nuclear localization signal ("NLS") functional in plants, operably linked to the DNA region encoding an enzyme of the NAD salvage pathway such as the SV40 NLS.

Having read this document, a person skilled in the art will immediately realize that similar effects with regard to increased stress resistance can be obtained whenever natural variants of plants are obtained wherein the endogenous genes coding for NAD salvage pathway enzymes are more active or expressed at a higher level. Such variant plants can be obtained by subjecting a population of plants to mutagenesis, such as, but not limited to EMS mutagenesis, followed by a screening for an increased activity of any one of the NAD salvage pathway enzymes, or a combination thereof.

It will also be immediately clear that a population of different varieties or cultivars can be screened for increased tolerance to the above mentioned stress conditions in general or particular selected abiotic stresses, followed by a correlation of the increased tolerance to stress conditions with the presence of a particular allele of any of the endogenous genes encoding an enzyme of the NAD salvage pathway enzyme. Such alleles can than be introduced into a plant of interest by crossing, if the species are sexually compatible, or they may be identified using conventional techniques as described herein (including hybridization or PCR amplification) and introduced using recombinant DNA technology. Introduction of particularly desired alleles using breeding techniques may be followed using molecular markers specific for the alleles of interest.

The methods and means described herein are believed to be suitable for all plant cells and plants, both dicotyledonous and monocotyledonous plant cells and plants including but not limited to cotton, Brassica vegetables, oilseed rape, wheat, corn or maize, barley, sunflowers, rice, oats, sugarcane, soybean, vegetables (including chicory, lettuce, tomato), tobacco, potato, sugarbeet, papaya, pineapple, mango, *Arabidopsis thaliana*, but also plants used in horticulture, floriculture or forestry.

As used herein "comprising" is to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not

preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. Thus, e.g., a nucleic acid or protein comprising a sequence of nucleotides or amino acids, may comprise more nucleotides or amino acids than the actually cited ones, i.e., be embedded in a larger nucleic acid or protein. A chimeric gene comprising a DNA region which is functionally or structurally defined, may comprise additional DNA regions etc.

The following non-limiting Examples describe the construction of chimeric genes to increase stress resistance in plant cells and plants and the use of such genes.

Unless stated otherwise in the Examples, all recombinant DNA techniques are carried out according to standard protocols as described in Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, NY and in Volumes 1 and 2 of Ausubel et al. (1994) Current Protocols in Molecular Biology, Current Protocols, USA. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfax (1993) by R.D.D. Croy, jointly published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications, UK. Other references for standard molecular biology techniques include Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual, Third Edition, Cold Spring Harbor Laboratory Press, NY, Volumes I and II of Brown (1998) Molecular Biology LabFax, Second Edition, Academic Press (UK). Standard materials and methods for polymerase chain reactions can be found in Dieffenbach and Dveksler (1995) PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, and in McPherson at al. (2000) PCR - Basics: From Background to Bench, First Edition, Springer Verlag, Germany.

Throughout the description and Examples, reference is made to the following sequences:

SEQ ID No. 1: nucleotide sequence of the nicotinamidase from *Saccharomyces cereviseae (PNC1).*

SEQ ID No. 2:. amino acid sequence of the nicotinamidase from *Saccharomyces cereviseae (PNC1).*

SEQ ID No. 3:. nucleotide sequence of the nicotinate phosphoribosyltransferase from *Saccharomyces cereviseae (NPT1)* (complement)

SEQ ID No. 4:. amino acid sequence of the nicotinate phosphoribosyltransferase from *Saccharomyces cereviseae (NPT1)*

SEQ ID No. 5: nucleotide sequence of the nicotinic acid mononucleotide adenyl transferase 1 (NMA1) from *Saccharomyces cereviseae.*

SEQ ID No. 6: amino acid sequence of the nicotinic acid mononucleotide adenyl transferase 1 (NMA1) from *Saccharomyces cereviseae*

SEQ ID No. 7: nucleotide sequence of the nicotinic acid mononucleotide adenyl transferase 2 (NMA2) from *Saccharomyces cereviseae.*

SEQ ID No. 8: amino acid sequence of the nicotinic acid mononucleotide adenyl transferase 2 (NMA2) from *Saccharomyces cereviseae.*

SEQ ID No. 9: nucleotide sequence of the NAD synthetase (QNS1) from *Saccharomyces cereviseae..*

SEQ ID No. 10: amino acid sequence of the NAD synthetase (QNS1) from *Saccharomyces cereviseae.*

SEQ ID No. 11:. nucleotide sequence of the nicotinamidase from *Arabidopsis thaliana (isoform 1).*

SEQ ID No. 12:. Amino acid sequence of the nicotinamidase from *Arabidopsis thaliana (isoform 1).*

SEQ ID No. 13:. nucleotide sequence of the nicotinamidase from *Arabidopsis thaliana (isoform 2)*

SEQ ID No. 14: Amino acid sequence of the nicotinamidase from *Arabidopsis thaliana (isoform 2).*

SEQ ID No. 15:. nucleotide sequence of the nicotinamidase from *Arabidopsis thaliana (isoform 3)*

SEQ ID No. 16: Amino acid sequence of the nicotinamidase from *Arabidopsis thaliana (isoform 3).*

SEQ ID No. 17: nucleotide sequence of the nicotinate phosphoribosyltransferase from *Arabidopsis thaliana (isoform 1).*

SEQ ID No. 18: amino acid sequence of the nicotinate phosphoribosyltransferase from *Arabidopsis thaliana (isoform 1).*

SEQ ID No. 19: nucleotide sequence of the nicotinate phosphoribosyltransferase from *Arabidopsis thaliana (isoform 2).*

SEQ ID No. 20: amino acid sequence of the nicotinate phosphoribosyltransferase from *Arabidopsis thaliana (isoform 2).*

SEQ ID No. 21: nucleotide sequence of the nicotinic acid mononucleotide adenyl transferase from *Arabidopsis thaliana.*

SEQ ID No. 22: amino acid sequence of the nicotinic acid mononucleotide adenyl transferase from *Arabidopsis thaliana.*

SEQ ID No. 23: nucleotide sequence of the NAD synthetase from *Arabidopsis thaliana.*

SEQ ID No. 24: amino acid sequence of the NAD synthetase from *Arabidopsis thaliana.*

SEQ ID No. 25: nucleotide sequence of T-DNA vector pTVE 467

SEQ ID No. 26: nucleotide sequence of T-DNA vector pTVE 468

SEQ ID No. 27: nucleotide sequence of T-DNA vector pTVE 469

SEQ ID No. 28: nucleotide sequence of T-DNA vector pTVE 470

SEQ ID No. 29: nucleotide sequence of T-DNA vector pTVE 496

SEQ ID No. 30: nucleotide sequence of T-DNA vector pTVE 497

SEQ ID No. 31: nucleotide sequence of T-DNA vector pTVE 500

SEQ ID No. 32: nucleotide sequence of T-DNA vector pTVE 501

SEQ ID No. 33: nucleotide sequence of T-DNA vector pTVE 502

SEQ ID No. 34: nucleotide sequence of T-DNA vector pTVE 503

Examples

Example 1: Assembly of stress resistant chimeric genes and introduction into plants.

pTVE467

To increase the stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower Mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;
- A DNA fragment encoding nicotinamidase from Saccharomyces cereviseae (SEQ ID NO 1);
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE467 (SEQ ID 25). T-DNA vector pTVE467 is schematically represented in Figure 2.

T-DNA vector pTVE467 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1181	534 (C)	PNC1 coding region
1250	1191(C)	cab22 leader
1781	1251(C)	P35S2 promoter
2293	2082 (C)	3'g7 transcription termination signal
2866	2315 (C)	bar coding region

4592	2867 (C)	PSSuAra promoter
4760	4784	Left T-DNA border
6352	5352 (C)	Sm/Sp resistance gene
6875	10645	pVS1origin of replication
10646	11709	ColE1 origin of replication

pTVE468

A similar chimeric gene as present in pTVE467 was constructed, wherein the nicotinamidase was equipped with a conventional nuclear localization signal. The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding nicotinamidase from Saccharomyces cereviseae (SEQ ID NO 1); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE468 (SEQ ID 26). T-DNA vector pTVE468 is schematically represented in Figure 3.

T-DNA vector pTVE468 comprises the following molecule features:
(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border

521	300 (C)	3'35S: transcription termination signal
1169	534 (C)	PNC1 coding region
1187	1167 (C)	Nuclear localization signal
1268	1209(C)	cab22 leader
1799	1269(C)	P35S2 promoter
2311	2100 (C)	3'g7 transcription termination signal
2884	2333 (C)	bar coding region
4610	2885 (C)	PSSuAra promoter
4778	4802	Left T-DNA border
6370	5370 (C)	Sm/Sp resistance gene
6893	10663	pVS1origin of replication
10664	11727	ColE1 origin of replication

pTVE469

To increase stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;
- A DNA fragment encoding nicotinate phosphoribosyltransferase from *Saccharomyces cereviseae* (NPT1; SEQ ID NO 3);
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE469 (SEQ ID 27). T-DNA vector pTVE469 is schematically represented in Figure 4.

T-DNA vector pTVE469 comprises the following molecule features:
(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1765	534 (C)	NPT1 coding region
1832	1773(C)	cab22 leader
2363	1833(C)	P35S2 promoter
2875	2664(C)	3'g7 transcription termination signal
3448	2897 (C)	bar coding region
5175	3449 (C)	PSSuAra promoter
5342	5366	Left T-DNA border
6934	5934(C)	Sm/Sp resistance gene
7457	11227	pVS1origin of replication
11228	12291	ColE1 origin of replication

pTVE470

A similar chimeric gene as present in pTVE469 was constructed, wherein the nicotinate phosphoribosyltransferase from *Saccharomyces cereviseae* was equipped with a conventional nuclear localization signal The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding nicotinate phosphoribosyltransferase from *Saccharomyces cereviseae* (NPT1; SEQ ID NO 3); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE470 (SEQ ID 28). T-DNA vector pTVE470 is schematically represented in Figure 5.

T-DNA vector pTVE470 comprises the following molecule features:
(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1787	534 (C)	NPT1 coding region
1775	1755 (C)	Nuclear localization signal SV40
1853	1794(C)	cab22 leader
2384	1854(C)	P35S2 promoter
2896	2685 (C)	3'g7 transcription termination signal
3469	2918(C)	bar coding region
5195	3470 (C)	PSSuAra promoter
5363	5387	Left T-DNA border
6955	5955 (C)	Sm/Sp resistance gene
7478	11248	pVS1origin of replication
11249	12312	ColE1 origin of replication

pTVE496

To increase stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;

- A DNA fragment encoding nicotinic acid mononucleotide adenyl transferase 1 from *Saccharomyces cereviseae* (NMA1; SEQ ID NO 5);
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE496 (SEQ ID 29). T-DNA vector pTVE496 is schematically represented in Figure 6.

T-DNA vector pTVE496 comprises the following molecule features:
(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1739	534 (C)	NMA1 coding region
1805	1746(C)	cab22 leader
2336	1806(C)	P35S2 promoter
2848	2637(C)	3'g7 transcription termination signal
3421	2870 (C)	bar coding region
5147	3422 (C)	PSSuAra promoter
5315	5339	Left T-DNA border
6907	5907(C)	Sm/Sp resistance gene
7430	11200	pVS1origin of replication
11201	12264	ColE1 origin of replication

pTVE497

A similar chimeric gene as present in pTVE496 was constructed, wherein the nicotinic acid mononucleotide adenyl transferase 1 from *Saccharomyces cereviseae* was equipped with a conventional nuclear localization signal The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding nicotinic acid mononucleotide adenyl transferase 1 from *Saccharomyces cereviseae* (NMA1; SEQ ID NO 5); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE497 (SEQ ID 30). T-DNA vector pTVE497 is schematically represented in Figure 7.

T-DNA vector pTVE497 comprises the following molecule features:
(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1757	534 (C)	NMA1 coding region
1748	1731 (C)	Nuclear localization signal SV40
1823	1764(C)	cab22 leader
2354	1824(C)	P35S2 promoter
2866	2655 (C)	3'g7 transcription termination signal
3439	2888(C)	bar coding region
5165	3440 (C)	PSSuAra promoter
5333	5357	Left T-DNA border
6925	5925 (C)	Sm/Sp resistance gene

7448	11218	pVS1origin of replication
11219	12282	ColE1 origin of replication

pTVE500

To increase stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;
- A DNA fragment encoding nicotinic acid mononucleotide adenyl transferase 2 from *Saccharomyces cereviseae* (NMA2; SEQ ID No. 7);
- A fragment of the 3' untranslated end from the 35S transcript of CaMV (3' 35S).

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE500 (SEQ ID 31). T-DNA vector pTVE500 is schematically represented in Figure 8.

T-DNA vector pTVE500 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
1721	534 (C)	NMA2 coding region
1787	1728(C)	cab22 leader
2318	1788(C)	P35S2 promoter
2830	2619(C)	3'g7 transcription termination signal
3403	2852 (C)	bar coding region
5129	3404 (C)	PSSuAra promoter

5297	5321	Left T-DNA border
6889	5889(C)	Sm/Sp resistance gene
7412	11182	pVS1origin of replication
11183	12246	ColE1 origin of replication

pTVE501

A similar chimeric gene as present in pTVE500 was constructed, wherein the nicotinic acid mononucleotide adenyl transferase 2 from *Saccharomyces cereviseae* was equipped with a conventional nuclear localization signal The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding nicotinic acid mononucleotide adenyl transferase 2 from *Saccharomyces cereviseae* (NMA2; SEQ ID No. 7); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE502 (SEQ ID 32). T-DNA vector pTVE501 is schematically represented in Figure 9.

T-DNA vector pTVE501 comprises the following molecule features:

(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border

521	300 (C)	3'35S: transcription termination signal
1739	534 (C)	NMA2 coding region
1733	1713 (C)	Nuclear localization signal SV40
1805	1746(C)	cab22 leader
2336	1806(C)	P35S2 promoter
2848	2637 (C)	3'g7 transcription termination signal
3421	2870(C)	bar coding region
5165	3440 (C)	PSSuAra promoter
5315	5339	Left T-DNA border
6907	5907 (C)	Sm/Sp resistance gene
7430	11200	pVS1origin of replication
11201	12264	ColE1 origin of replication

pTVE502

To increase stress resistance in plants, a chimeric gene was constructed using conventional techniques comprising the following DNA fragments in order:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 bp corresponding to the untranslated leader Cab22L;
- A DNA fragment encoding NAD synthase from *Saccharomyces cereviseae* (QNS1; SEQ ID No. 9);
- A fragment of the 3' untranslated end from the 35S transcript of CaMV (3' 35S).

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE502 (SEQ ID 33). T-DNA vector pTVE502 is schematically represented in Figure 10.

