

TRANSFORMED CELL CO-EXPRESSING CYTOKININ RECEPTOR AND CYTOKININ
BIOSYNTHESIS ENZYME

BACKGROUND OF THE INVENTION

5 FIELD OF THE INVENTION

The present invention relates to a method for analyzing agonist-activity and antagonist-activity of an examinee to cytokinin receptor.

DESCRIPTION OF THE RELATED ART

10 Cytokinins are plant hormones relevant to cell division and differentiation of higher plants and are known as important physiologically active substances having functions of inducing division of cells of higher plants, differentiating callus or pith to stems and leaves, preventing ethiolating and defoliating,
15 preventing dropping of fruits, breaking dominance of apical buds, and the like [Cytokinins: Chemistry, Activity, and Function, CRC press (1994)]. In recent years, it has been found that the key enzyme of cytokinin biosynthesis of plants is isopentenyltransferase and that this enzyme transfers an
20 isopentenyl group of dimethylallyl diphosphate to adenosine-5'-triphosphate and/or adenosine-5'-diphosphate [Plant & Cell Physiol. (2001) 42, 677-685; J. Biol. Chem. (2001) 276, 26405-26410; and WO2002072818A1].

Substances having the activity of controlling
25 biosynthesis of cytokinins can be used as plant growth regulators,

for example, agents for preventing the dropping of fruits such as apples and oranges, agents for preventing plants such as rice plants and wheat from falling down by regulating the height of such plants, agents for increasing sweetness of fruits after
5 harvest, and agents for suppressing axillary buds of tobaccos or roses.

As a method for finding such substances having the activity of controlling biosynthesis of cytokinins, a method, in which examinee substances are directly sprayed to plants to observe
10 and evaluate the physiological changes of the plants, can be employed.

The aforementioned method has a problem in that it is necessary to prepare the examinee substances in amounts sufficient to directly spray them to plants and it takes a great
15 deal of time to grow the plants and to observe and evaluate the physiological changes of the plants after spraying of the examinee substances. Therefore, there has been a demand for development of various methods for quickly finding substances having the activity of controlling biosynthesis of cytokinins
20 with small amounts of examinee substances.

SUMMARY OF THE INVENTION

Inventors of the present invention have intensively investigated in such situations and they have found a transformed
25 cell co-expressing a cytokinin receptor and a cytokinin

biosynthesis enzyme for use in analyzing the activity of
controlling biosynthesis of cytokinins of examinee substances
and quickly searching substances having the activity of
controlling biosynthesis of cytokinins with small amounts of
5 samples, leading to the completion of the present invention.

The present invention provides:

1. A cell transformed with
a polynucleotide comprising a nucleotide sequence encoding an
amino acid sequence of a cytokinin receptor and
10 a polynucleotide comprising a nucleotide sequence encoding an
amino acid sequence of a cytokinin biosynthesis enzyme
(hereinafter sometimes referred to as a transformed cell of the
present invention);
2. The transformed cell according to claim 1, wherein said
15 cytokinin receptor is selected from the group consisting of:
 - (a) a cytokinin receptor having the amino acid sequence
represented by SEQ ID No: 2;
 - (b) a cytokinin receptor having the amino acid sequence
represented by SEQ ID No: 4;
 - 20 (c) a cytokinin receptor having the amino acid sequence
represented by SEQ ID No: 6;
 - (d) a partially transmembrane region-deleted type
cytokinin receptor;
 - (e) a cytokinin receptor having the amino acid sequence
25 represented by amino acids 196 to 1176 of SEQ ID No: 4;

(f) a cytokinin receptor having the amino acid sequence represented by amino acids 50 to 1176 of SEQ ID No: 4;

(g) a cytokinin receptor having the amino acid sequence represented by amino acids 32 to 1036 of SEQ ID No: 6;

5 (h) a chimera-type cytokinin receptor comprising an extracellular region of the cytokinin receptor, transmembrane regions of the cytokinin receptor, a histidine kinase region of the cytokinin receptor, and a receiver region of the histidine kinase, wherein said extracellular region, said transmembrane
10 regions and said histidine kinase region are homogeneous to one another and the receiver region is heterogeneous thereto;

(i) a cytokinin receptor having the amino acid sequence of (a), (b), (c), (e), (f), or (g) with deletion, substitution, or addition of one or a plurality of amino acids; and

15 (j) a cytokinin receptor having an amino acid sequence encoded by a nucleotide sequence of a polynucleotide, wherein said polynucleotide hybridizes under a stringent condition to a polynucleotide that has a nucleotide sequence complementary to a nucleotide sequence encoding the amino acid sequence
20 represented by SEQ ID Nos: 2, 4, or 6;

3. The transformed cell according to claim 1, wherein said cytokinin biosynthesis enzyme is a isopentenyltransferase;

4. The transformed cell according to claim 1, wherein said cytokinin biosynthesis enzyme is selected from the group
25 consisting of:

(a) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 8;

(b) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 10;

5 (c) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 12;

(d) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 14;

(e) a cytokinin biosynthesis enzyme having the amino acid
10 sequence represented by SEQ ID No: 16;

(f) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 18;

(g) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 20;

15 (h) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID Nos: 8, 10, 12, 14, 16, 18, or 20 with deletion, substitution, or addition of one or a plurality of amino acids; and

(i) a cytokinin biosynthesis enzyme having an amino acid
20 sequence encoded by a nucleotide sequence of a polynucleotide, wherein said polynucleotide hybridizes under a stringent condition to a polynucleotide that has a nucleotide sequence complementary to a nucleotide sequence encoding the amino acid sequence represented by SEQ ID Nos: 8, 10, 12, 14, 16, 18, or
25 20;

5. The transformed cell according to claim 1, wherein said cell is yeast; and

6. The transformed cell according to claim 1, wherein said cell is budding yeast.

5

Further scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred
10 embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Throughout this specification and the claims which follow,
15 unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step.

20

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, the present invention will be described in detail.

A cytokinin biosynthetic pathway in plants has been
25 considered to include isopentenylation of some kind of adenine

structure, because cytokinins contain an isopentenyl group or a hydroxylated isopentenyl group. In recent years, it has been found that the key step of cytokinin biosynthesis in plants is isopentenylation of adenosine-5'-triphosphate and/or
5 adenosine-5'-diphosphate with dimethylallyl diphosphate as isopentenyl donor, more concretely, transfer of an isopentenyl group from dimethylallyl diphosphate to N6 of adenosine-5'-triphosphate and/or adenosine-5'-diphosphate. An enzyme which can catalyze the isopentenylation, more
10 concretely, an enzyme which can catalyze the transfer of the isopentenyl group, such as isopentenyltransferase, is a representative example of cytokinin biosynthesis enzyme to be used in the present invention.