T-DNA vector pTVE502 comprises the following molecule features:
(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
2678	534 (C)	QNS1 coding region
2744	2685(C)	cab22 leader
3275	2745(C)	P35S2 promoter
3787	3576 (C)	3'g7 transcription termination signal
4360	3809 (C)	bar coding region
6086	4361 (C)	PSSuAra promoter
6254	6278	Left T-DNA border
7846	6846 (C)	Sm/Sp resistance gene
8369	12139	pVS1origin of replication
12140	13203	ColE1 origin of replication

pTVE503

A similar chimeric gene as present in pTVE502 was constructed, wherein the NAD synthase from *Saccharomyces cereviseae* was equipped with a conventional nuclear localization signal The chimeric gene thus comprises the following operably linked DNA fragments:

- A promoter region from Cauliflower mosaic Virus (CaMV 35S);
- A DNA fragment of about 60 nt corresponding to the untranslated leader Cab22L;
- A DNA fragment of about 20 nt encoding a peptide comprising a nuclear localization signal (NLS),
- A DNA fragment encoding NAD synthase from *Saccharomyces cereviseae* (QNS1; SEQ ID No. ç); whereby the NLS signal is fused in frame;
- A fragment of the 3' untranslated end from the 35 S transcript of CaMV (3' 35S)

This chimeric gene was introduced in a T-DNA vector, between the left and right border sequences from the T-DNA, together with a selectable marker gene providing resistance to the herbicide phosphinotricin, to yield pTVE503 (SEQ ID No. 34). T-DNA vector pTVE503 is schematically represented in Figure 11.

T-DNA vector pTVE503 comprises the following molecule features:
(C) indicates complementary strand.

Start (nt)	End (nt)	
198	222	RB: right T-DNA border
521	300 (C)	3'35S: transcription termination signal
2699	534 (C)	QNS1 coding region
2690	2670(C)	Nuclear localization signal SV40
2765	2706(C)	cab22 leader
3296	2766(C)	P35S2 promoter
3808	3597 (C)	3'g7 transcription termination signal
4381	3830 (C)	bar coding region
6107	4382 (C)	PSSuAra promoter
6275	6299	Left T-DNA border
7867	6867 (C)	Sm/Sp resistance gene
8390	12610	pVS1origin of replication
12161	13224	ColE1 origin of replication

The T-DNA vectors were introduced into Agrobacterium strains comprising a helper Ti-plasmid using conventional methods. The chimeric genes were introduced into Arabidopsis plants by Agrobacterium mediated transformation as described in the art.

Example 2. Analysis of transgenic Arabidopsis lines comprising the chimeric genes described in Example 1.

Seed of transgenic Arabidopsis lines (T1-generation) expressing the yeast genes of the NAD-salvage pathway, obtained as described in Example 1 were germinated and grown on medium containing 15 mg L^{-1} phosphinotricin (PPT). Arabidopsis thaliana cv Col-0 was used as a control.

All plants were subjected to high light stress. Two week old plants grown at 30 µEinstein m^{-2} sec^{-1} were transferred to 250 µEinstein m^{-2} sec^{-1} (high light) for 6 hours, followed by 8 hours in the dark and again 8 hours high light.

After this treatment, NADH content and superoxide radicals content were determined for all lines and compared to measurement of the same compounds in transgenic and control lines grown under low light conditions. The results are summarized in Table 1.

Transgenic plants exhibited a higher NADH content under high light than control plants, and produced less reactive oxygen species under high light than control plants. No difference was observed between constructs wherein the encoded NAD salvage pathway enzyme was equipped with a nuclear localization signal or not.

Transgenic plant lines were also phenotypically scored for tolerance to high light stress conditions. To this end, plants were grown in vitro at low light conditions (30 µEinstein m^{-2} sec^{-1}) for two weeks and transferred for 3 days to high light conditions (250 µEinstein m^{-2} sec^{-1}; 16 hrs light -8hrs dark). After the high light treatment the plants were returned to low light conditions and grown for another three days before scoring the phenotype.

Whereas control plants were small, and had started flowering (stress-induced), the plants of the transgenic lines comprising the chimeric genes as described in Example 1 were larger than the control plants and only had started to bolt.

Table 1. High light tolerance of transgenic Arabidopsis lines over-expressing the chimeric yeast genes as described in Example 1.

Chimeric genes	Segregation for PPT tolerance	% NADH versus low light control		% superoxides versus low light control	
		Low light	High light	Low light	High light
Control	-	100	68	100	145
PNC1 (NLS) line 1	3:1	108	128	80	73
PNC1 (NLS) line 2	3:1	139	128	82	76
NPT1 line 1	6:1	128	147	66	70
NPT1 line 2	6:1	122	135	82	76
NPT1 (NLS)	12:1	106	150	61	80

STANDARD ERROR OF MEAN < 10%

Example 3: protocols for measurement of NADH content and superoxide content.

Intracellular NAD(P)H quantification using a water-soluble tetrazolium salt

Reference

Jun Nakamura, Shoji Asakura, Susan D. Hester, Gilbert de Murcia, Keith W. Caldecott and James A. Swenberg (2003) Quantitation of intracellular NAD(P)H can monitor an imbalance of DNA single strand break repair in base excision repair deficient cells in real time. Nucleic Acids Research 31(17), e104.

Plant material

Most plant material can be used:

- In vitro grown Arabidopsis shoots 14-18 days old but NOT flowering
- Hypocotyl explants of oilseed rape

Cell Counting Kit-8 (CCK-8)

Sopachem n.v./Belgium
72A, Avenue du Laarbeeklaan - 1090 Brussels
Belgium

Contents:

5mL bottles containing 5mMol/L WST-8 (tetrazolium salt), 0.2mMol/L 1-Methoxy PMS, 150mMol/L NaCl

Reaction solution:

- 10mL 25mM K-phosphate buffer pH7.4
- 0.5mL CCK-8

- 0.1mM 1-Methoxy-5-methylphenazinium methyl sulfate (= 1-Methoxyphenazine methosulfate): 1µL/mL of 100mM stock (MW= 336.4; 100mg in 2.973mL water)
- 1 drop Tween20/25mL

Procedure

- Harvest plant material and put in 25mM K-phosphate buffer pH7.4
  e.g.: 150 oilseed rape hypocotyl explants
  1gr Arabidopsis shoots (without roots)

- Replace buffer with reaction solution
  15mL for 1gr Arabidopsis shoots
  15mL for 150 oilseed rape hypocotyl explants

- Incubate at 26°C in the dark for about 1/2 hour (follow reaction)

- Measure the absorbance of the reaction solution at 450nm

Measuring superoxide production by quantifying the reduction of XTT

Ref.: De Block, M., De Brouwer, D. (2002) A simple and robust in vitro assay to quantify the vigour of oilseed rape lines and hybrids. Plant Physiol. Biochem. 40, 845-852

A. BRASSICA NAPUS

Media and reaction buffers

Sowing medium (medium 201):

Half concentrated Murashige and Skoog salts

2% sucrose

pH 5.8

0.6% agar (Difco Bacto Agar)

250mg/l triacillin

Callus inducing medium A2S3:

MS medium, 0.5g/l Mes (pH 5.8), 3% sucrose, 40mg/l adenine-SO_4, 0.5% agarose, 1mg/l 2,4-D, 0.25mg/l NAA, 1mg/l BAP, 250mg/l triacillin

Reaction buffer:

25mM K-phosphate buffer pH 8

1mM sodium,3'-{1-[phenylamino-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro) = XTT (BioVectra, Canada) (MW 674.53)

Dissolve XTT by careful warming solution (± 37°C) (cool down to room temperature before use)

1 drop Tween20 for 25ml buffer

Sterilization of seeds - pregermination of seeds - growing of the seedlings

Seeds are soaked in 70% ethanol for 2 min, then surface-sterilized for 15 min in a sodium hypochlorite solution (with about 6% active chlorine) containing 0.1% Tween20. Finally, the seeds are rinsed with 1l of sterile tap water.

Incubate seeds for at least one hour in sterile tap water (to allow diffusion from seeds of components that may inhibit germination).

Seeds are put in 250ml erlenmeyer flasks containing 50ml of sterile tap water (+ 250mg/l triacillin). Shake for about 20 hours.

Seeds from which the radicle is protruded are put in Vitro Vent containers from Duchefa containing about 125ml of sowing medium (10 seeds/vessel, not too many to reduce loss of seed by contamination). The seeds are germinated at ±24°C and 10-30μEinstein $s^{-1}m^{-2}$ with a daylength of 16h.

P.S.: For calculating the amount of seeds that have to be sawn: 5 hypocytyl segments/seedling

Preculture of the hypocotyl explants and induction of stress

- 12-14 days after sowing, the hypocotyls are cut in about 7-10mm segments.
- The hypocotyl explants (25 hypocotyls/Optilux Petridish, Falcon S1005, Denmark) are cultured for 5 days on medium A2S3 at 25°C (at 10-30μEinstein $s^{-1}m^{-2}$).

P.S.: 150 hypocotyl explants are used per condition.

- Induction of stress:
  Transfer hypocotyl explants to A2S3 medium containing respectively 0, 25 and 50mg/l acetylsalicylic acid.
  Incubate for about 24 hours at 25°C and10-30μEinstein $s^{-1}m^{-2}$ with a

daylength of 16h.

XTT-assay

- Transfer 150 hypocotyl explants to a 50ml Falcon tube.
- Wash with reaction buffer (without XTT).
- Add 20mL reaction buffer + XTT.
  (explants have to be submerged, but do not vacuum infiltrate)
- Incubate in the dark at 26°C
- Follow the reaction by measuring the absorption of the reaction medium at 470nm

B. ARABIDOPSIS THALIANA

Media and reaction buffers

Plant medium:

Half concentrated Murashige and Skoog salts

B5 vitamins

1.5% sucrose

pH 5.8

0.7% Difco agar

Incubation medium:

1/2 concentrated MS-salts

1% sucrose

0.5g/L MES pH 5.8

1 drop Tween20 for 25ml medium

Reaction buffer:

25mM K-phosphate buffer pH 8

1mM sodium,3'-{1-[phenylamino-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro) = XTT (BioVectra, Canada) (MW 674.53)

Dissolve XTT by careful warming solution (± 37°C) (cool down to room temperature before use)

1 drop Tween20 for 25ml buffer

***Arabidopsis* plants**

- Arabidopsis lines: control (mother line from which tested lines were derived)
  lines to test

- Sterilization of *Arabidopsis* seeds:

2min. 70% ethanol

10 min. bleach (6% active chlorine) + 1drop Tween 20 for 20ml solution

wash 5 times with sterile tap water

P.S.: sterilization is done in 2ml eppendorf tubes

Arabidopsis seeds sink to the bottom of the tube, allowing removal of the liquids by means of a 1ml pipetman

- Pregermination of seeds:

In 9cm Optilux Petridishes (Falcon) containing 12ml sterile tap water.

Low light overnight to 24 hours.

- Growing of *Arabidopsis* plants

Seeds are sown in Intergrid Tissue Culture disks of Falcon (nr. 3025) containing ±125ml of plant medium: 1 seed/grid.

Plants are grown at 24°C

30µEinstein $s^{-1}m^{-2}$

16hours light - 8hours dark

for about 18 days (before bolting)

P.S.: 1g of plant material (shoots without roots)/line/condition are needed to cary out the asssay. 1g shoots corresponds wilth 40-60 plants.

Induction of stress

Paraquat

- Harvest *Arabidopsis* shoots (without roots)
- Put 1g shoots in incubation medium (shoots have to be submerged, but do not vacuum infiltrate) containing respectively 0, 5 and 10µM paraquat

Incubation medium: ±150ml in Intergrid Tissue Culture disks of Falcon (nr. 3025)

- Incubate at 24°C in the dark for ±24 hours and 30-50µEinstein $s^{-1}m^{-2}$ with a daylength of 16h.

High light

- Transfer half of the plates to high light (250µEinstein $s^{-1}m^{-2}$) and incubate for 4 to 20 hours

XTT-assay

- Harvest shoots (without roots) from agar plates (high light stress) or from liquid incubation medium (paraquat stress) and put them in 50ml Falcon tubes containing reaction buffer (without XTT)
- Replace reaction buffer with buffer containing XTT (15mL/gr)
- Shoots have to be submerged, but do not vacuum infiltrate
- Incubate in the dark at 26°C
- Follow the reaction by measuring the absorption of the reaction medium at 470nm (about one hour)

Example 4: Increased ozone tolerance of Arabidopsis thaliana plants over-expressing the yeast nicotineamidase (Pnc1) gene.

The chimeric vector pTVE467 (Example 1) was used for transformation of *A. thaliana* ecotype Columbia. Primary transformants were analyzed by Southern-DNA- and Northern-RNA-blot analysis. One transgenic line was identified to carry a single copy of the Pnc1-transgene construct and to have a high steady state level of transgenic full-length Pnc1-mRNA (20 pg/5 µg total RNA).

6 weeks after germination 100 individual plants each of the single copy transgenic line and of wild-type Columbia as a control, were exposed to ozone in fumigation chambers. During 2 consecutive days the plants were treated for 5h/day with ozone concentrations of 250, 350 and 500 ppb respectively. After

treatment all plants were visually screened for ozone injury manifested as necrotic lesions. The results are summarized in Table 2. At 500 ppb ozone exposure nearly all plants showed necrotic lesions whereas at the 2 lower ozone concentrations a significantly lower percentage of transgenic plants were injured.

In addition, the evolution of the vitality performance index (PI) was determined for all plants of the transgenic line and of the wild-type plants under increasing ozone concentration. PI can be calculated by the formula: PI = (ABS/CS) x (TR/CS) x (ET/CS). (ABS = flux of photons absorbed by the antenna pigments Chl*; CS = cross section; TR = energy trapped by the reaction centre and converted into redox energy; ET = electron flux further downstream leading to CO_2 fixation) In the transgenic line, the vitality performance index PI significantly increased with increasing ozone concentrations whereas this index remains constant in wild-type plants treated with increasing ozone concentrations. This can be explained by a physiological compensation response within the transgenic line to counteract the ozone damage.

Table 2. Increased ozone tolerance of Arabidopsis thaliana plants over-expressing the yeast nicotineamidase (Pnc1) gene.

	250 ppb O_3	**350 ppb O_3**	**500 ppb O_3**
Wild-type	45%*	50%	100%
Pnc1	20%	25%	100%

* percentage of the plants exhibiting necrotic lesions

Furthermore, control plants, homozygous transgenic populations of plants comprising the chimeric Pnc1 gene as well as a heterozygous transgenic population, were subjected to ozone fumigations and scored for visible injury and various physiological responses compared to non-fumigated plants. The assessment included measurement of non-modulated fluorescence, modulated fluorescence, chlorophyll measurement and fresh weight determination.

Based on the visible injury and physiological responses, a ranking was made for each population indicating the degree of the ozone impact. The more negative the evaluation, the more sensitive the population's response to ozone.

Whereas the control non-transgenic population and the heterozygous transgenic population had a cumulative score of -13, the two homozygous transgenic populations had a score of -6 and -2 respectively. It is therefore clear that the homozygous transgenic populations performed statistically significantly better than the control plants.

Claims

1. A method for obtaining a plant with increased stress resistance comprising
    a. introducing a chimeric gene into a cells of a plant to obtain transgenic cells, said chimeric gene comprising the following operably linked DNA fragments:
        i. A plant-expressible promoter;
        ii. A DNA region coding for a plant-functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase;
        iii. A 3'end region involved in transcription termination and polyadenylation;
    b. regenerating said transgenic cells to obtain a population of transgenic plants; and
    c. selecting a plant from said population of transgenic plants which exhibits increased stress resistance or selecting a plant which exhibits a reduced level of reactive oxygen species or maintains a high level of NADH under stress conditions when compared to a similar non-transgenic plant.

2. The method according to claim 1, wherein said DNA region codes for an enzyme comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway.