15 Practical examples of the cytokinin biosynthesis enzymes are a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 8; a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 10; a cytokinin biosynthesis enzyme having the amino acid
20 sequence represented by SEQ ID No: 12; a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 14; a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 16; a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No:
25 18; a cytokinin biosynthesis enzyme having the amino acid

sequence represented by SEQ ID No: 20; a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID Nos: 8, 10, 12, 14, 16, 18, or 20 with deletion, substitution, or addition of one or a plurality of amino acids; a cytokinin
5 biosynthesis enzyme having an amino acid sequence encoded by a nucleotide sequence of a polynucleotide, wherein said polynucleotide hybridizes under a stringent condition to a polynucleotide that has a nucleotide sequence complementary to a nucleotide sequence encoding the amino acid sequence
10 represented by SEQ ID Nos: 8, 10, 12, 14, 16, 18, or 20; a cytokinin biosynthesis enzyme having an amino acid sequence encoded by a nucleotide sequence of a polynucleotide, wherein said polynucleotide hybridizes under a stringent condition to a polynucleotide that has a nucleotide sequence complementary to
15 the nucleotide sequence represented by SEQ ID Nos: 7, 9, 11, 13, 15, 17, or 19; and the like. Incidentally, the phrase, "a plurality of amino acids", means more particularly about 2 to 20 amino acids and for example, 2 to 10 amino acids and 2 to 5 amino acids may be exemplified. Also, the phrase, "the amino
20 acid sequence ... with deletion, substitution, or addition of one or a plurality of amino acids", means as examples those having the amino acid sequences of which 80% or higher, preferably 90% or higher, and more preferably 95% or higher are identical with the sequences of the amino acids before the deletion,
25 substitution or addition of amino acids (i.e. amino acid sequence

identity of 80% or higher, particularly 90% or higher, and more preferably 95% or higher).

Those phrases, "the amino acid sequence ... with deletion, substitution, or addition of one or a plurality of amino acids" or "the amino acid sequences of which 80% or higher ... are identical" include amino acid sequences of the proteins provided by the intracellular processing to which proteins having the amino acid sequences represented by SEQ ID Nos: 8, 10, 12, 14, 16, 18, or 20 are subjected, and the natural variations caused by differences in type of organisms from which the proteins are derived, differences in individual bodies, differences in tissues, and the like.

A cytokinin receptor is a protein having functions of controlling the propagation and differentiation of cells of higher plants based on the intracellular signal transduction mechanism so-called Two-Component regulatory system (or Histidine to Aspartic acid phosphorelay system) while being specifically bonded with cytokinins such as purine type cytokinins, e.g. kinetine, zeatine, and the like, and urea type cytokinins, e.g. N-phenyl-N'-(4-pyridyl)urea and the like. The cytokinin receptor to be used in the present invention belongs to the histidine kinase family and is protein composed of extracellular regions, transmembrane regions, histidine kinase regions (regions having histidine kinase activity in the cell

and holding Histidine residue to be an active site), and receiver regions (regions having a reception part for phosphate group transfer and holding Aspartic acid residue to be an active site).

Practical examples of the cytokinin receptor are a
5 cytokinin receptor having the amino acid sequence represented
by SEQ ID No: 2; a cytokinin receptor having the amino acid sequence
represented by SEQ ID No: 4; a cytokinin receptor having the
amino acid sequence represented by SEQ ID No: 6; a cytokinin
receptor having the amino acid sequence represented by SEQ ID
10 No: 2 with deletion, substitution, or addition of one or a
plurality of amino acids; a cytokinin receptor having the amino
acid sequence represented by SEQ ID No: 4 with deletion,
substitution, or addition of one or a plurality of amino acids;
a cytokinin receptor having the amino acid sequence represented
15 by SEQ ID No: 6 with deletion, substitution, or addition of one
or a plurality of amino acids; a cytokinin receptor having an
amino acid sequence encoded by a nucleotide sequence of a
polynucleotide, wherein said polynucleotide hybridizes under
a stringent condition to a polynucleotide that has a nucleotide
20 sequence complementary to a nucleotide sequence encoding the
amino acid sequences represented by SEQ ID No: 2; a cytokinin
receptor having an amino acid sequence encoded by a nucleotide
sequence of a polynucleotide, wherein said polynucleotide
hybridizes under a stringent condition to a polynucleotide that
25 has a nucleotide sequence complementary to a nucleotide sequence

encoding the amino acid sequences represented by SEQ ID No: 4;
a cytokinin receptor having an amino acid sequence encoded by
a nucleotide sequence of a polynucleotide, wherein said
polynucleotide hybridizes under a stringent condition to a
5 polynucleotide that has a nucleotide sequence complementary to
a nucleotide sequence encoding the amino acid sequences
represented by SEQ ID No: 6; a cytokinin receptor having an amino
acid sequence encoded by a nucleotide sequence of a
polynucleotide, wherein said polynucleotide hybridizes under
10 a stringent condition to a polynucleotide that has a nucleotide
sequence complementary to the nucleotide sequence represented
by SEQ ID Nos: 1, 3, or 5; cytokinin receptors of partially
transmembrane region-deleted type which will be described later;
chimera-type cytokinin receptors which will be described later;
15 and the like. Incidentally, the phrase, "a plurality of amino
acids", means more particularly about 2 to 20 amino acids and
for example, 2 to 10 amino acids and 2 to 5 amino acids may be
exemplified. Also, the phrase, "the amino acid sequences ... with
deletion, substitution, or addition of one or a plurality of
20 amino acids", means as examples those having the amino acid
sequences of which 80% or higher, preferably 90% or higher, and
more preferably 95% or higher are identical with the sequences
of the amino acids before the deletion, substitution or addition
of amino acids (i.e. amino acid sequence identity of 80% or higher,
25 particularly 90% or higher, and more preferably 95% or higher).

Those phrases, "the amino acid sequence ... with deletion, substitution or addition of amino acids" or "the amino acid sequences of which 80% or higher ... are identical" include amino acid sequences of the proteins provided by the intracellular
5 processing to which proteins having the amino acid sequences represented by SEQ ID Nos: 2, 4, and 6 are subjected, and the natural variations caused by differences in type of organisms from which the proteins are derived, differences in individual bodies, differences in tissues, and the like.

10

The phrase, "sequence identity", in the present invention means the identity and homology between two DNA sequences and between two protein sequences. The sequence identity may be determined by comparing two sequences aligned in the optimum
15 states in a region of the sequences of the comparison objects. The DNA or proteins, the comparison objects, may have addition or deletion (e.g. gap and the like) in the optimum alignment of two sequences. Regarding such sequence identity, computation may be performed using, for example, Vector NTI by
20 producing alignment by utilizing Clustal W algorithm [Nucleic Acid Res., 22 (22): 4673-4680 (1994)]. Incidentally, the sequence identity may be measured by a sequence analysis software, practically Vector NTI, GENETYX-MAC and analysis tools provided in public databases. The aforementioned public databases may
25 be, in general, accessible in, for example, the webpage

(<http://www.ddbj.nig.ac.jp>) of the DNA Data Bank of Japan (the international databank operated within the Center for Information Biology and DNA Data Bank of Japan).

Regarding the phrase, "hybridizes under a stringent
5 condition", hybridization in this case may be performed according
to a conventional method described in, for example, Molecular
Cloning 2nd edition, written by Sambrook J., Frisch E. F.,
Maniatis T., issued by Cold Spring Harbor Laboratory press. As
the "stringent condition", there can be mentioned, for example,
10 the conditions under which a hybrid is formed in a solution of
6 x SSC (a solution containing 1.5 M NaCl and 0.15 M trisodium
citrate is defined as 10 x SSC) at 65°C and then the hybrid is
washed with 1 x SSC at a room temperature. The salt concentration
in the washing step may be selected, for example, from the
15 condition of 1 x SSC at a room temperature (a low stringent
condition) to 0.1 x SSC at a room temperature (a high stringent
condition). The temperature in the washing step may be selected,
for example, from a room temperature (a low stringent condition)
to 68°C (a high stringent condition). Further, both of the salt
20 concentration and the temperature may be changed.

Production of a cell that is transformed with a
polynucleotide comprising a nucleotide sequence encoding an
amino acid sequence of a cytokinin receptor and a polynucleotide
25 comprising a nucleotide sequence encoding an amino acid sequence

of a cytokinin biosynthesis enzyme will be described below.

A cell transformed with a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and a polynucleotide comprising a nucleotide
5 sequence encoding an amino acid sequence of a cytokinin biosynthesis enzyme may be obtained by introducing into a host cell, for example, in the following manner, a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and a polynucleotide comprising a
10 nucleotide sequence encoding an amino acid sequence of a cytokinin biosynthesis enzyme.

(1) Preparation of cDNA

At first, the total RNA is prepared from plants such as
15 higher plants according to the method described in, for example, Molecular Cloning 2nd edition written by J., Sambrook, E., F., Frishch, T., Maniatis.

Concretely, for example, after a part of tissues are sampled from a higher plant, the tissues are frozen in liquified
20 nitrogen and successively physically milled using a mortar and pestle or the like. The total RNA may then be obtained by methods in which (a) the resulting milled product is mixed with a solution containing guanidine hydrochloride with phenol or a solution containing SDS with phenol to obtain the total RNA or (b) the
25 resulting milled product is mixed with a solution containing

guanidine thiocyanate and further with CsCl and then subjected to centrifugal separation to obtain the total RNA. Examples of the higher plant include a monocotyledonous plant, e.g. rice, corn, barley, wheat and the like and a dicotyledonous plant, e.g. tobacco, soybean, Arabidopsis, and the like. For the
5 aforementioned process, commercialized kits such as ISOGEN (produced by Nippon Gene Co.), RNeasy Total RNA Purification Kit (produced by QIAGEN Co.), and the like may be employed.

Next, mRNA is prepared from the total RNA. For example,
10 the preparation may be carried out by a method utilizing the hybridization of oligo-dT chains bonded with cellulose or latex and poly(A) chains of mRNA. For the operation, for example, commercialized kits such as mRNA Purification Kit (produced by Amersham Pharmacia Co.), OLIGOTEX™ dT30 super (Oligo(dT)latex
15 beads) (produced by Takara Shuzo Co. Ltd.), and the like may be employed.

Further, cDNA is produced using the mRNA (mRNA having poly(A) chains) may be prepared in the following manner. For example, oligo-dT chains or random primers are annealed with
20 mRNA and then reacted with reverse transcriptase to produce cDNA. Further, the cDNA is reacted with, for example, RNaseH, DNA polymerase I to produce double-stranded cDNA. For the operation, for example, the following commercialized kits may be employed: SMART™ PCR cDNA Synthesis Kit (produced by Clontech Co.), cDNA
25 Synthesis Kit (produced by Takara Shuzo Co. Ltd.), cDNA Synthesis

Kit (produced by Amersham Pharmacia Co.), ZAP-cDNA Synthesis Kit (produced by Stratagene Co.) and the like.

(2) Cloning

5 A polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor may be obtained by a polymerase chain reaction (hereinafter referred as to PCR) from the produced cDNA using, for example, a polynucleotide having partial nucleotide sequence of the
10 nucleotide sequence of SEQ ID Nos: 1, 3 or 5 as a primer or by a hybridization method using a polynucleotide having partial nucleotide sequence of the nucleotide sequence of SEQ ID Nos: 1, 3 or 5 as a probe. In a similar manner, a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence
15 of a cytokinin biosynthesis enzyme may be obtained by a PCR using, for example, a polynucleotide having partial nucleotide sequence of the nucleotide sequence of SEQ ID Nos: 7, 9, 11, 13, 15, 17 or 19 as a primer or by a hybridization method using a polynucleotide having partial nucleotide sequence of the
20 nucleotide sequence of SEQ ID Nos: 7, 9, 11, 13, 15, 17 or 19 as a probe.

 In the case of employing PCR, polynucleotides usable as a primer set is those designed and synthesized based on the
25 nucleotide sequences of about 20 bp to 40 bp, for example, the

nucleotide sequences selected respectively from 5'-non-coding regions and 3'-non-coding regions of the nucleotide sequence represented by SEQ ID Nos: 1, 3, or 5. Examples of the primer set are sets of the polynucleotide of nucleotide sequence
5 represented by SEQ ID No: 23 and the polynucleotide of nucleotide sequence represented by SEQ ID No: 24. The PCR solution to be used may be prepared by adding reaction solutions instructed by the kit to 250 ng of cDNA. The conditions of the PCR may properly be changed depending on the primer set to be used and,
10 for example, concrete conditions include as follows: keeping at 94°C for 2 minutes, and then at about 8°C for 3 minutes, and further repeating 40 cycles each of which comprises steps of keeping at 94°C for 30 seconds, at 55°C for 30 seconds, and at 72°C for 4 minutes; and repeating 5 to 10 cycles each of which
15 comprises steps of keeping at 94°C for 5 seconds and at 72°C for 4 minutes, and further repeating about 20 to 40 cycles each of which comprises steps of keeping at 94°C for 5 seconds and at 70°C for 4 minutes. For the operation, the following commercialized kits may be, for example, employed: HERCULASE™
20 (DNA polymerase) (produced by Stratagene Co., Ltd.), DNA polymerase contained in Advantage cDNA PCR Kit (Clontech Co.), TAKARA Ex Taq (Takara Shuzo Co., Ltd.), PLATINUM™ PCR SUPER (thermostable DNA polymerase in a PCR reaction mix) (Lifetech Oriental Co.), and the like.