3. The method according to claim 1, wherein said DNA region codes for nicotinamidase.

4. The method according to claim 3, wherein said DNA region encodes a protein comprising an amino acid sequence selected from the amino acid sequence of SEQ ID No.:2, SEQ ID No.:12, SEQ ID No.:14 or SEQ ID No.:16.

5. The method according to claim 3, wherein said DNA region comprises the nucleotide sequence of SEQ ID No.: 1, SEQ ID No.:3, SEQ ID No.:11, SEQ ID No.:13 or SEQ ID No.: 15.

6. The method according to claim 1, wherein said DNA region codes for nicotinate phosphoribosyltransferase.

7. The method according to claim 6, wherein said DNA region encodes a protein comprising an amino acid sequence selected from the amino acid sequence of SEQ ID No.: 4, SEQ ID No.: 18 or SEQ ID No.: 20.

8. The method according to claim 6 wherein said DNA region comprises the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.: 17 or SEQ ID No.: 19.

9. The method according to claim 1, wherein said DNA region codes for nicotinic acid mononucleotide adenyltransferase.

10. The method according to claim 9, wherein said DNA region encodes a protein comprising an amino acid sequence selected from the amino acid sequence of SEQ ID No.: 6, SEQ ID No.: 8 or SEQ ID No.: 22.

11. The method according to claim 9, wherein said DNA region comprises the nucleotide sequence of SEQ ID No.: 5, SEQ ID No.: 7 or SEQ ID No.: 21.

12. The method according to claim 1, wherein said DNA region codes for NAD synthetase.

13. The method according to claim 12, wherein said DNA region encodes a protein comprising an amino acid sequence selected from the amino acid sequence of SEQ ID No.: 10 or SEQ ID No.: 24.

14. The method according to claim 12, wherein said DNA region comprises the nucleotide sequence of SEQ ID No.: 9 or SEQ ID No.: 23.

15. The method according to any one of claims 1 to 14, further comprising the step of crossing said plant with another plant.

16. A chimeric gene as described in any one of claims 1 to 14.

17. A plant cell comprising a chimeric gene as described in claim 16.

18. A plant comprising a chimeric gene as described in claim 16.

19. The plant of claim 18 which is a plant selected from cotton, Brassica vegetables, oilseed rape, wheat, corn or maize, barley, sunflower, rice, oats, sugarcane, soybean, vegetables, chicory, lettuce, tomato, tobacco, potato, sugarbeet, papaya, pineapple, mango or *Arabidopsis thaliana.*

20. The plant according to claim 18 or 19, further characterized in that it has a lower level of reactive oxygen species under stress conditions than a similar plant not comprising such a chimeric gene.

21. A seed of a plant according to claim 18 or claim 19 comprising a chimeric gene according to claim 16.

22. Use of a chimeric gene according to claim 16 to increase the stress resistance of a plant.

23. Use of a chimeric gene according to claim 16 to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions or to maintain the level of NAD in a plant or plant cell under stress conditions.

24. Use of a DNA sequence encoding a plant functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase to increase the stress resistance of a plant.

25. Use of a DNA sequence encoding a plant functional enzyme of the nicotinamide adenine dinucleotide salvage synthesis pathway selected from nicotinamidase, nicotinate phosphoribosyltransferase, nicotinic acid mononucleotide adenyl transferase or nicotinamide adenine dinucleotide synthetase to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions or to maintain the level of NAD in a plant or plant cell under stress conditions.

26. Use of a DNA sequence encoding a protein comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway to increase the stress resistance of a plant.

27. Use of a DNA sequence encoding a protein comprising an amino acid sequence selected from the aminoacid sequence of SEQ ID No.:2, SEQ ID No.:4, SEQ ID No.:6; SEQ ID No.:8, SEQ ID No.:10, SEQ ID No.:12; SEQ ID No.:14; SEQ ID No.:16, SEQ ID No.:18, SEQ ID No.:20, SEQ ID No.: 22, SEQ ID No.:24 or a protein having about 60% sequence identity and having the enzymatic activity of nicotinamide adenine dinucleotide salvage synthesis pathway to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions or to maintain the level of NAD in a plant or plant cell under stress conditions.

28. Use of a DNA sequence comprising an nucleotide sequence selected from the nucleotide sequence of SEQ ID No.:1, SEQ ID No.:3, SEQ ID No.:5; SEQ ID No.:7, SEQ ID No.:9, SEQ ID No.:11; SEQ ID No.:13; SEQ ID No.:15, SEQ ID No.:17, SEQ ID No.:19, SEQ ID No.:21 or SEQ ID No.:23 to increase the stress resistance of a plant.

29. Use of a DNA sequence comprising an nucleotide sequence selected from the nucleotide sequence of SEQ ID No.:1, SEQ ID No.:3, SEQ ID No.:5; SEQ ID No.:7, SEQ ID No.:9, SEQ ID No.:11; SEQ ID No.:13; SEQ ID No.:15, SEQ ID No.:17, SEQ ID No.:19, SEQ ID No.:21 or SEQ ID No.:23 to increase the stress resistance of a plant. to decrease the level of reactive oxygen species in a plant or a plant cell under stress conditions or to maintain the level of NAD in a plant or plant cell under stress conditions.

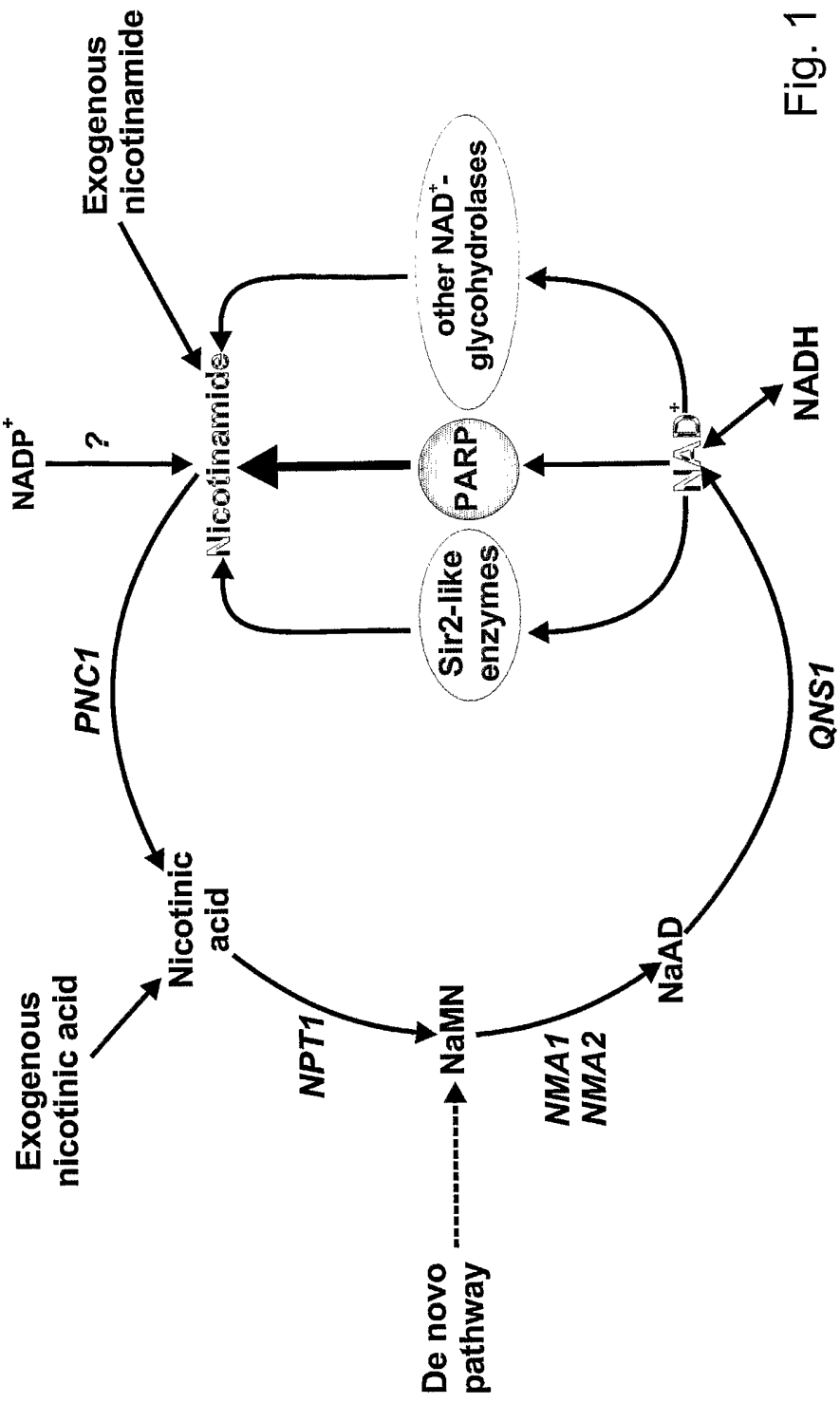

Fig. 1

NaMN: nicotinic acid mononucleotide
NaAD: desamino-NAD^+

PNC1: nicotinamidase
NPT1: nicotinate phosphoribosyl transferase
NMA: NAD^+ pyrophosphorylase
QNS1: NAD^+ synthetase