In the case of employing hybridization, cloning may be carried out according to a method described in, for example, "Cloning and Sequence", Experimental Manual of Plant Biotechnology (edited by Watanabe and Sugiura, Noson Bunka
5 Publisher, 1989)

The probe to be used may be obtained by preparing polynucleotide (with the chain length of about 200 nucleotides to 500 nucleotides) having partial nucleotide sequences of the nucleotide sequence represented by SEQ ID Nos: 1, 3 or 5 and
10 labelling the polynucleotide with radioisotope markers or fluorescent markers according to the known methods using, for example, RandomPrimedDNA Labelling Kit (Boehringer Co.), Random Primer DNA Labelling Kit Ver. 2 (Takara Shuzo Co., Ltd.), ECL Direct Nucleic Acid Labelling and Detection System (Amersham
15 Pharmacia Co.), Megaprime DNA-labelling system (Amersham Pharmacia Co.) and the like.

Examples of the hybridization conditions include stringent conditions and the following conditions may be exemplified: keeping at 65°C in the presence of 6 x SSC (0.9
20 M NaCl and 0.09 M trisodium citrate), 5 x Denhard's solution [0.1% (w/v) ficoll 400, 0.1% (w/v) polyvinylpyrrolidone, 0.1% BSA], 0.5% (w/v) SDS and 100 µg/ml degenerated salmon sperm DNA or in DIG EASY Hyb solution (Boeringer-Mannheim Co.) containing 100 µg/ml of degenerated salmon sperm DNA, successively keeping
25 at a room temperature for 15 minutes two times in the presence

of 1 x SSC (0.15 M NaCl and 0.015 M trisodium citrate) and 0.5% (w/v) SDS, and further keeping at 68°C for 30 minutes in the presence of 0.1 x SSC (0.015 M NaCl and 0.0015 M trisodium citrate) and 0.5% SDS.

5 In order to obtain a polynucleotide coding the cytokinin receptor of Arabidopsis, PCR can be carried out employing TAKARA LA taqTM (thermostable DNA polymerase) (Takara Shuzo Co., Ltd.) and using a solution containing cDNA library phage of Arabidopsis (about 1,000,000 pfu) as a template and a polynucleotide having
10 the nucleotide sequence represented by SEQ ID No: 25 and a polynucleotide having the nucleotide sequence represented by SEQ ID No: 26 as a primer set to amplify and obtain DNA to be a probe. In order to obtain a polynucleotide coding the cytokinin biosynthesis enzyme of Arabidopsis, PCR can be carried out
15 employing TAKARA LA taqTM (thermostable DNA polymerase) (Takara Shuzo Co., Ltd.) and using a solution containing cDNA library phage of Arabidopsis (about 1,000,000 pfu) as a template and polynucleotides having partial nucleotide sequence of the nucleotide sequence of SEQ ID Nos: 7, 9, 11, 13, 15, 17 or 19
20 as a primer set to amplify and obtain DNA to be a probe. The PCR solution to be used may be prepared by adding to 250 ng of cDNA library, the reaction solutions instructed by the kit.

 The PCR conditions may be as follows: keeping at 94°C for 2 minutes, and then at 8°C for 3 minutes, and further repeating
25 40 cycles each of which comprising keeping at 94°C for 30 seconds,

at 55°C for 30 seconds, and at 68°C for 5 minutes.

Using the amplified and obtained DNA as a template, a ³²P-labeled probe may be produced employing Megaprime DNA-labelling system kit (Amersham Pharmacia Co.) and using the reaction solutions instructed by the kit. Using the probe obtained in such a manner, colony hybridization is carried out by a conventional method, practically the hybridization is carried out keeping at 65°C in the presence of 6 x SSC (0.9 M NaCl and 0.09 M trisodium citrate), 5 x Denhard's solution [0.1% (w/v) ficoll 400, 0.1% (w/v) polyvinylpyrrolidone, 0.1% BSA], 0.5% (w/v) SDS and 100 µg/ml degenerated salmon sperm DNA or in the DIG EASY Hyb solution (Boeringer-Mannheim Co.) containing 100 µg/ml of degenerated salmon sperm DNA, successively keeping at a room temperature for 15 minutes two times in the presence of 1 x SSC (0.15 M NaCl and 0.015 M trisodium citrate) and 0.5% (w/v) SDS, and further keeping at 68 °C for 30 minutes in the presence of 0.1 x SSC (0.015 M NaCl and 0.0015 M sodium citrate) and 0.5% SDS to obtain clone hybridized with the probe.

Further, a polynucleotide coding the cytokinin receptor may be prepared based on, for example, the nucleotide sequence represented by SEQ ID No: 1, 3 or 5 by chemical synthesis of polynucleotides according to a conventional method such as phosphite-triester method (Hunkapiller, M. et al., Nature, 310, 105, 1984). In a similar manner, a polynucleotide coding the

cytokinin biosynthesis enzyme may be prepared based on, for example, the nucleotide sequence represented by SEQ ID No: 7, 9, 11, 13, 15, 17 or 19 by chemical synthesis of polynucleotides.

5 The polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor and the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin biosynthesis enzyme, obtained in such a manner, may be cloned in a vector by a
10 conventional method described in, "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN0-471-50338-X, and the like. The vector to be used may be, for example, pBlue Script II vector
15 (produced by Stratagene Co.), pUC18/19 vector (produced by Takara Shuzo Co., Ltd.), TA cloning vector (produced by Invitrogen Co.), and the like

Incidentally, the nucleotide sequence of cloned polynucleotide may be confirmed by, the Maxam Gilbert method
20 (described in, for example, Maxam, A., M & W. Gilbert, Proc. Natl. Acad. Sci. USA, 74, 560, 1977, and the like), the Sanger method (described in, for example, Sanger, F. & A.R. Coulson, J. Mol. Biol., 94, 441, 1975, Sanger, F. & Nicklen and A.R. Coulson., Proc. Natl. Acad. Sci. USA, 74, 5463, 1977, and the like). For
25 the process, the following commercialized kits may be, for

example, employed: Thermo Sequenase II dye terminator cycle sequencing kit (produced by Amersham Pharmacia Co.), BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (produced by PE Biosystems Japan Co.), and the like.

5

(3) Construction of expression vector

An expression vector of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor and an expression vector of a polynucleotide
10 comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin biosynthesis enzyme may be constructed according to a conventional method described in, for example, Molecular Cloning 2nd edition written by J., Sambrook, E., F., Frisch, & T., Maniatis, published by Cold Spring Harbor Laboratory
15 Press.

Usable are vectors to be used in host cells to be transformed, for example, independently replicating vectors which contain genetic information possible to be duplicated in the host cells and further are possible to be isolated from the host cells and
20 purified and may have detectable marker. More practically, in the case of using bacteria such as E. coli as the host cells, for example, Plasmid pUC119 (produced by Takara Shuzo Co., Ltd.), Phagemid pBluescript II (Stratagene Co.) and the like may be used. In the case of using yeast as the host cells, for example,
25 Plasmid pACT2 (Clontech Co.) and the like may be used. In the

case of using plant cells as the host cells, for example, a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor or a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence
5 of the cytokinin biosynthesis enzyme may be integrated with plasmid pBI221 (Clontech Co.) to construct the vectors.

An expression vector possible to express a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence
10 of the cytokinin receptor in a host cell may be constructed by integrating a promoter with the aforementioned vectors in the upstream of the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor in a binding manner of enabling to function in the host cell. In
15 a similar manner, an expression vector possible to express a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin biosynthesis enzyme in a host cell may be constructed by integrating a promoter with the aforementioned vectors in the upstream of the polynucleotide
20 comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin biosynthesis enzyme in a binding manner of enabling to function in the host cell. In these cases, the phrase, "in a binding manner of enabling to function", means that the polynucleotide comprising a nucleotide sequence encoding an
25 amino acid sequence of the cytokinin receptor or the

polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin biosynthesis enzyme, and the promoter are bonded as to express the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor or the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin biosynthesis enzyme in the host cell under the control of the promoter. Usable as the promoter possible to function in the host cell in the case of using *E. coli* as the host cell are, for example, a promoter (*lacP*) of lactose operon of *E. coli*, a promoter (*trpP*) of triptophan operon, a promoter (*argP*) of arginine operon, a promoter (*galP*) of galactose operon, a *tac*-promoter, T7-promoter, T3-promoter, λ -phage promoter, (λ -pL, λ -pR) and the like. In the case of using yeast as the host cell, it may be prepared by a conventional genetic engineering method [described in Method in Enzymology 101 part (p.192-201) by Ammerer, et. al.] from *ADH1* promoter (the *ADH1* promoter is available from the yeast expression vector pAAH5 which contains the *ADH1* promoter and its terminator and which may be obtained from Washington Research Foundation). The *ADH1* promoter is included in US patent application number 299,733 of Washington Research Foundation and in the case that it is used for industrial and commercial purposes in USA., it is required to obtain permission from the patent holder. In the case of using a plant cell as the host cell, usable examples

are a nopaline synthesis enzyme gene (*NOS*) promoter, an octopine synthesis enzyme gene (*OCT*) promoter, a cauliflower mosaic virus (CaMV) -derived 19S promoter, a CaMV-derived 35S promoter and the like.

5

Further, in the case of integrating a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor or a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the
10 cytokinin biosynthesis enzyme into a vector already having a promoter possible to function in a host cell, the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor or the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the
15 cytokinin biosynthesis enzyme is inserted in the downstream of the promoter and the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor or the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin biosynthesis
20 enzyme is inserted in a manner of enabling to function. For example, the aforementioned plasmid pACT 2 for yeast comprises the *ADH1* promoter and therefore, an expression vector possible to express the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor or
25 the polynucleotide comprising a nucleotide sequence encoding

an amino acid sequence of the cytokinin biosynthesis enzyme in yeast, such as CG1945 (Clontech Co.) may be constructed by inserting the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin receptor or
5 the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin biosynthesis enzyme in the downstream of the *ADH1* promoter of the plasmid pACT2.

(4) Production of a transformed cell

10 A transformed cell to be used in the present invention may be produced by introducing the constructed expression vector into a host cell by a conventional method. As the host cell to be used for production of the transformed cell, examples are bacteria, yeast, plant cell and the like. As bacteria, examples
15 are bacteria belonging to *E. coli*, *Serratia*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Microbacterium* and the like. As yeast, examples are budding yeast and fission yeast. More particularly, examples are yeast belonging to *Saccharomyces*, *Schizosaccharomyces* and the like. As plant cell, examples are
20 BY-2 strain, which is a cultured cell of tobacco, and BMS strain, which is a cultured cell of corn (Black Mexican Sweet), and the like.

As the method for introducing the expression vector into the aforementioned host cell, conventional introduction methods
25 are employed according to the host cell to be transformed. For

example, in the case of using bacteria as the host cell, the
aforementioned expression vector may be introduced into a host
cell by employing a conventional introduction method such as
a calcium chloride method and an electroporation method described
5 in, "Molecular Cloning" (by J. Sambrook, et. al Cold Spring Harbor
1989). In the case of using yeast as a host cell, the
aforementioned expression vector may be introduced into the host
cell by employing Yeast transformation kit (produced by Clontech
Co.) based on a lithium method. Further, in the case of using
10 a plant cell as a host cell, the aforementioned expression vector
may be introduced into the host cell by a conventional
introduction method, for example, Agrobacterium infection
method (Japanese Examined Patent Application No. 2 - 58917,
Japanese Laid-Open Patent Application No. 60 - 70080), an
15 electroporation method into a protoplast (Japanese Laid-Open
Patent Application No. 60 - 251887, Japanese Laid-Open Patent
Application No. 5 - 68575), a particle gun method (Japanese
Laid-Open Patent Application No. 6 - 508316, Japanese Laid-Open
Patent Application No. 63 - 258525).

20

A transformed cell in which a cytokinin receptor of
partially transmembrane region-deleted type, i.e. a cytokinin
receptor of a transmembrane quantity variation type is introduced,
will be described below.

25 A cytokinin receptor to be used in the present invention

includes a cytokinin receptor wherein said cytokinin receptor has at least one transmembrane region but less than that in its natural form (commonly 2 to 4 transmembrane region) (incidentally, in the present invention, such cytokinin receptors are sometimes referred as to cytokinin receptors of partially transmembrane region-deleted type.) In this case, the phrase, "its natural form" means cytokinin receptors having an amino acid sequence most frequently existing among organisms having the similar nomenclature and generally called also as wild-type cytokinin receptor.

Such cytokinin receptors of partially transmembrane regions-deleted type are cytokinin receptors whose transmembrane region structure may be determined by employing structure assumption software available in http://www.ch.embnet.org/software/TMPRED_form.html and whose transmembrane regions are partially deleted, for example, in 1 to 2 sites, and are less in number than the number of the transmembrane regions of the natural type cytokinin receptors (i.e. natural form).

More particularly, examples of such cytokinin receptors include a cytokinin receptor having the amino acid sequence from amino acid number 196 to 1176 among the amino acid sequence represented by SEQ ID No: 4 (2 transmembrane regions); a cytokinin receptor having the amino acid sequence from amino acid number 50 to 1176 among the amino acid sequence represented by SEQ ID

No: 4 (3 transmembrane regions); a cytokinin receptor having the amino acid sequence from amino acid number 32 to 1036 among the amino acid sequence represented by SEQ ID No: 6 (2 transmembrane regions); a cytokinin receptor having the amino acid sequence derived from the amino acid sequences of these cytokinin receptors wherein one or a plurality of amino acids are deleted, substituted or added, for example, cytokinin receptors having the amino acid sequence derived from the amino acid sequences in which one methionine is added to the amino-terminal and the like.