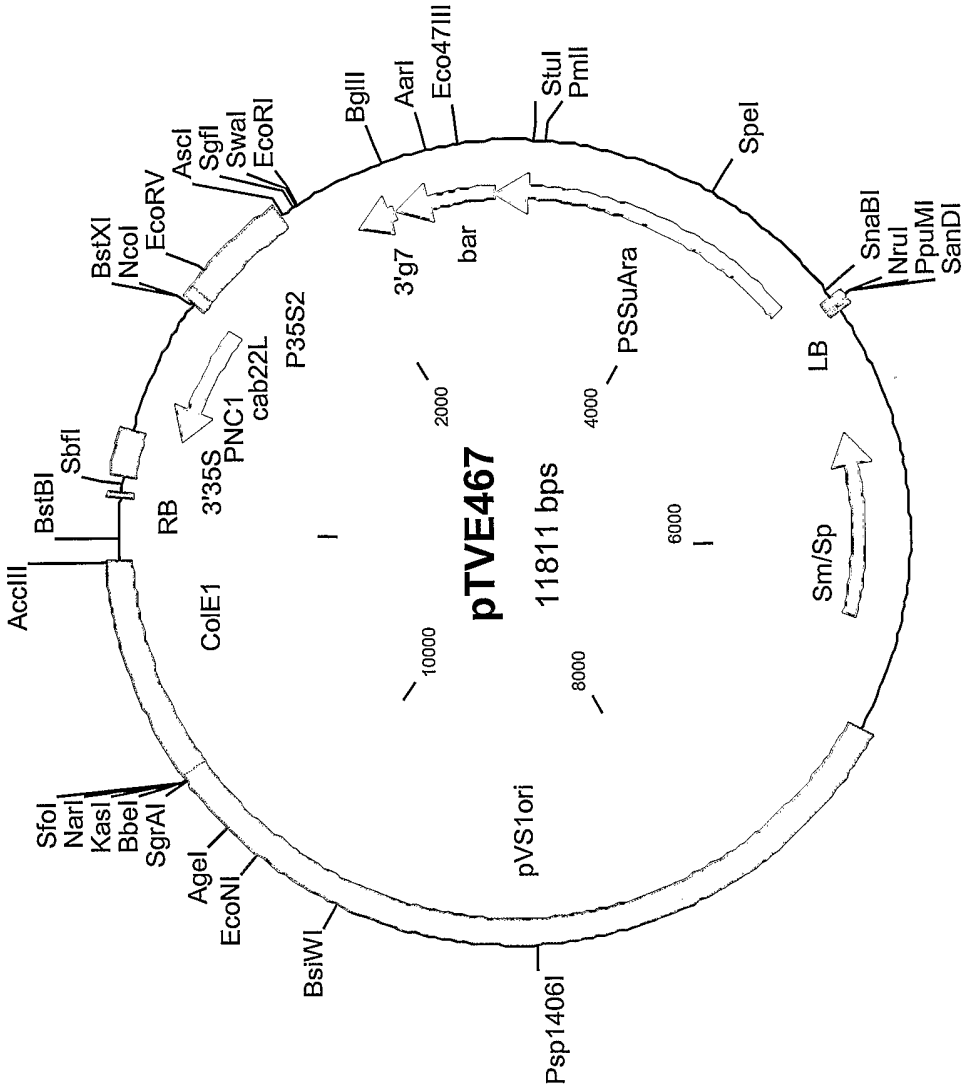
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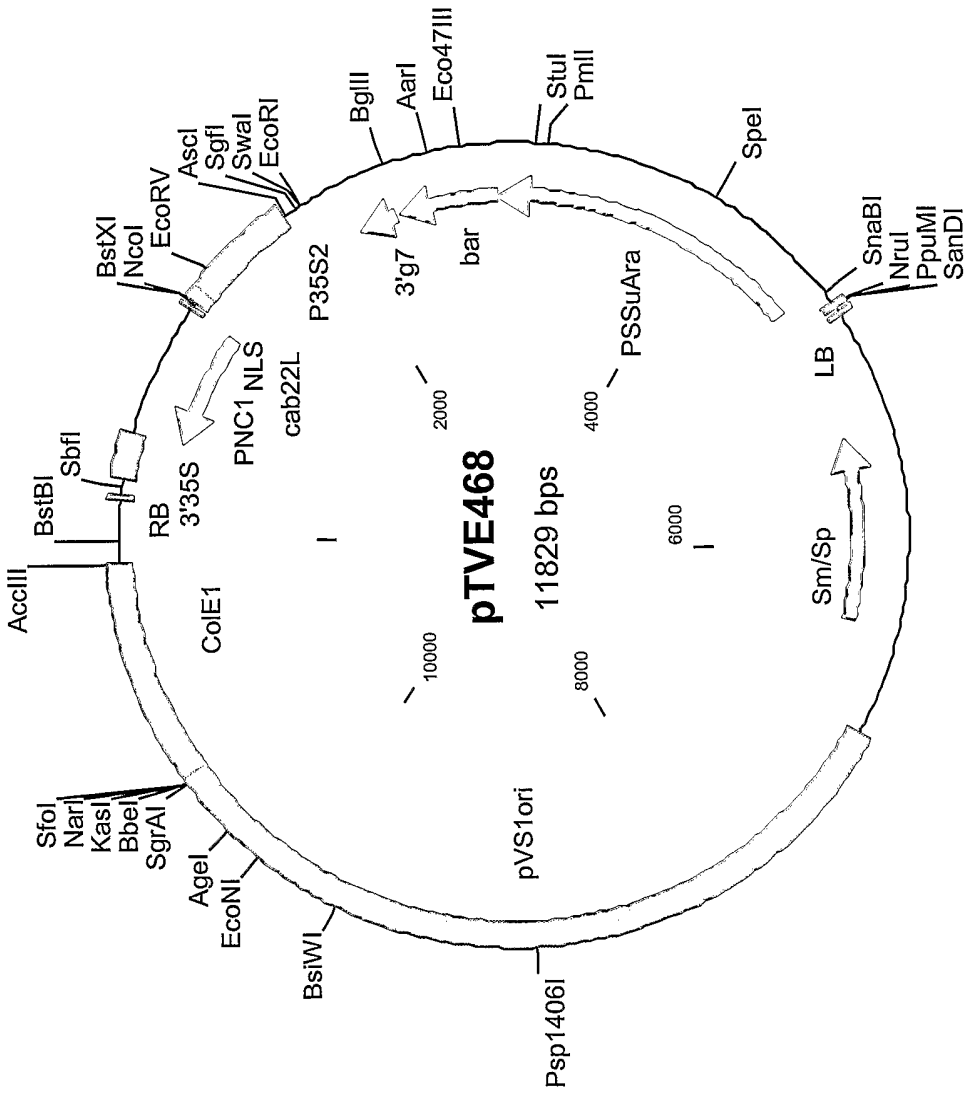
Fig. 2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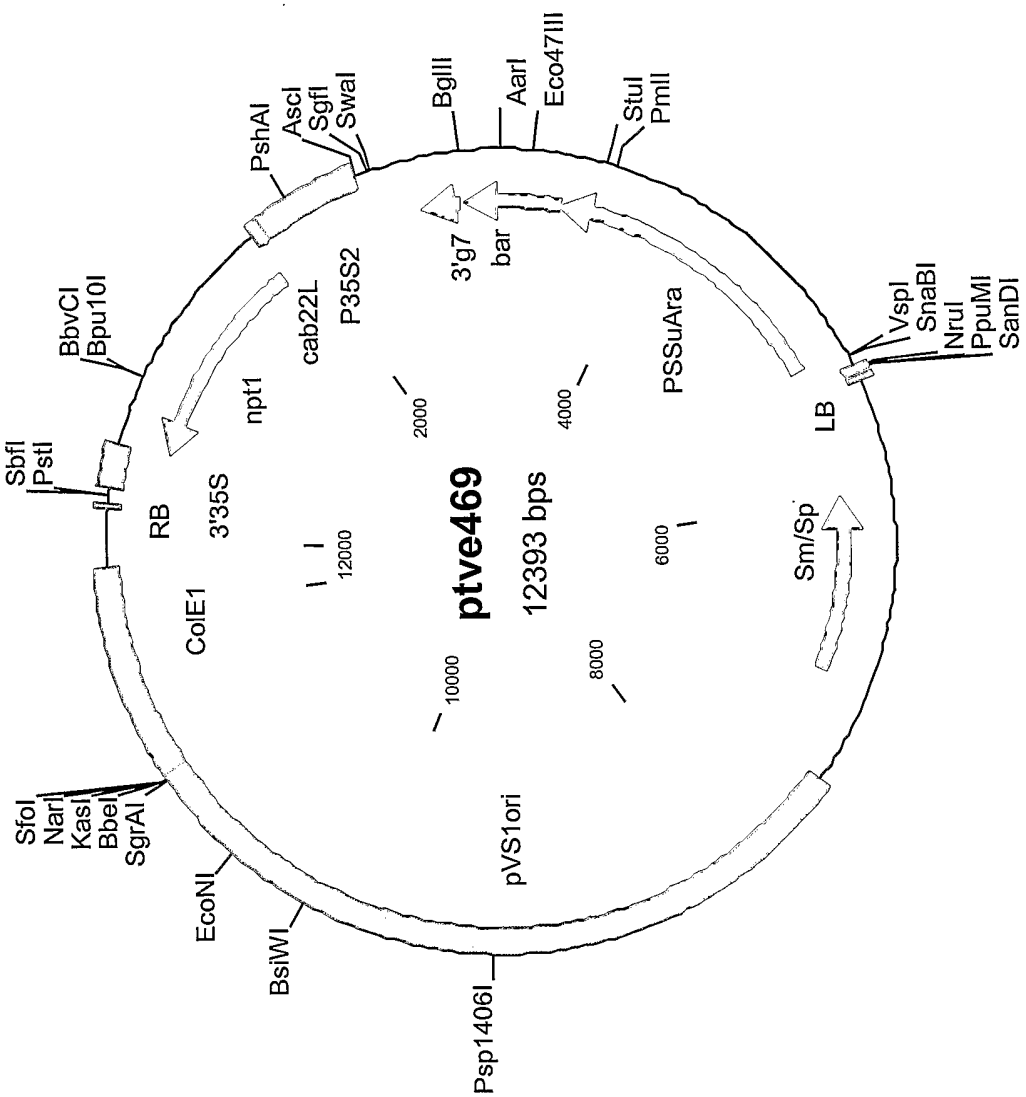
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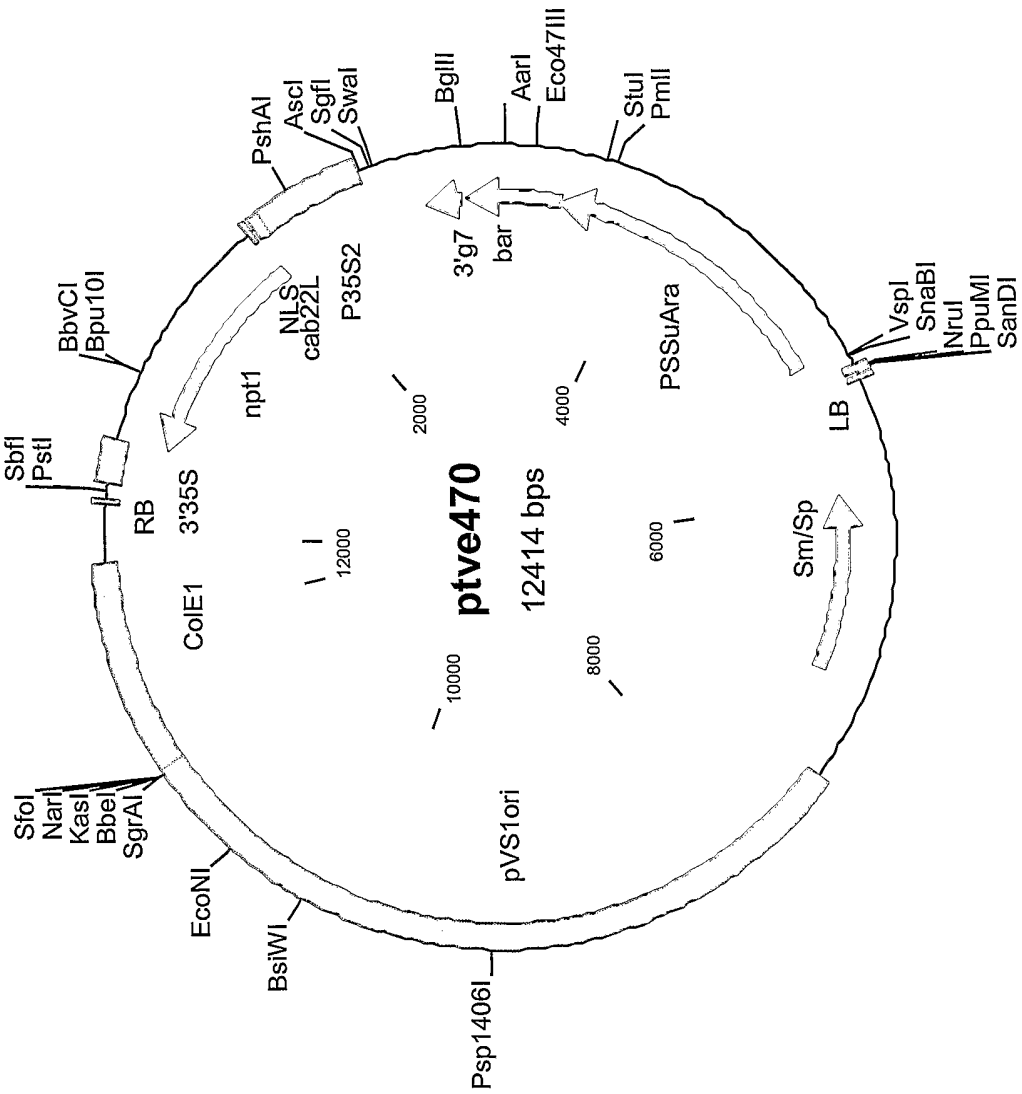
Fig. 4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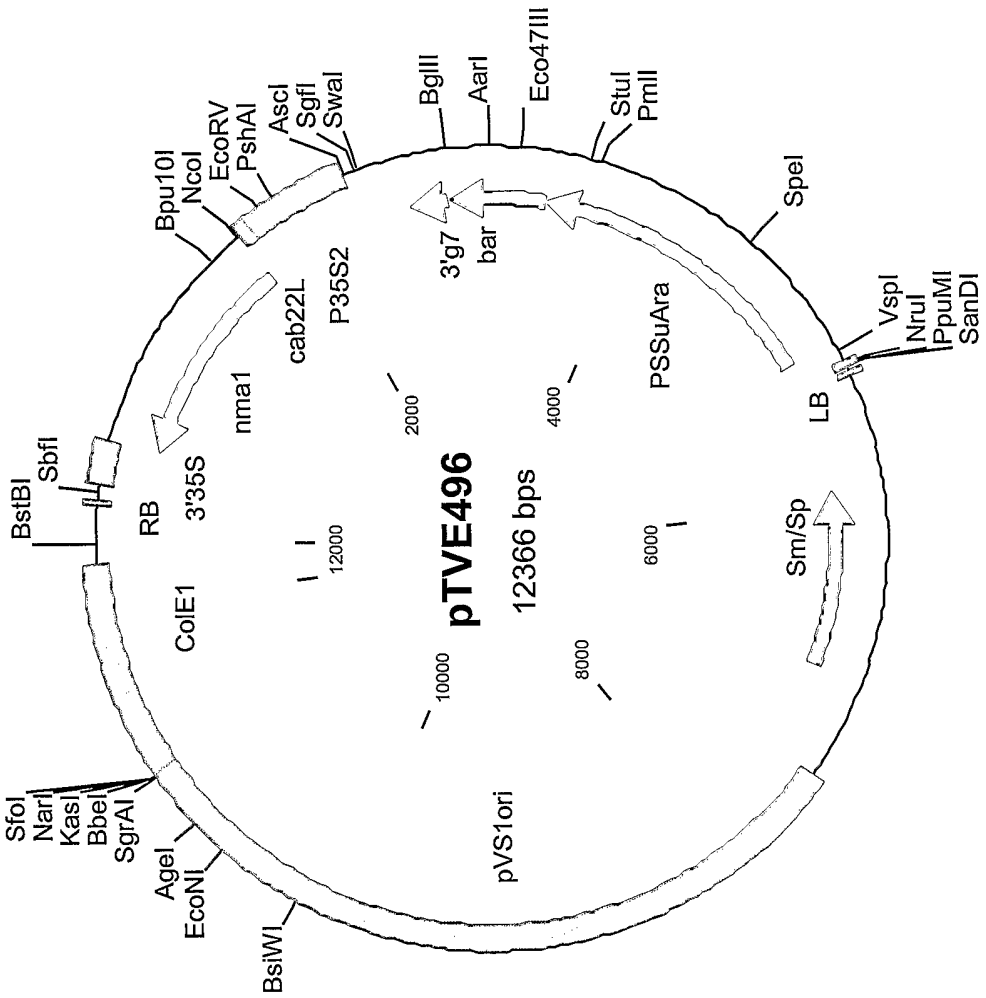
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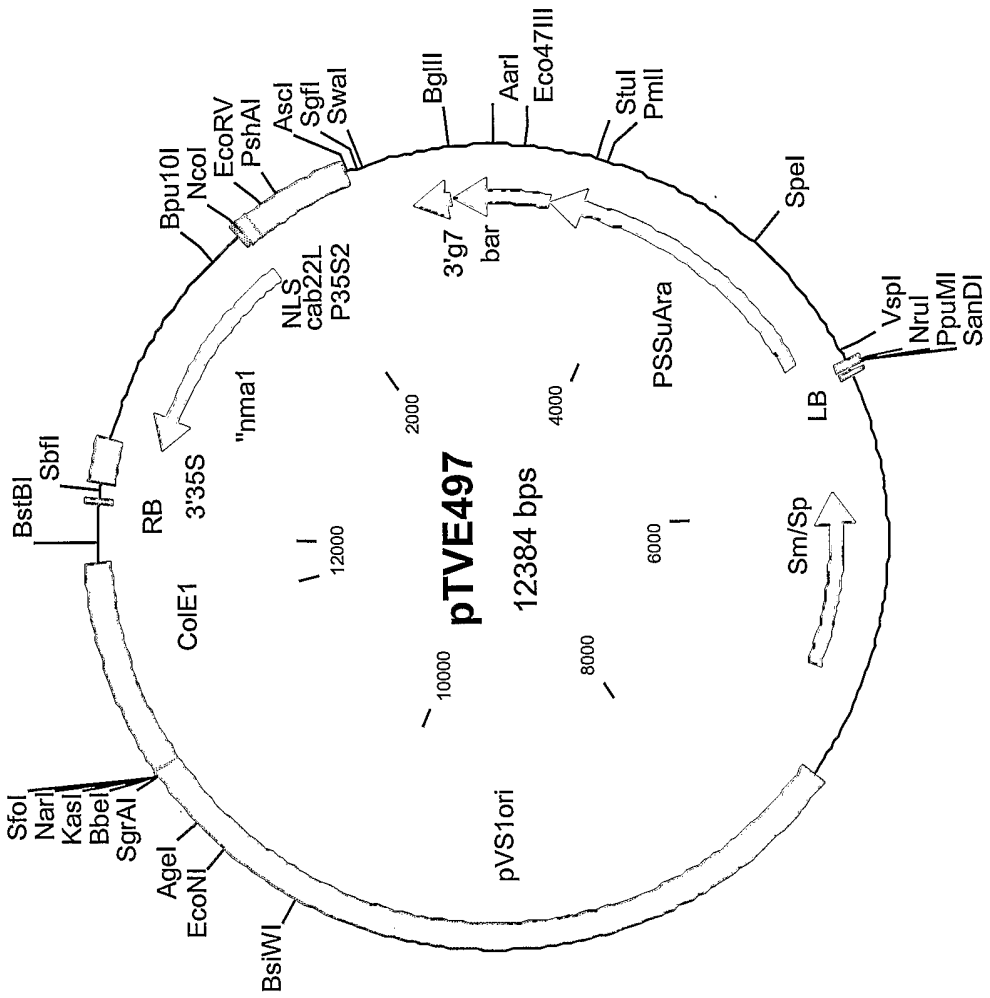
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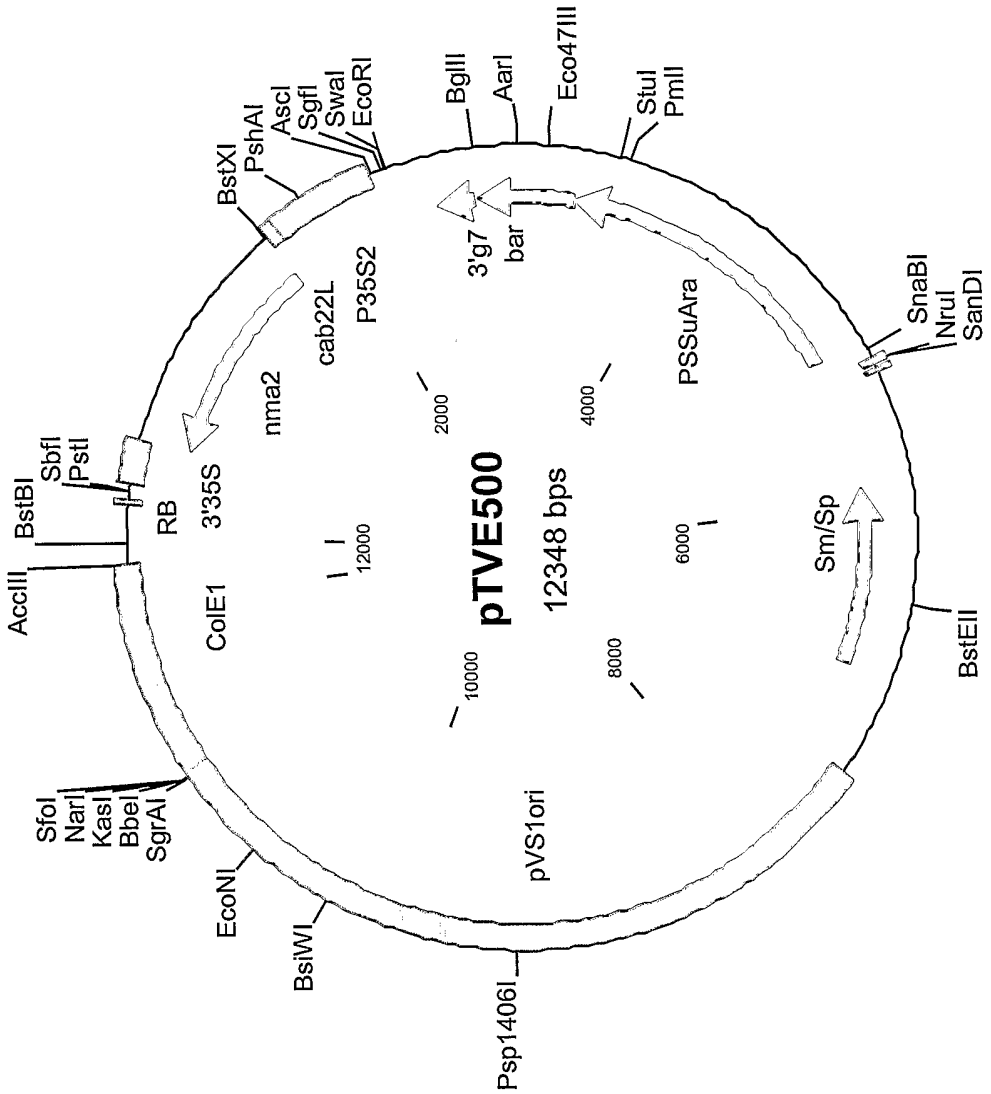
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Fig. 8