The DNA coding the cytokinin receptor may be constructed as to hold transmembrane regions in a less number than the number of the transmembrane regions of the natural type cytokinin receptors by partially deleting the transmembrane regions by a conventional genetic engineering technique.

A transformed cell in which the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the partially transmembrane region-deleted type cytokinin receptor and the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin biosynthesis enzyme are introduced may be produced according to the aforementioned method, "Production of a cell that is transformed with a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a

cytokinin biosynthesis enzyme".

A transformed cell to express chimera-type cytokinin receptor will be described below.

5 A cytokinin receptor to be used in the present invention also includes a chimera-type cytokinin receptor comprising an extracellular region of the cytokinin receptor, transmembrane regions of the cytokinin receptor, a histidine kinase region of the cytokinin receptor, and a receiver region for a histidine
10 kinase. The chimera-type cytokinin receptor has the extracellular region, the transmembrane regions, and the histidine kinase region homogeneous to one another and the receiver region for the histidine kinase heterogeneous to these regions. For example, the chimera-type cytokinin receptor may
15 have said extracellular region, transmembrane regions, and histidine kinase region derived from a cytokinin receptor selected from CRE1, AHK2, and AHK3, and have the receiver region derived from the osmosensor SLN1 in budding yeast.

20 The histidine kinase protein has the following sequence in common in plants, e.g. higher plants, and microorganism. That is, histidine kinase protein is composed of an extracellular region, transmembrane region (generally about 2 to 4), histidine kinase region having histidine kinase activity and holding
25 Histidine residue to be an active site, and receiver region having

a reception part for a phosphoryl group transfer and holding Aspartic acid residue to be an active site. In the chimera-type cytokinin receptor, it is preferable that the extracellular region, the transmembrane regions, and the histidine kinase
5 region are all derived from the identical type of cytokinin receptor, whereas the receiver regions are derived from a histidine kinase protein that is different from type of the former cytokinin receptor.

It is sufficient for the receiver region of the
10 chimera-type cytokinin receptor to have a function of receiving signals transmitted from the histidine kinase region and transmitting them to the next step and any of receiver region may be usable as long as they can complement or improve the intrinsic functions of the receiver region of a histidine kinase
15 protein comprising the homogeneously derived extracellular region, transmembrane regions, and histidine kinase region.

As such a receiver region, usable are, for example, a receiver region of histidine kinase proteins derived from microorganism (e.g. a receiver region of histidine kinase protein
20 derived from a microorganism such as yeast, *E. coli*) and more particularly receiver region of histidine kinase protein coded in *SLN1* gene derived from budding yeast (e.g. the amino acid sequence represented by SEQ ID No: 21), receiver region of histidine kinase protein coded in *Chey* gene derived from
25 *Salmonella*, receiver region of histidine kinase protein coded

in *RcsC* gene, which is a hybrid sensor of *E. coli* [Maeda T, et al. *Nature*: 369 242-245, (1994): e.g. the amino acid sequence represented by SEQ ID No: 22], receiver region of histidine kinase protein coded in *Phks* gene relevant to cell cycle control of
5 fission yeast [Shieh, JC, et al., *Gene Dev.* 11, 1008-1022 (1997)].

In the above, histidine kinase regions of the cytokinin receptor may include the region present C-terminally downstream from the particular transmembrane region which is the most
10 C-terminally downstream transmembrane region of the cytokinin receptor. Said histidine kinase region typically has five conserved motifs which are common to generic histidine kinases as described in *Annual Review of Genetics* 23:311-336 (1989), *Microbiological Reviews* 53(4):450-490 (1989), *Science*
15 262:539-544 (1993), and the like. Examples of the region includes a region having the amino acid sequence from amino acid number 587 to 844 among the amino acid sequence represented by SEQ ID No: 4 in a case of AHK2, a region having the amino acid sequence from amino acid number 450 to 700 among the amino acid
20 sequence represented by SEQ ID No: 6 in a case of AHK3 and a region having the amino acid sequence from amino acid number 449 to 714 among the amino acid sequence represented by SEQ ID No: 2 in a case of CRE1.

Receiver regions of the cytokinin receptor may include
25 the region existing between the histidine kinase region and the

C-terminal end of the cytokinin receptor. Said region having three conserved motifs which are common to generic histidine kinases as described in Annual Review of Genetics 23:311-336 (1989), Science 262:539-544(1993), and the like. Examples of
5 the region includes a region having the amino acid sequence from amino acid number 891 to 1163 among the amino acid sequence represented by SEQ ID No: 4 in a case of AHK2, a region having the amino acid sequence from amino acid number 746 to 1018 among the amino acid sequence represented by SEQ ID No: 6 in a case
10 of AHK3 and a region having the amino acid sequence from amino acid number 763 to 1038 among the amino acid sequence represented by SEQ ID No: 2 in a case of CRE1.

In addition, a sensor region for cytokinin means, for example, a region which is a part of any of the extracellular
15 regions of the cytokinin receptor, wherein said sensor region is present between a transmembrane region next to the histidine kinase region and a transmembrane region secondary close to the histidine kinase region. Said sensor region typically has 50% or more identity and homology between three cytokinin receptors
20 of AHK2, AHK3 and CRE1 as described in Plant and Cell Physiology 42(2):231-235(2001) and the like. Examples of the region includes a region having the amino acid sequence from amino acid number 259 to 536 among the amino acid sequence represented by SEQ ID No: 4 in a case of AHK2, a region having the amino acid
25 sequence from amino acid number 120 to 399 among the amino acid

sequence represented by SEQ ID No: 6 in a case of AHK3 and a region having the amino acid sequence from amino acid number 132 to 398 among the amino acid sequence represented by SEQ ID No: 2 in a case of CRE1.

5

A polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the chimera-type cytokinin receptor may be constructed by respectively producing polynucleotides encoding each of the extracellular region of
10 the cytokinin receptor, transmembrane regions of the cytokinin receptor, histidine kinase region of the cytokinin receptor, and receiver region for the histidine kinase protein, joining the polynucleotides by a conventional genetic engineering technique so as to prevent appearance of any termination codon
15 in the middle while using a proper linker so as to prevent frame shift. Incidentally, one polynucleotide may be utilized to provide the polynucleotide encoding the extracellular region of the cytokinin receptor and transmembrane regions of the cytokinin receptor, or the polynucleotide encoding the
20 extracellular region of the cytokinin receptor, transmembrane regions of the cytokinin receptor, and histidine kinase region of the cytokinin receptor.

The polynucleotides coding amino acid sequences of the aforementioned respective regions may respectively be produced
25 by known methods. For example, in the case of production by

PCR, at first, the oligonucleotides (5' side primers) having the nucleotide sequences of 5' terminal regions of respective regions to be amplified and oligonucleotides (3' side primers) having complementary nucleotide sequences to the nucleotide sequences of 3' terminal are designed and synthesized. The primers may be oligonucleotides of about 14 nucleotides to about 35 nucleotides in general and are preferable to contain in the 5' side of the primers, restriction enzyme recognition sequences usable at the time of ligating the polynucleotides amplified by the PCR to one another or these polynucleotides to vectors. Then, using the primers and the cDNA library as a template, amplification reactions may be carried out in the conventional reaction conditions employed for the PCR. As the template to be used in the case of producing the polynucleotide coding amino acid sequence of the extracellular region, the transmembrane regions of the cytokinin receptor or the histidine kinase region of the cytokinin receptor, usable is cDNA library derived from plants such as higher plants. Also as the template to be used in the case of producing the polynucleotides coding amino acid sequence of the receiver regions of the histidine kinase, usable is the total DNA or cDNA library derived from microorganism prepared by a conventional method.

The transformed cell in which the polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of the chimera-type cytokinin receptor and the polynucleotide

comprising a nucleotide sequence encoding an amino acid sequence of the cytokinin biosynthesis enzyme are introduced may be produced according to the aforementioned, "Production of a cell that is transformed with a polynucleotide comprising a nucleotide
5 sequence encoding an amino acid sequence of a cytokinin receptor and a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin biosynthesis enzyme".

Intracellular signal transduction system relevant to
10 cytokinin will be described below.

Measurement of the existence or the quantity of intracellular signal transduction from the cytokinin receptor expressed in the transformed cell produced by the above-described manner may be carried out by utilizing the intracellular signal
15 transduction system originally present in the host cell used for production of the transformed cell. The existence or the quantity of intracellular signal transduction can be determined by employing the quantity of the cell growth of the transformed cell as an indicator. Alternatively, a regulator (and/or a
20 mediator), which is a downstream signal transducer in the so-called Two-Component regulatory system, can be additionally introduced and expressed in the host cell and the expressed system may be used in the intracellular signal transduction system. Usable as the Two-Component regulatory system, for example, are
25 Two-Component regulatory systems corresponding to 5 types of

ethylene receptors; ETR1, ETR2, ERS1, ERS2, and EIN4; present in Arabidopsis [Chang et al., Science 262: 539-544 (1993), Hua et al., Science 269: 1712-1714 (1995), Sakai et al., Plant Cell Physiol 39: 1232-1239 (1998)] and AtHK1 having sensor functions
5 to the osmotic pressure [Urao, Plant cell 11: 1743-1754 (1999)].

As the host cell to be used for production of such a transformed cell, usable are host cells improved as to have histidine kinase activity lower than the intrinsic histidine kinase activity of the host cells. For example, it includes
10 the host cells improved as to have histidine kinase activity lower than the intrinsic histidine kinase activity of the host cell by deleting one or more of histidine kinases. The phrase, "histidine kinase activity lower than" means that the quantity of a phosphoryl group transfer from Histidine residue (the active
15 site of histidine kinase regions having histidine kinase activity) to Aspartic acid residue (the active site of receiver regions having a reception part) is decreased. Such improvements in the host cell may provide a change of the quantity of the cell growth, change of the morphology, change of the shape,
20 change of the quantity of the biosynthesis of specific compound, change of the quantity of the metabolism of specific compound in the transformed cell. More particularly, the following strain [Maeda T et al., Nature 369: 242-245 (1994)] may be exemplified: a strain obtained by defecting the *SLN1* gene coding
25 the protein having the osmotic pressure sensor function and

derived from the budding yeast such as *Saccharomyces cerevisiae* and the like. Since the strain is defected in the histidine kinase protein existing in *Saccharomyces cerevisiae*, there can be more clearly detected the existence or the quantity of the
5 intracellular signal transduction from the cytokinin receptor expressed in the transformed cell by using the quantity of the cell growth of the transformed cell as an indicator. Further, other preferable example includes an *E. coli* strain defective in the *RcsC* gene that encodes a hybrid sensor, and a fission
10 yeast strain defective in *Phks* gene that is relevant to the cell cycle.

Method for analyzing the activity of controlling biosynthesis of cytokinin will be described below.

15 In the method for analyzing the activity of controlling biosynthesis of cytokinin, examples of the first step of bringing an examinee substance into contact with a transformed cell into which a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and a
20 polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin biosynthesis enzyme are introduced include a method for culturing the transformed cell in a culture medium containing the examinee substance. In order to culture the transformed cell, the following cultures are
25 usable: liquid-phase culture for culturing the transformed cell

in a liquid culture medium and a solid-phase culture for culturing the transformed cell in a solid culture medium produced by adding agar or the like to the liquid culture medium. The concentration of an examinee substance in the culture medium is about 1 nM to about 1 mM and preferably about 10 nM to about 100 μ M. The culture time is, for example, 1 hour to 3 days and preferably 25 hours to 2 days.

Following the above-described first step, the existence or the quantity of intracellular signal transduction from the cytokinin receptor expressed in the transformed cell may be measured. Using the measured value as an indicator, the activity of the examinee substance to control biosynthesis of cytokinin may be analyzed.

More particularly, for example, in the case of using a transformed cell of the present invention (that is, a transformed cell, wherein growth of said cell is directly controlled by intracellular signal transduction from the cytokinin receptor) produced using TM182 (*sln1* Δ) [Maeda T et al, Nature 369: 242-245 (1994)], a *SLN1* genetically defected strain in which *PTP2* Tyrosine phosphatase gene [Ota et al. Proc. N. A. Sci., USA, 89, 2355-2359 (1992)] is introduced, as a host cell, the activity of controlling biosynthesis of cytokinin may be measured using, as an indicator, the quantity of the cell growth of the transformed cell in a culture medium (an agar culture medium or a liquid culture medium) containing glucose as a carbon source, for

example, a DOHLU + Glu culture medium. In the case of the DOHLU + Glu culture medium to which the examinee substance is added, the examinee substance found capable of changing growth of the transformed cell can be evaluated as a substance having the activity of controlling biosynthesis of cytokinin, as compared with growth of the transformed cell in the DOHLU + Glu culture medium to which the examinee substance is not added (a culture medium containing no substance having the activity of controlling biosynthesis of cytokinin). Incidentally, as a control to verify that the change of cell growth of the transformed cell is not resulted from activity of the examinee substance directly controlling signal transduction from cytokinin receptor, such as agonist-activity or antagonist-activity to the cytokinin receptor, TM182 (*sln1* Δ) transformed with only a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor may be cultured in DOLU + Glu culture medium with or without the examinee substance. In a case where the examinee substance dose not affect growth of the control cell, the examinee substance may not directly affect signal transduction system from cytokinin receptor. As a control to verify that the change of cell growth of the transformed cell is not resulted from activity of the examinee substance which affects somewhere not involved in biosynthesis of cytokinin nor signal transduction from cytokinin receptor, TM182 (*sln1* Δ) may be cultured in culture medium containing galactose as the carbon

source in place of glucose, such as DOLU + Gal culture medium with or without the examinee substance. In a case where the examinee substance dose not affect growth of the control cell [TM182 (*sln1Δ*)], the examinee substance may not affect somewhere
5 not involved in biosynthesis of cytokinin or signal transduction from cytokinin receptor.