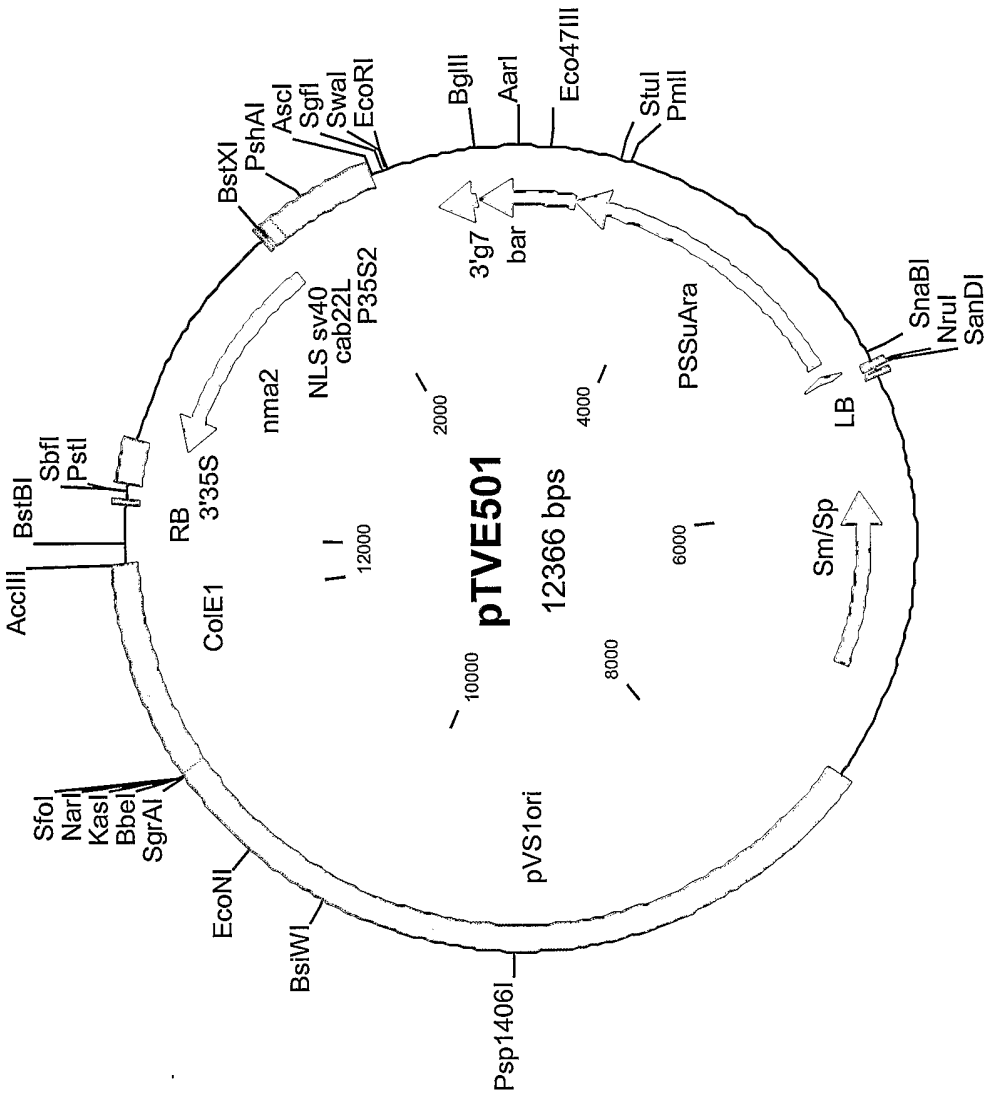
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Fig. 9

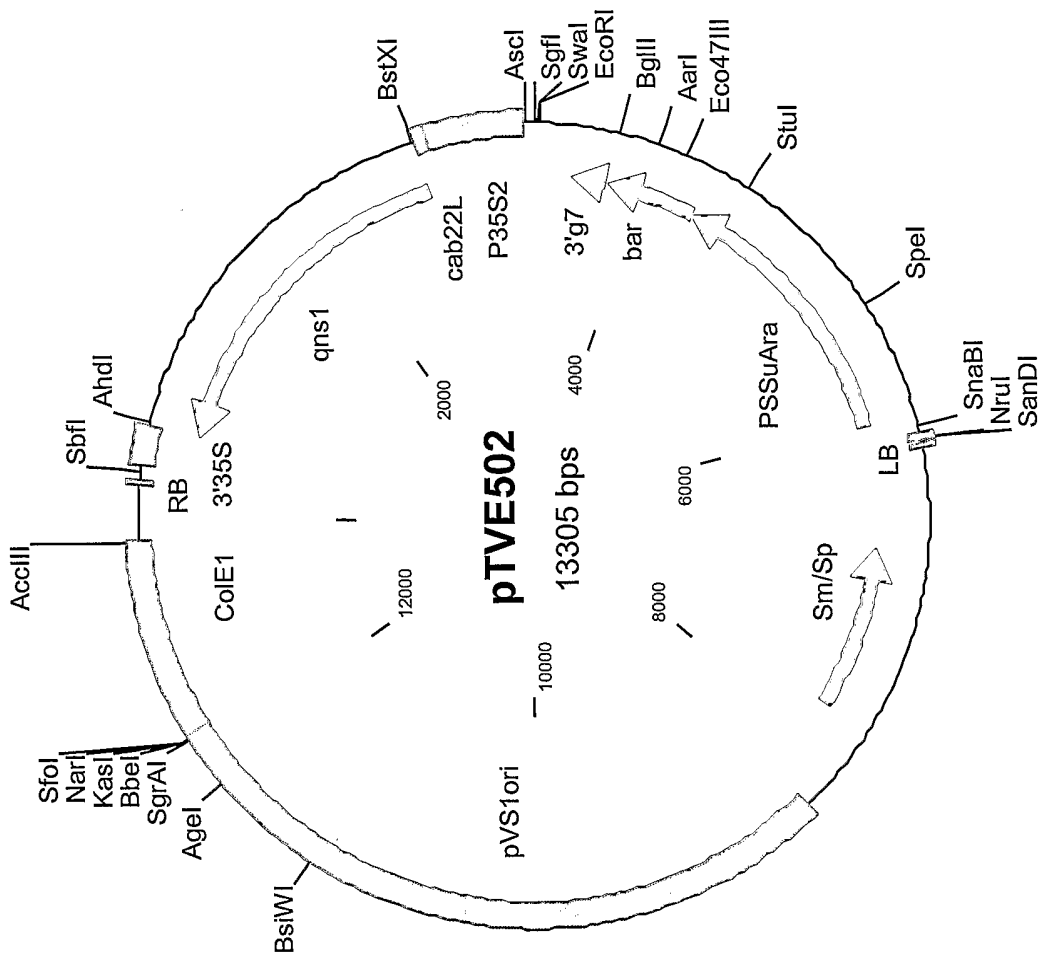
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Fig. 10

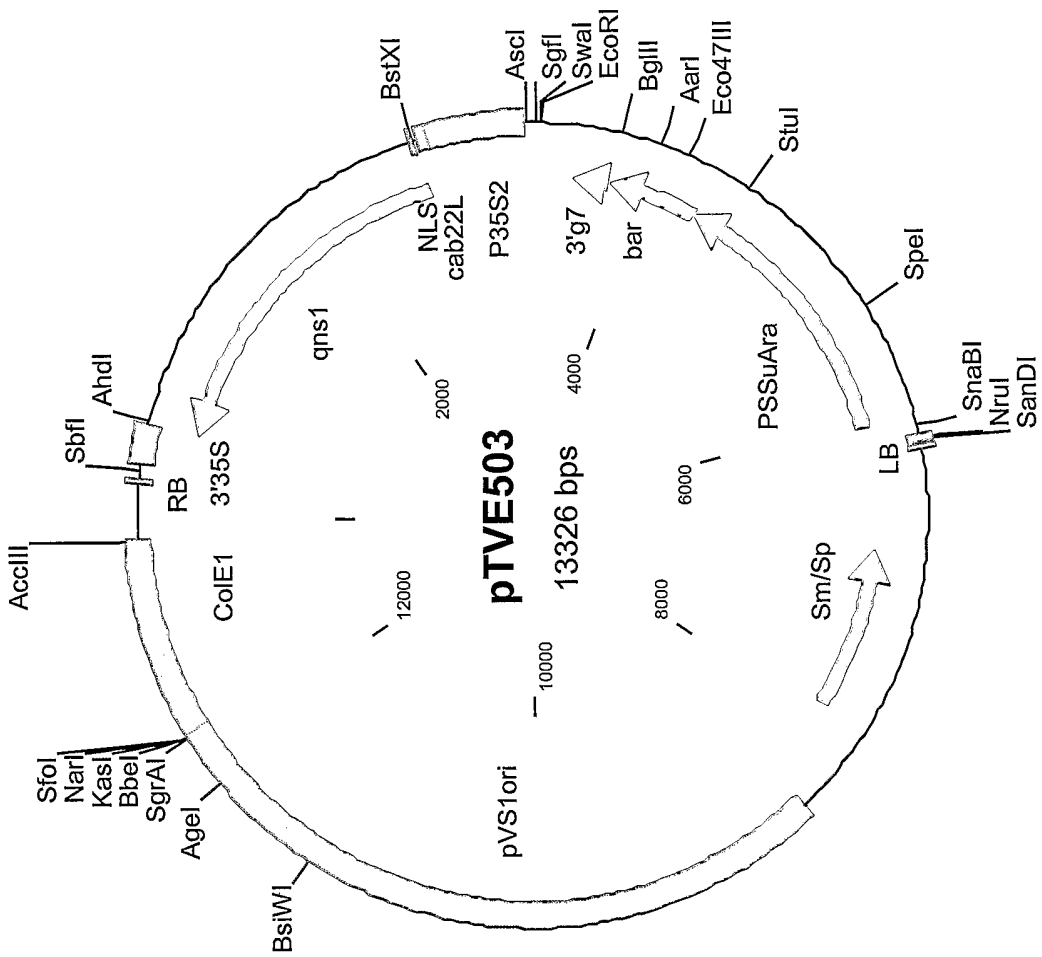
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Fig. 11

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aatggcttcc ttattgaagg tcaattggga cgacctgttg gtatatttat aagtaactgt 1200
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<210> 4

<211> 429

<212> PRT

<213> Saccharomyces cerevisiae

<400> 4
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20 25 30
Thr Tyr Lys Tyr Thr Asn Arg Ser Ser Gln Leu Thr Phe Asn Lys Glu
35 40 45
Ala Ile Asn Trp Leu Lys Glu Gln Phe Ser Tyr Leu Gly Asn Leu Arg
50 55 60
Phe Thr Glu Glu Glu Ile Glu Tyr Leu Lys Gln Glu Ile Pro Tyr Leu
65 70 75 80
Pro Ser Ala Tyr Ile Lys Tyr Ile Ser Ser Ser Asn Tyr Lys Leu His
85 90 95
Pro Glu Glu Gln Ile Ser Phe Thr Ser Glu Glu Ile Glu Gly Lys Pro
100 105 110
Thr His Tyr Lys Leu Lys Ile Leu Val Ser Gly Ser Trp Lys Asp Thr
115 120 125
Ile Leu Tyr Glu Ile Pro Leu Leu Ser Leu Ile Ser Glu Ala Tyr Phe
130 135 140
Lys Phe Val Asp Ile Asp Trp Asp Tyr Glu Asn Gln Leu Glu Gln Ala
145 150 155 160

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Glu Lys Lys Ala Glu Thr Leu Phe Asp Asn Gly Ile Arg Phe Ser Glu
                165                 170                 175
Phe Gly Thr Arg Arg Arg Arg Ser Leu Lys Ala Gln Asp Leu Ile Met
            180                 185                 190
Gln Gly Ile Met Lys Ala Val Asn Gly Asn Pro Asp Arg Asn Lys Ser
        195                 200                 205
Leu Leu Leu Gly Thr Ser Asn Ile Leu Phe Ala Lys Lys Tyr Gly Val
    210                 215                 220
Lys Pro Ile Gly Thr Val Ala His Glu Trp Val Met Gly Val Ala Ser
225                 230                 235                 240
Ile Ser Glu Asp Tyr Leu His Ala Asn Lys Asn Ala Met Asp Cys Trp
                245                 250                 255
Ile Asn Thr Phe Gly Ala Lys Asn Ala Gly Leu Ala Leu Thr Asp Thr
            260                 265                 270
Phe Gly Thr Asp Asp Phe Leu Lys Ser Phe Arg Pro Pro Tyr Ser Asp
        275                 280                 285
Ala Tyr Val Gly Val Arg Gln Asp Ser Gly Asp Pro Val Glu Tyr Thr
    290                 295                 300
Lys Lys Ile Ser His His Tyr His Asp Val Leu Lys Leu Pro Lys Phe
305                 310                 315                 320
Ser Lys Ile Ile Cys Tyr Ser Asp Ser Leu Asn Val Glu Lys Ala Ile
                325                 330                 335
Thr Tyr Ser His Ala Ala Lys Glu Asn Gly Met Leu Ala Thr Phe Gly
            340                 345                 350
Ile Gly Thr Asn Phe Thr Asn Asp Phe Arg Lys Lys Ser Glu Pro Gln
        355                 360                 365
Val Lys Ser Glu Pro Leu Asn Ile Val Ile Lys Leu Leu Glu Val Asn
    370                 375                 380
Gly Asn His Ala Ile Lys Ile Ser Asp Asn Leu Gly Lys Asn Met Gly
385                 390                 395                 400
Asp Pro Ala Thr Val Lys Arg Val Lys Glu Glu Leu Gly Tyr Thr Glu
                405                 410                 415
Arg Ser Trp Ser Gly Asp Asn Glu Ala His Arg Trp Thr
            420                 425
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<210> 5

<211> 1206

<212> DNA

<213> Saccharomyces cerevisiae

<400> 5

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gatgcgaatt cctctataga tgcacctttt aatattaaga ggaagaaaaa gcatcctaag    180
catcatcatc accatcatca cagtcgtaaa gaaggcaatg ataaaaaaca tcagcatatt    240
ccattgaacc aagacgactt tcaaccactt tccgcagaag tgtcttccga agatgatgac    300
gcggatttta gatccaagga gagatacggt tcagattcaa ccacagaatc agaaactaga    360
ggtgttcaga aatatcagat tgctgattta gaagaagttc cacatggaat cgttcgtcaa    420
gcaagaacct tggaagacta cgaattcccc tcacacagat tatcgaaaaa attactggat    480
ccaaataaac tgccgttagt aatagtagca tgtgggtctt tttcaccaat cacctacttg    540
catctaagaa tgtttgaaat ggctttagat gcaatctctg aacaaacaag gtttgaagtc    600
ataggtggat attactcccc tgttagtgat aactatcaaa agcaaggctt ggccccatcc    660
taccatagag tacgtatgtg tgaattggcc tgcgaaagaa cctcatcttg gttgatggtg    720
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gatgcatggg agtcattgca accttcatac acaagaactg ccaaggtctt ggatcatttc    780
aatcacgaaa tcaatattaa gagaggtggt gtagctactg ttactggaga aaaaattggt    840
gtgaaaataa tgttgctggc tggtggtgac ctaatagagt caatgggtga accaaacgtt    900
tgggcggacg ccgatttaca tcacattctc ggtaattacg gttgtttgat tgtcgaacgt    960
actggttctg atgtaaggtc ttttttgtta tcccatgata ttatgtatga acatagaagg   1020
aatattctta tcatcaagca actcatctat aatgatattt cttccacgaa agttcgtcta   1080
tttatcagac gcgccatgtc tgtacaatat ttgttaccta attcggtcat caggtatatc   1140
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gaatga                                                              1206
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<210> 6

<211> 401

<212> PRT

<213> Saccharomyces cerevisiae

<400> 6

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

```
Gly Asp Leu Ile Glu Ser Met Gly Glu Pro Asn Val Trp Ala Asp Ala
    290                 295                 300
Asp Leu His His Ile Leu Gly Asn Tyr Gly Cys Leu Ile Val Glu Arg
305                 310                 315                 320
Thr Gly Ser Asp Val Arg Ser Phe Leu Leu Ser His Asp Ile Met Tyr
                325                 330                 335
Glu His Arg Arg Asn Ile Leu Ile Ile Lys Gln Leu Ile Tyr Asn Asp
            340                 345                 350
Ile Ser Ser Thr Lys Val Arg Leu Phe Ile Arg Arg Ala Met Ser Val
        355                 360                 365
Gln Tyr Leu Leu Pro Asn Ser Val Ile Arg Tyr Ile Gln Glu His Arg
    370                 375                 380
Leu Tyr Val Asp Gln Thr Glu Pro Val Lys Gln Val Leu Gly Asn Lys
385                 390                 395                 400
Glu
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<210> 7

<211> 1188

<212> DNA

<213> Saccharomyces cerevisiae

<400> 7

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ccgccagatc caacacatac gataccaaaa tctggaccca tagttccata tgttttagct    120
gattataatt cttcgatcga tgctcctttc aatctcgaca tttacaaaac cctgtcgtca    180
aggaaaaaaa acgccaactc aagcaaccga atggaccata ttccattaaa tactagtgac    240
ttccagccac tatctcggga tgtatcatcg gaggaggaaa gtgaagggca atcgaatgga    300
attgacgcta ctctacagga tgttacgatg actgggaatt tgggggtact gaagagccaa    360
attgctgatt tggaagaagt tcctcacaca attgtaagac aagccagaac tattgaagat    420
tacgaatttc ctgtacacag attgacgaaa aagttacaag atcctgaaaa actgcctctg    480
atcatcgttg cttgtggatc attttctccc ataacatacc tacatttgag aatgtttgaa    540
atggctttag atgatatcaa tgagcaaacg cgttttgaag tggttggtgg ttatttttct    600
ccagtaagtg ataactatca aaagcgaggg ttagccccag cttatcatcg tgtccgcatg    660
tgcgaattag catgcgagcg gacatcatct tggttaatgg ttgatgcctg ggaatcttta    720
caatcaagtt atacaaggac agcaaaagtc ttggaccatt tcaatcatga aataaatatc    780
aagagaggtg gaatcatgac tgtagatggt gaaaaaatgg gcgtaaaaat catgttattg    840
gcaggcggtg atcttatcga atccatgggc gagcctcatg tgtgggctga ttcagacctg    900
caccatattt tgggtaatta tggatgtttg atcgtggaaa ggactggttc tgatgttagg    960
tccttcttgc tttcccatga tatcatgtat gaacacagaa gaaatatcct tattatcaaa   1020
caacttattt acaatgatat ttcctctacg aaagtgcggc ttttcatcag acgtggaatg   1080
tcagttcaat atcttcttcc aaactctgtc atccgttaca tccaagagta taatctatac   1140
attaatcaaa gtgaaccggt caagcaggtc ttggatagca aagagtga                1188
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<210> 8

<211> 395

<212> PRT

<213> Saccharomyces cerevisiae

<400> 8

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Glu Leu Gln Pro Pro Pro Asp Pro Thr His Thr Ile Pro Lys Ser Gly
            20                  25                  30
Pro Ile Val Pro Tyr Val Leu Ala Asp Tyr Asn Ser Ser Ile Asp Ala
        35                  40                  45
Pro Phe Asn Leu Asp Ile Tyr Lys Thr Leu Ser Ser Arg Lys Lys Asn
    50                  55                  60
Ala Asn Ser Ser Asn Arg Met Asp His Ile Pro Leu Asn Thr Ser Asp
65                  70                  75                  80
Phe Gln Pro Leu Ser Arg Asp Val Ser Ser Glu Glu Glu Ser Glu Gly
                85                  90                  95
Gln Ser Asn Gly Ile Asp Ala Thr Leu Gln Asp Val Thr Met Thr Gly
            100                 105                 110


Asn Leu Gly Val Leu Lys Ser Gln Ile Ala Asp Leu Glu Glu Val Pro
        115                 120                 125
His Thr Ile Val Arg Gln Ala Arg Thr Ile Glu Asp Tyr Glu Phe Pro
    130                 135                 140
Val His Arg Leu Thr Lys Lys Leu Gln Asp Pro Glu Lys Leu Pro Leu
145                 150                 155                 160
Ile Ile Val Ala Cys Gly Ser Phe Ser Pro Ile Thr Tyr Leu His Leu
                165                 170                 175
Arg Met Phe Glu Met Ala Leu Asp Asp Ile Asn Glu Gln Thr Arg Phe
            180                 185                 190
Glu Val Val Gly Gly Tyr Phe Ser Pro Val Ser Asp Asn Tyr Gln Lys
        195                 200                 205
Arg Gly Leu Ala Pro Ala Tyr His Arg Val Arg Met Cys Glu Leu Ala
    210                 215                 220
Cys Glu Arg Thr Ser Ser Trp Leu Met Val Asp Ala Trp Glu Ser Leu
225                 230                 235                 240
Gln Ser Ser Tyr Thr Arg Thr Ala Lys Val Leu Asp His Phe Asn His
                245                 250                 255
Glu Ile Asn Ile Lys Arg Gly Gly Ile Met Thr Val Asp Gly Glu Lys
            260                 265                 270
Met Gly Val Lys Ile Met Leu Leu Ala Gly Gly Asp Leu Ile Glu Ser
        275                 280                 285
Met Gly Glu Pro His Val Trp Ala Asp Ser Asp Leu His His Ile Leu
    290                 295                 300
Gly Asn Tyr Gly Cys Leu Ile Val Glu Arg Thr Gly Ser Asp Val Arg
305                 310                 315                 320
Ser Phe Leu Leu Ser His Asp Ile Met Tyr Glu His Arg Arg Asn Ile
                325                 330                 335
Leu Ile Ile Lys Gln Leu Ile Tyr Asn Asp Ile Ser Ser Thr Lys Val
            340                 345                 350
Arg Leu Phe Ile Arg Arg Gly Met Ser Val Gln Tyr Leu Leu Pro Asn
        355                 360                 365
Ser Val Ile Arg Tyr Ile Gln Glu Tyr Asn Leu Tyr Ile Asn Gln Ser
    370                 375                 380
Glu Pro Val Lys Gln Val Leu Asp Ser Lys Glu
385                 390                 395
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<210> 9

<211> 2145

<212> DNA

<213> Saccharomyces cerevisiae

<400> 9

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cgtgtcggcc cagaactgga aataactggc tacggatgtt tagatcattt tttagaaaat 180
gacgtttgcc ttcattcatg ggaaatgtat gctcaaatca ttaagaataa agaaacccat 240
ggattaatac ttgacattgg tatgcccgtt ctacacaaga atgttcgtta taattgtcgt 300
ttgttatcct tggatggtga gatattgttc ataagaccta agatttggtt agctaatgat 360
ggtaactata gggaaatgag atttttcaca ccttggatga aacctggcgt ggtggaggac 420
tttatccttc cacctgagat tcagaaagtt accggccaga gacttgtgcc atttggggac 480
gctgtgataa attcattgga tacatgcatt ggtacagaaa cttgtgaaga attgtttaca 540
cctcaatccc cccacatcgc catgtcttta gatggtgtgg aaatcatgac aaactcatct 600
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aaacgttgtg gtggtgttta cttgtatgca aatcaaagag gttgtgatgg tgacagatta 720
tattatgatg gctgtgcact aattgccatc aatggtacaa ttgtagccca aggttcacaa 780
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aatgcaattg gatcttacca cgtggattta aagatggact cattggtatc cagtgtggtg 1380
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atgttcttaa aacttcttca tcaatggtcc ccaaagttaa cacctcgtca aatatctgaa 1920
aaggtgaaaa gatttttctt cttctatgcc atcaacagac acaagcaaac tgttttaact 1980
cctagttatc atgctgaaca gtattcacca gaagacaaca gatttgactt acgtcctttc 2040
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<210> 10

<211> 714

<212> PRT

<213> Saccharomyces cerevisiae

<400> 10

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            20                  25                  30
Ala Lys Glu Arg Gly Ala Arg Leu Arg Val Gly Pro Glu Leu Glu Ile
        35                  40                  45
Thr Gly Tyr Gly Cys Leu Asp His Phe Leu Glu Asn Asp Val Cys Leu
    50                  55                  60
His Ser Trp Glu Met Tyr Ala Gln Ile Ile Lys Asn Lys Glu Thr His
65                  70                  75                  80
Gly Leu Ile Leu Asp Ile Gly Met Pro Val Leu His Lys Asn Val Arg
                85                  90                  95
Tyr Asn Cys Arg Leu Leu Ser Leu Asp Gly Glu Ile Leu Phe Ile Arg
            100                 105                 110
Pro Lys Ile Trp Leu Ala Asn Asp Gly Asn Tyr Arg Glu Met Arg Phe
        115                 120                 125
Phe Thr Pro Trp Met Lys Pro Gly Val Val Glu Asp Phe Ile Leu Pro
    130                 135                 140
Pro Glu Ile Gln Lys Val Thr Gly Gln Arg Leu Val Pro Phe Gly Asp
145                 150                 155                 160
Ala Val Ile Asn Ser Leu Asp Thr Cys Ile Gly Thr Glu Thr Cys Glu
                165                 170                 175
Glu Leu Phe Thr Pro Gln Ser Pro His Ile Ala Met Ser Leu Asp Gly
            180                 185                 190
Val Glu Ile Met Thr Asn Ser Ser Gly Ser His His Glu Leu Arg Lys
        195                 200                 205
Leu Asn Lys Arg Leu Asp Leu Ile Leu Asn Ala Thr Lys Arg Cys Gly
    210                 215                 220
Gly Val Tyr Leu Tyr Ala Asn Gln Arg Gly Cys Asp Gly Asp Arg Leu
225                 230                 235                 240
Tyr Tyr Asp Gly Cys Ala Leu Ile Ala Ile Asn Gly Thr Ile Val Ala
                245                 250                 255
Gln Gly Ser Gln Phe Ser Leu Asp Asp Val Glu Val Val Thr Ala Thr
            260                 265                 270
Val Asp Leu Glu Glu Val Arg Ser Tyr Arg Ala Ala Val Met Ser Arg
        275                 280                 285
Gly Leu Gln Ala Ser Leu Ala Glu Ile Lys Phe Lys Arg Ile Asp Ile
    290                 295                 300
Pro Val Glu Leu Ala Leu Met Thr Ser Arg Phe Asp Pro Thr Val Cys
305                 310                 315                 320
Pro Thr Lys Val Arg Glu Pro Phe Tyr His Ser Pro Glu Glu Glu Ile
                325                 330                 335
Ala Leu Gly Pro Ala Cys Trp Met Trp Asp Tyr Leu Arg Arg Cys Asn
            340                 345                 350
Gly Thr Gly Phe Phe Leu Pro Leu Ser Gly Gly Ile Asp Ser Cys Ala
        355                 360                 365
Thr Ala Met Ile Val His Ser Met Cys Arg Leu Val Thr Asp Ala Ala
    370                 375                 380
Gln Asn Gly Asn Glu Gln Val Ile Lys Asp Val Arg Lys Ile Thr Arg
385                 390                 395                 400
Ser Gly Asp Asp Trp Ile Pro Asp Ser Pro Gln Asp Leu Ala Ser Lys
                405                 410                 415
Ile Phe His Ser Cys Phe Met Gly Thr Glu Asn Ser Ser Lys Glu Thr
            420                 425                 430
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Arg Asn Arg Ala Lys Asp Leu Ser Asn Ala Ile Gly Ser Tyr His Val
        435                 440                 445
Asp Leu Lys Met Asp Ser Leu Val Ser Ser Val Val Ser Leu Phe Glu
    450                 455                 460
Val Ala Thr Gly Lys Lys Pro Ile Tyr Lys Ile Phe Gly Gly Ser Gln
465                 470                 475                 480
Ile Glu Asn Leu Ala Leu Gln Asn Ile Gln Ala Arg Leu Arg Met Val
                485                 490                 495
Leu Ser Tyr Leu Phe Ala Gln Leu Leu Pro Trp Val Arg Gly Ile Pro
            500                 505                 510
Asn Ser Gly Gly Leu Leu Val Leu Gly Ser Ala Asn Val Asp Glu Cys
        515                 520                 525
Leu Arg Gly Tyr Leu Thr Lys Tyr Asp Cys Ser Ser Ala Asp Ile Asn
    530                 535                 540
Pro Ile Gly Gly Ile Ser Lys Thr Asp Leu Lys Arg Phe Ile Ala Tyr
545                 550                 555                 560
Ala Ser Lys Gln Tyr Asn Met Pro Ile Leu Asn Asp Phe Leu Asn Ala
                565                 570                 575
Thr Pro Thr Ala Glu Leu Glu Pro Met Thr Lys Asp Tyr Val Gln Ser
            580                 585                 590
Asp Glu Ile Asp Met Gly Met Thr Tyr Glu Glu Leu Gly Val Phe Gly
        595                 600                 605
Tyr Leu Arg Lys Val Glu Lys Cys Gly Pro Tyr Ser Met Phe Leu Lys
    610                 615                 620
Leu Leu His Gln Trp Ser Pro Lys Leu Thr Pro Arg Gln Ile Ser Glu
625                 630                 635                 640
Lys Val Lys Arg Phe Phe Phe Phe Tyr Ala Ile Asn Arg His Lys Gln
                645                 650                 655
Thr Val Leu Thr Pro Ser Tyr His Ala Glu Gln Tyr Ser Pro Glu Asp
            660                 665                 670
Asn Arg Phe Asp Leu Arg Pro Phe Leu Ile Asn Pro Arg Phe Pro Trp
        675                 680                 685
Ala Ser Arg Lys Ile Asp Glu Val Val Glu Gln Cys Glu Ala His Lys
    690                 695                 700
Gly Ser Thr Leu Asp Ile Met Ser Ile Asp
705                 710
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<210> 11

<211> 597

<212> DNA

<213> Arabidopsis thaliana

<400> 11

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aacaacgttc tcaccaccat cgacatctgc cgacgcgcct cagtccccgt attctttacg    180
cgtcacaacc acaaatcccc gaccgaccac ggcatgctcg gcgagtggtg taacggcgat    240
gtaatccttg acggaaccac cgattctgaa atcatccagg agatacaagg ccaagtaacc    300
ggaccagacg agatggtgga gaagaacacg tacagtgcgt ttaacaaaac ccgcctccag    360
gaaaacctgg aaaagatcgg agtaaaggag gtgatcgtga tcggagtgat gacgaacttg    420
tgctgtgaga caacggcgcg tgaagcgttt attaagggtt ttagggtttt tttctcgacg    480
gacgcgactg cgacgtttaa tgaggagctt cacgaggcta cgctaatgaa tctcgctttt    540
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ggcttcgctt atctcgtcga ttgcgataaa ctccggcgaa gtctactcgg taactaa 597

<210> 12

<211> 198

<212> PRT

<213> Arabidopsis thaliana

<400> 12

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Asn Ser Lys Ile Ala Ala Leu Leu Val Ile Asp Met Gln Asn His Phe
            20                  25                  30
Ser Ser Met Ala Lys Pro Ile Leu Asn Asn Val Leu Thr Thr Ile Asp
        35                  40                  45
Ile Cys Arg Arg Ala Ser Val Pro Val Phe Phe Thr Arg His Asn His
    50                  55                  60
Lys Ser Pro Thr Asp His Gly Met Leu Gly Glu Trp Cys Asn Gly Asp
65                  70                  75                  80
Val Ile Leu Asp Gly Thr Thr Asp Ser Glu Ile Ile Gln Glu Ile Gln
                85                  90                  95
Gly Gln Val Thr Gly Pro Asp Glu Met Val Glu Lys Asn Thr Tyr Ser
            100                 105                 110
Ala Phe Asn Lys Thr Arg Leu Gln Glu Asn Leu Glu Lys Ile Gly Val
        115                 120                 125
Lys Glu Val Ile Val Ile Gly Val Met Thr Asn Leu Cys Cys Glu Thr
    130                 135                 140
Thr Ala Arg Glu Ala Phe Ile Lys Gly Phe Arg Val Phe Phe Ser Thr
145                 150                 155                 160
Asp Ala Thr Ala Thr Phe Asn Glu Glu Leu His Glu Ala Thr Leu Met
                165                 170                 175
Asn Leu Ala Phe Gly Phe Ala Tyr Leu Val Asp Cys Asp Lys Leu Arg
            180                 185                 190
Arg Ser Leu Leu Gly Asn
        195
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<210> 13

<211> 597

<212> DNA

<213> Arabidopsis thaliana

<400> 13

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gcagctcttc tcgtcatcga tatgcagaat cacttctact ctatggctga accaatcctc   120
caaaacgctc tcaccaccat cgacatctgc cgacgcgctt caatccccgt attcttcacg   180
cgccacaacc acaaatcccc aaccgaccac ggcatgctcg gagagtggtg gaacggcgat   240
ctaatcctcg acggaaccac tgattccgaa atcatcccgg aaatcaatcg ccaggtcacc   300
ggaccagacg aaatcgtgga gaagagcacg tacagtgcgt ttaacaacac gcaccttcag   360
gagaagctgg acaagatcgg agtgaaggag gtgatcgtta tcggagtgat gacgaaccta   420
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tgctgtgaga cgacggcgcg tgaagcgttt gtaaaggggt ttagggtttt tttctcgacg 480
gacgcgactg cgacggttaa tgaagagctt cacgaggcta ctctaatgaa tctcgcgtat 540
ggctttgctt atctcgtcga ttgcgataga ctccggcgag gtctactcag tagttaa 597

<210> 14

<211> 198

<212> PRT

<213> Arabidopsis thaliana

<400> 14
Met Ala Ser Ser Ser Ser Arg Thr Tyr Glu Thr Arg Lys Arg Glu Pro
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20 25 30
Tyr Ser Met Ala Glu Pro Ile Leu Gln Asn Ala Leu Thr Thr Ile Asp
35 40 45
Ile Cys Arg Arg Ala Ser Ile Pro Val Phe Phe Thr Arg His Asn His
50 55 60
Lys Ser Pro Thr Asp His Gly Met Leu Gly Glu Trp Trp Asn Gly Asp
65 70 75 80
Leu Ile Leu Asp Gly Thr Thr Asp Ser Glu Ile Ile Pro Glu Ile Asn
85 90 95
Arg Gln Val Thr Gly Pro Asp Glu Ile Val Glu Lys Ser Thr Tyr Ser
100 105 110
Ala Phe Asn Asn Thr His Leu Gln Glu Lys Leu Asp Lys Ile Gly Val
115 120 125
Lys Glu Val Ile Val Ile Gly Val Met Thr Asn Leu Cys Cys Glu Thr
130 135 140
Thr Ala Arg Glu Ala Phe Val Lys Gly Phe Arg Val Phe Phe Ser Thr
145 150 155 160
Asp Ala Thr Ala Thr Val Asn Glu Glu Leu His Glu Ala Thr Leu Met
165 170 175
Asn Leu Ala Tyr Gly Phe Ala Tyr Leu Val Asp Cys Asp Arg Leu Arg
180 185 190
Arg Gly Leu Leu Ser Ser
195

<210> 15

<211> 591

<212> DNA

<213> Arabidopsis thaliana

<400> 15
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gtcgtcgaac tcgcgaggca gcgtggtatt ctcgtaattt gggttgttcg agaacatgat 180
cgtcaaggaa gagatgttga attattcagg cgccataact acagttctga gaaagtcggg 240
ccagttatta aaggcaccgt aggagcagaa ttggttgatg gattgatgat caacgaagaa 300

gatgactata agattgtgaa aactcgtttc agtgctttct ttagtaccaa tcttcattcc 360
ttcttgcaaa cttcaggggt taccaagtta gtgattgctg gtgtgcaaac gccgaactgt 420
atccggcaaa cggtgtttga tgcagtggcg ctggattatc ccaatgtgac tgttattaca 480
gatgccacag ctgctgcaac accagagatc catactgcga atattcttga catgaagaat 540
attggagtca agactcctac attacacgag tggtccgaag aacttgcttg a 591

<210> 16

<211> 196

<212> PRT

<213> Arabidopsis thaliana

<400> 16

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Asn	Asp	Phe	Ile	Glu	Glu	Gly	Ala	Val	Thr	Gln	Val	Lys	Gly	Gly	Lys
			20					25					30		
Ser	Ile	Val	Pro	Asn	Val	Ile	Arg	Val	Val	Glu	Leu	Ala	Arg	Gln	Arg
		35					40					45			
Gly	Ile	Leu	Val	Ile	Trp	Val	Val	Arg	Glu	His	Asp	Arg	Gln	Gly	Arg
	50					55					60				
Asp	Val	Glu	Leu	Phe	Arg	Arg	His	Asn	Tyr	Ser	Ser	Glu	Lys	Val	Gly
65					70					75					80
Pro	Val	Ile	Lys	Gly	Thr	Val	Gly	Ala	Glu	Leu	Val	Asp	Gly	Leu	Met
				85					90					95	
Ile	Asn	Glu	Glu	Asp	Asp	Tyr	Lys	Ile	Val	Lys	Thr	Arg	Phe	Ser	Ala
			100					105					110		
Phe	Phe	Ser	Thr	Asn	Leu	His	Ser	Phe	Leu	Gln	Thr	Ser	Gly	Val	Thr
		115					120					125			
Lys	Leu	Val	Ile	Ala	Gly	Val	Gln	Thr	Pro	Asn	Cys	Ile	Arg	Gln	Thr
	130					135					140				
Val	Phe	Asp	Ala	Val	Ala	Leu	Asp	Tyr	Pro	Asn	Val	Thr	Val	Ile	Thr
145					150					155					160
Asp	Ala	Thr	Ala	Ala	Ala	Thr	Pro	Glu	Ile	His	Thr	Ala	Asn	Ile	Leu
				165					170					175	
Asp	Met	Lys	Asn	Ile	Gly	Val	Lys	Thr	Pro	Thr	Leu	His	Glu	Trp	Ser
			180					185					190		
Glu	Glu	Leu	Ala												
		195													

<210> 17

<211> 1680

<212> DNA

<213> Arabidopsis thaliana

<400> 17
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actaacccga tggtcacacc tctgctcaac gatctttacc aattcaccat ggcttatgct 120

```
tattggaaag ctggcaaaca atctgagcga tctgtgtttg atctgtattt tcgtaagaat    180
ccttttggtg gagaatacac tatctttgct ggtttagaag aatgcatcaa atttctcgct    240
aatttcaatt tgactgatga agagatcgat ttcgttcgtg attcgttacc tggatgtgag    300
gaagctttct gtgattatct tcgagggctt gattgttctg acattgaagt gtatgccatt    360
tcggaaggat cagttgtttt tcctaaagtt cctttactca gaatcgaagg tcctgttgct    420
gtggtgcaat tgttggaaac tccattcctc aatctcatca attacgcatc tttggttgct    480
acaaatgcag caagacatcg gtttgttgca ggaaaatcta agcttctgct tgagtttggt    540
gctagaagag ctcagggacc cgatggtgca ataagcgcat caaagtattg ctaccttgga    600
ggttttgatg caacaagtaa tgttgcagcg ggaaaactgt ttgggatacc cctccgtggt    660
actcattccc atgcttttgt tagctcattc atgagccttg atgaaattgt tgacaaagtg    720
cttcgaagtt ctgatgggaa aagcacttgt aaggatttta tatgtttggt ccaaacttgc    780
ctaacaaaga ttcagaattc atcttcatta caaggaattt tttccgagac aaatcaaagc    840
gagcttgcag cgttcatttc atatgcactg gcattcccaa actcctttct cgctcttgta    900
gacacttatg atgtgatgaa gagtggtatt ccaaacttct gtgctgttgc tctagcactt    960
aatgaattgg gatacaaagc agtaggcatt agactggatt caggtgactt agcctatctt   1020
tctactgagg tcaggaaatt cttttgtgcc atagagagag acctcaaagt tcctgatttc   1080
gggaagatga tcgtcactgc tagtaacgat ctaaacgaag agacagtcga tgctctaaat   1140
aaacagggtc atgaagtaga tgcatttgga attggaacca acttagtgac ttgctatgcg   1200
caagctgcgt taggttgtgt tttcaaactt gtggaaataa acaatcagcc tcggatcaaa   1260
ctttctgaag atgttactaa ggtatcgatt ccatgtaaaa agcgtactta cagattgttc   1320
ggaaaagagg gttaccctct tgttgatata atgactggag agaacgaacc acctccaaag   1380
gtcggtgaaa ggttactttg ccgtcatcca ttcaatgaat caaaaagggc ttatgtggtt   1440
ccacaacgcg ttgaagagct tctgaaatgt tattggcgtg gcaatgcaga tgaagctagg   1500
gaagagctag agccattgaa agagctaaga aatcgttgca tcaaacagct cgaaaatatg   1560
cgacccgatc atatgagaag attaaaccct actccttata aggttagtgt cagcgccaag   1620
ttgtatgact tcatccactt cctctggctc aacgaagctc ctgtcggtga actgcattga   1680
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<210> 18

<211> 559

<212> PRT

<213> Arabidopsis thaliana

<400> 18

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Ile Asn Gly Pro Thr Asn Pro Met Val Thr Pro Leu Leu Asn Asp Leu
            20                  25                  30
Tyr Gln Phe Thr Met Ala Tyr Ala Tyr Trp Lys Ala Gly Lys Gln Ser
        35                  40                  45
Glu Arg Ser Val Phe Asp Leu Tyr Phe Arg Lys Asn Pro Phe Gly Gly
    50                  55                  60
Glu Tyr Thr Ile Phe Ala Gly Leu Glu Glu Cys Ile Lys Phe Leu Ala
65                  70                  75                  80
Asn Phe Asn Leu Thr Asp Glu Glu Ile Asp Phe Val Arg Asp Ser Leu
                85                  90                  95
Pro Gly Cys Glu Glu Ala Phe Cys Asp Tyr Leu Arg Gly Leu Asp Cys
            100                 105                 110
Ser Asp Ile Glu Val Tyr Ala Ile Ser Glu Gly Ser Val Val Phe Pro
        115                 120                 125
Lys Val Pro Leu Leu Arg Ile Glu Gly Pro Val Ala Val Val Gln Leu
    130                 135                 140
Leu Glu Thr Pro Phe Leu Asn Leu Ile Asn Tyr Ala Ser Leu Val Ala
```

```
145                 150                 155                 160
Thr Asn Ala Ala Arg His Arg Phe Val Ala Gly Lys Ser Lys Leu Leu
                165                 170                 175
Leu Glu Phe Gly Ala Arg Arg Ala Gln Gly Pro Asp Gly Ala Ile Ser
            180                 185                 190
Ala Ser Lys Tyr Cys Tyr Leu Gly Gly Phe Asp Ala Thr Ser Asn Val
        195                 200                 205
Ala Ala Gly Lys Leu Phe Gly Ile Pro Leu Arg Gly Thr His Ser His
    210                 215                 220
Ala Phe Val Ser Ser Phe Met Ser Leu Asp Glu Ile Val Asp Lys Val
225                 230                 235                 240
Leu Arg Ser Ser Asp Gly Lys Ser Thr Cys Lys Asp Phe Ile Cys Leu
                245                 250                 255
Val Gln Thr Cys Leu Thr Lys Ile Gln Asn Ser Ser Ser Leu Gln Gly
            260                 265                 270
Ile Phe Ser Glu Thr Asn Gln Ser Glu Leu Ala Ala Phe Ile Ser Tyr
        275                 280                 285
Ala Leu Ala Phe Pro Asn Ser Phe Leu Ala Leu Val Asp Thr Tyr Asp
    290                 295                 300
Val Met Lys Ser Gly Ile Pro Asn Phe Cys Ala Val Ala Leu Ala Leu
305                 310                 315                 320
Asn Glu Leu Gly Tyr Lys Ala Val Gly Ile Arg Leu Asp Ser Gly Asp
                325                 330                 335
Leu Ala Tyr Leu Ser Thr Glu Val Arg Lys Phe Phe Cys Ala Ile Glu
            340                 345                 350
Arg Asp Leu Lys Val Pro Asp Phe Gly Lys Met Ile Val Thr Ala Ser
        355                 360                 365
Asn Asp Leu Asn Glu Glu Thr Val Asp Ala Leu Asn Lys Gln Gly His
    370                 375                 380
Glu Val Asp Ala Phe Gly Ile Gly Thr Asn Leu Val Thr Cys Tyr Ala
385                 390                 395                 400
Gln Ala Ala Leu Gly Cys Val Phe Lys Leu Val Glu Ile Asn Asn Gln
                405                 410                 415
Pro Arg Ile Lys Leu Ser Glu Asp Val Thr Lys Val Ser Ile Pro Cys
            420                 425                 430
Lys Lys Arg Thr Tyr Arg Leu Phe Gly Lys Glu Gly Tyr Pro Leu Val
        435                 440                 445
Asp Ile Met Thr Gly Glu Asn Glu Pro Pro Pro Lys Val Gly Glu Arg
    450                 455                 460
Leu Leu Cys Arg His Pro Phe Asn Glu Ser Lys Arg Ala Tyr Val Val
465                 470                 475                 480
Pro Gln Arg Val Glu Glu Leu Leu Lys Cys Tyr Trp Arg Gly Asn Ala
                485                 490                 495
Asp Glu Ala Arg Glu Glu Leu Glu Pro Leu Lys Glu Leu Arg Asn Arg
            500                 505                 510
Cys Ile Lys Gln Leu Glu Asn Met Arg Pro Asp His Met Arg Arg Leu
        515                 520                 525
Asn Pro Thr Pro Tyr Lys Val Ser Val Ser Ala Lys Leu Tyr Asp Phe
    530                 535                 540
Ile His Phe Leu Trp Leu Asn Glu Ala Pro Val Gly Glu Leu His
545                 550                 555
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<210> 19

<211> 1674

<212> DNA

<213> Arabidopsis thaliana

<400> 19

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ccaatggtca cacctttact caatgatctt tatcaattca ccatggctta tgcttattgg    120
aaagctggca aacacaaaga acgatccgtt ttcgatctgt attttcgtaa gaacccattt    180
ggtggtgagt acactgtgtt tgctggatta gaagagtgtg ttaagttctt agccaatttc    240
aaattgactg atgaagaaat cgatttcgtt caagagtgtt tgcctggatc tgaggaagct    300
ttttgtgatt atcttagagg gcttgattgt tctgatgttg aagtttatgc aattccggaa    360
ggatcagttg tttttcctaa agtacctctc atgagagttg aaggacctgt tggtgttgtt    420
caattgttgg aaactccatt cctcaatctt gtcaattttg catctttggt agctactaac    480
gcagctaggc atcgctttgt tgccggaaaa tctaagagtc tactcgagtt tggtgctcga    540
agggctcagg gtccggatgg tgcaataagc gcatcaaaat attgctacct tggaggtttt    600
gatgcaacaa gtaatgtagc agctggaaaa ctttttggga ttcctcttcg tggaacacac    660
tctcatgctt atgttagctc attcatgagt actgatgaga ttgttgacaa agtacttcgt    720
agtgctgatg ggaaaaccac gtgcgaggat tttgttagtc atgttcagac atggttaaaa    780
aagattcagt attcaccatc tctaagtggc attttctctg agacaaatca aagcgagcta    840
gcagctttca cctcatatgc actggcattc cccaaaactt ttcttgccct cgtagataca    900
tacgatgtga tgaagagtgg aatccctaac ttctgtgcag ttgctttagc actcaatgac    960
tttggatata aagcattagg tattagactg gattcaggtg atttagctta tctatctaga   1020
gaggccagaa atttcttctg cacggtagag agagaactaa aagtgcctgg ttttgggaag   1080
atggtcgtca ctgctagtaa tgatctaaat gaagagacga ttgacgcttt aaataaacag   1140
ggacatgagg tggatgcttt tggcatcggg acctacttgg tcacttgcta ttcacaagcg   1200
gccttaggtt gcgttttcaa acttgtggag ataaacaatc agcctcggat taaactttct   1260
gaagatgtta caaaggtatc aataccgtgt aaaaagcgaa gttacagatt atacggcaaa   1320
gaaggttacc ctctggtaga tataatgact ggagagaacg aaccacctcc aaaggttggt   1380
gagcgtttac tttgtcgtca cccattcaac gaatccaaaa gagcatatgt agtgccacaa   1440
cgtgtcgaag agctcctcaa atgttattgg cgtggaagtg cagatgaagc aagagaagta   1500
ttaccgcctt tgaaagagat aagagaccgt tgcatcaaac agctcgaaaa catgcgacct   1560
gatcatatga ggagattaaa cccaactcct tataaggtta gtgtaagcgc aaagctgtac   1620
gatttcatcc acttcttatg gctaaacgaa gcacctgttg gtgaattgca gtga          1674
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<210> 20

<211> 557

<212> PRT

<213> Arabidopsis thaliana

<400> 20

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Met Glu Pro Lys Glu Asn Gly Ser Glu Leu Gly Gln Lys Ile Ile Asp
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Gly Pro Thr Asn Pro Met Val Thr Pro Leu Leu Asn Asp Leu Tyr Gln
            20                  25                  30
Phe Thr Met Ala Tyr Ala Tyr Trp Lys Ala Gly Lys His Asn Glu Arg
        35                  40                  45
Ser Val Phe Asp Leu Tyr Phe Arg Lys Asn Pro Phe Gly Gly Glu Tyr
    50                  55                  60
Thr Val Phe Ala Gly Leu Glu Glu Cys Val Lys Phe Leu Ala Asn Phe
65                  70                  75                  80
```

```
Lys Leu Thr Asp Glu Glu Ile Asp Phe Val Gln Glu Cys Leu Pro Gly
                85                  90                  95
Ser Glu Glu Ala Phe Cys Asp Tyr Leu Arg Gly Leu Asp Cys Ser Asp
            100                 105                 110
Val Glu Val Tyr Ala Ile Pro Glu Gly Ser Val Val Phe Pro Lys Val
        115                 120                 125
Pro Leu Met Arg Val Glu Gly Pro Val Gly Val Val Gln Leu Leu Glu
    130                 135                 140
Thr Pro Phe Leu Asn Leu Val Asn Phe Ala Ser Leu Val Ala Thr Asn
145                 150                 155                 160
Ala Ala Arg His Arg Phe Val Ala Gly Lys Ser Lys Ser Leu Leu Glu
                165                 170                 175
Phe Gly Ala Arg Arg Ala Gln Gly Pro Asp Gly Ala Ile Ser Ala Ser
            180                 185                 190
Lys Tyr Cys Tyr Leu Gly Gly Phe Asp Ala Thr Ser Asn Val Ala Ala
        195                 200                 205
Gly Lys Leu Phe Gly Ile Pro Leu Arg Gly Thr His Ser His Ala Tyr
    210                 215                 220
Val Ser Ser Phe Met Ser Thr Asp Glu Ile Val Asp Lys Val Leu Arg
225                 230                 235                 240
Ser Ala Asp Gly Lys Thr Thr Cys Glu Asp Phe Val Ser His Val Gln
                245                 250                 255
Thr Trp Leu Lys Lys Ile Gln Tyr Ser Pro Ser Leu Ser Gly Ile Phe
            260                 265                 270
Ser Glu Thr Asn Gln Ser Glu Leu Ala Ala Phe Thr Ser Tyr Ala Leu
        275                 280                 285
Ala Phe Pro Lys Thr Phe Leu Ala Leu Val Asp Thr Tyr Asp Val Met
    290                 295                 300
Lys Ser Gly Ile Pro Asn Phe Cys Ala Val Ala Leu Ala Leu Asn Asp
305                 310                 315                 320
Phe Gly Tyr Lys Ala Leu Gly Ile Arg Leu Asp Ser Gly Asp Leu Ala
                325                 330                 335
Tyr Leu Ser Arg Glu Ala Arg Asn Phe Phe Cys Thr Val Glu Arg Glu
            340                 345                 350
Leu Lys Val Pro Gly Phe Gly Lys Met Val Val Thr Ala Ser Asn Asp
        355                 360                 365
Leu Asn Glu Glu Thr Ile Asp Ala Leu Asn Lys Gln Gly His Glu Val
    370                 375                 380
Asp Ala Phe Gly Ile Gly Thr Tyr Leu Val Thr Cys Tyr Ser Gln Ala
385                 390                 395                 400
Ala Leu Gly Cys Val Phe Lys Leu Val Glu Ile Asn Asn Gln Pro Arg
                405                 410                 415
Ile Lys Leu Ser Glu Asp Val Thr Lys Val Ser Ile Pro Cys Lys Lys
            420                 425                 430
Arg Ser Tyr Arg Leu Tyr Gly Lys Glu Gly Tyr Pro Leu Val Asp Ile
        435                 440                 445
Met Thr Gly Glu Asn Glu Pro Pro Pro Lys Val Gly Glu Arg Leu Leu
    450                 455                 460
Cys Arg His Pro Phe Asn Glu Ser Lys Arg Ala Tyr Val Val Pro Gln
465                 470                 475                 480
Arg Val Glu Glu Leu Leu Lys Cys Tyr Trp Arg Gly Ser Ala Asp Glu
                485                 490                 495
Ala Arg Glu Val Leu Pro Pro Leu Lys Glu Ile Arg Asp Arg Cys Ile
            500                 505                 510
Lys Gln Leu Glu Asn Met Arg Pro Asp His Met Arg Arg Leu Asn Pro
        515                 520                 525
Thr Pro Tyr Lys Val Ser Val Ser Ala Lys Leu Tyr Asp Phe Ile His
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```
    530                 535                 540
Phe Leu Trp Leu Asn Glu Ala Pro Val Gly Glu Leu Gln
545                 550                 555
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<210> 21

<211> 717

<212> DNA

<213> Arabidopsis thaliana

<400> 21

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atggatgtcc cgttaccagt cgagaaatta tcttatggat caaacactga ggacaaaact     60
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tttgagctgg cgagagatga attacgctca aaaggatttc atgttcttgg aggatatatg    180
tctcctgtta atgatgcata taagaagaag ggccttttat ctgcagaaca tcgtttagag    240
atgtgtaatg tatcatgtca aagctctgac tttgtaatgg ttgatccgtg ggaggcatct    300
caaagcaact accaacgaac tttgacggtt ttatcaaggg tcaagacttt cttaacaaca    360
aatcgacatg tacccgagga atctctcaaa gtcatgctac tatgtggctc ggatttactg    420
ctatctttct gcactcccgg tgtttggatc cctgaacagt taagaactat ttgcaaagat    480
tatggcattg tgtgcatccg tagagaagga caagatgttg aaaatatgat ctctggtgac    540
gaaatcttaa acgaaaactg tgctaacgtc aaaatcgttg acaatactgt tcctaatcaa    600
atcagttcga gtagattaag gcaatgcatt tcgcgagggt tatcggttaa atacttgact    660
gaagatggag taatagatta tatcagacaa catcaactat acactgagct cacatga       717
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<210> 22

<211> 238

<212> PRT

<213> Arabidopsis thaliana

<400> 22

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            20                  25                  30
Pro Thr Phe Met His Leu Arg Met Phe Glu Leu Ala Arg Asp Glu Leu
        35                  40                  45
Arg Ser Lys Gly Phe His Val Leu Gly Gly Tyr Met Ser Pro Val Asn
    50                  55                  60
Asp Ala Tyr Lys Lys Lys Gly Leu Leu Ser Ala Glu His Arg Leu Glu
65                  70                  75                  80
Met Cys Asn Val Ser Cys Gln Ser Ser Asp Phe Val Met Val Asp Pro
                85                  90                  95
Trp Glu Ala Ser Gln Ser Asn Tyr Gln Arg Thr Leu Thr Val Leu Ser
            100                 105                 110
Arg Val Lys Thr Phe Leu Thr Thr Asn Arg His Val Pro Glu Glu Ser
        115                 120                 125
Leu Lys Val Met Leu Leu Cys Gly Ser Asp Leu Leu Leu Ser Phe Cys
    130                 135                 140
```

```
Thr Pro Gly Val Trp Ile Pro Glu Gln Leu Arg Thr Ile Cys Lys Asp
145                 150                 155                 160
Tyr Gly Ile Val Cys Ile Arg Arg Glu Gly Gln Asp Val Glu Asn Met
                165                 170                 175
Ile Ser Gly Asp Glu Ile Leu Asn Glu Asn Cys Ala Asn Val Lys Ile
            180                 185                 190
Val Asp Asn Thr Val Pro Asn Gln Ile Ser Ser Ser Arg Leu Arg Gln
        195                 200                 205
Cys Ile Ser Arg Gly Leu Ser Val Lys Tyr Leu Thr Glu Asp Gly Val
    210                 215                 220
Ile Asp Tyr Ile Arg Gln His Gln Leu Tyr Thr Glu Leu Thr
225                 230                 235
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<210> 23

<211> 2178

<212> DNA

<213> Arabidopsis thaliana

<400> 23

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cttggacccg agctcgaggt cactggctat ggttgcgagg atcacttctt ggaactcgac    180
actgtcactc atgcgtggga gtgtttgaag gaattgctgc ttggtgattg gacggatgat    240
attttgtgca gcataggaat gcctgtgatt aaaggagcag agcgttataa ctgccaggtt    300
ctctgtatga acagaagaat catcatgatt cgaccgaaaa tgtggctcgc aaacgatgga    360
aactataggg agctacggtg gttcacagct tggaagcaga gagaagagct agaggaattt    420
cagctcccca ttgaaatttc agaggctttg gagcagaaat cagtcccttt tggttatggt    480
tacatccagt ttatcgacac ggctgttgca gctgaagtct gtgaggaact gtttagtcca    540
cttcctcctc atgccgagct cgcattgaat ggtgttgaag tatttatgaa tgcaagtggg    600
agtcatcacc aacttaggaa actagatatt cgtctgaatg cttttatggg ggctactcat    660
gctcgtggtg gggtgtatat gtacagtaat caacaaggat gcgatggtag ccgcttatac    720
tacgatggat gtgcatgtat tgttgtaaac gggaatgttg ttgctcaagg ctcacaattc    780
tcgttgagag acgttgaggt catcatttca caagtggatc ttgatgcggt tgctagcctt    840
cgtggatcta taagtagctt tcaggaacaa gcaagctgca aggttaaagt atcttcagta    900
gctgtgccct gtagacttac acagtccttc aacctgaaaa tgacactaag cagtccgaag    960
aagatcattt accactctcc acaagaagaa atagcctttg gtcccgcttg ctggatgtgg   1020
gactatttga gaagaagtgg cgcttcagga tttttgcttc ctctttctgg cggagcagac   1080
agctcctccg tggcagctat tgttggctgc atgtgccaac ttgttgttaa agagattgca   1140
aagggagatg agcaagtaaa agctgatgcg aaccgaattg ggaattatgc taatgggcag   1200
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