Further, in the case of using a transformed cell of the present invention (that is, a transformed cell, wherein growth of said cell is directly controlled by the intracellular signal
10 transduction from the cytokinin receptor) produced by employing fission yeast as a host cell, such as a Phks genetically defected strain, the fission pattern of the fission yeast may be observed with a microscope. In this case, when the examinee substance is added to the culture medium, the examinee substance found
15 capable of changing fission and propagation of the transformed cell can be evaluated as a substance having the activity of controlling biosynthesis of cytokinin, as compared with fission and propagation of the transformed cell in a medium to which the examinee substance is not added.

20 Furthermore, in the case of using a transformed cell of the present invention produced by employing as the host cell an *E. coli* defective in *RcsC* gene into which *cps-LacZ* is introduced, the X-Gal coloring may be observed in an agar culture medium or a liquid culture medium [Suzuki et al. Plant Cell Physiol
25 42: 107-113 (2001)]. In this case, when the culture medium

contains the examinee substance, the examinee substance found capable of changing coloring of the transformed cell can be evaluated as a substance having the activity of controlling biosynthesis of cytokinin, as compared with coloring of the
5 transformed cell in a medium to which the examinee substance is not added.

The activity of the examinee substance for controlling biosynthesis of cytokinin can be detected by evaluating the
10 activity of two or more examinee substances. The activity of controlling biosynthesis of cytokinin can be provided for the evaluation by conducting the aforementioned method for analyzing the activity of controlling biosynthesis of cytokinin. For example, the evaluation of the activity of controlling
15 biosynthesis of cytokinin can be based on the difference between the two or more examinee substances, which are obtained by comparison of the existence or the quantity of the intracellular signal transduction. In utilizing the two or more different examinee substances, the examinee substances are typically
20 utilized independently in different systems. It is preferable that at least one of the two or more examinee substances has no activity of controlling biosynthesis of cytokinin.

Moreover, a substance with the activity of controlling biosynthesis of cytokinin may be searched by selecting substances
25 having the activity of controlling biosynthesis of cytokinin

based on the activity evaluated by the aforementioned detection methods.

Further, a substance selected by the above described
5 detection methods may be utilized as an active ingredient of a plant growth regulator.

The plants to be the objects to be treated with the aforementioned plant growth regulator are, for example, decorative plants such as flowering plants and ornamental foliage
10 plants; cultivating plants such as crop, vegetable, fruit and the like; fibrous plants; trees; lawn and the like.

The growth regulator is generally mixed with a solid carrier, a liquid carrier, and the like and further, if needed, mixed with a surfactant and other auxiliary agents for the
15 formulation of an agricultural and horticultural agent and formulated in an emulsion agent, a hydrating agent, a suspension agent, a solution agent and the like. In these agricultural and horticultural agents, an agonist-active substance or an antagonist-active substance to the cytokinin receptor may be
20 contained generally in 0.5 to 90 % by weight and preferably in 1 to 80 % by weight.

Usable as the solid carrier to be used for the formulation of an agricultural and horticultural agent are, for example, clays (kaolinite, kieselguhr, synthesized hydrated silicon
25 oxide, intercalated clays, bentonite, acidic white clay, and

the like), talc, other inorganic minerals (sericite, quartz powder, sulfur powder, activated carbon, calcium carbonate, and the like), chemical fertilizers (ammonium sulfate, ammonium phosphate, ammonium nitrate, ammonium chloride, urea, and the
5 like) in finely powdered state or in granular state and usable as the liquid carrier are, for example, water, alcohols (methanol, ethanol and the like), ketones (acetone, methyl ethyl ketone, cyclohexanone and the like), aromatic hydrocarbons (toluene, xylene, ethylbenzene, methyl naphthalene and the like),
10 non-aromatic hydrocarbons (hexane, cyclohexane, kerosene and the like), esters (ethyl acetate, butyl acetate and the like), nitriles (acetonitrile, isobutylnitrile and the like), ethers (dioxane, diisopropyl ether and the like), acid amides (dimethylformamide, dimethylacetamide and the like),
15 halohydrocarbons (dichloroethane, trichloroethane and the like), etc.

As the surfactant, usable are, for example, alkylsulfuric acid esters, alkylsulfonic acid salts, alkylarylsulfonic acid salts, alkylaryl ethers and their polyoxyethylene compounds,
20 polyethylene glycols, polyhydric alcohol esters, sugar alcohols and the like.

As other auxiliary agents for the formulation of agricultural and horticultural agents, usable are solidification agents and dispersants such as casein, gelatin,
25 polysaccharides (starch, acacia, cellulose derivatives,

alginic acid and the like), lignin derivatives, bentonite, synthesized water-soluble polymer [poly(vinyl alcohol), poly(vinyl pyrrolidone), poly(acrylic acid) and the like] and the like, and stabilizers such as PAP (acidic isopropyl phosphate), BHT (2,6-tert-butyl-4-methylphenol), BHA (2-/3-tert-butyl-4-methoxyphenol), plant oils, mineral oils, aliphatic acids, aliphatic acid esters and the like.

The substance with the activity of controlling biosynthesis of cytokinin made to be agricultural and horticultural agents is used as it is or diluted with water to carry out treatment for the stem and leave parts, branch and leave parts, and flower and fruit parts of plants by spraying, for fruits by immersion, and for fruits by application. The plant growth regulator is used for the object plants to carry out the treatment once or a plurality of times.

In the case of using the plant growth regulator for the purpose of suppressing the dropping of fruits, the plant growth regulator is diluted with water and the resulting diluted agent is sprayed to the fruit parts and branch and leave parts before harvest.

In the case of using the plant growth regulator for the purpose of suppressing the ball dropping of cotton, the plant growth regulator is diluted with water and the resulting diluted agent is sprayed to the balls and stem and leave parts of cotton before harvest.

The plant growth regulator may be used for the treatment of growing plants or of plants after harvest.

The application amount of the substance with the activity of controlling biosynthesis of cytokinin in an agricultural and horticultural agent is generally 1 to 8000 g per 1 hectare, although it is changed depending on the state of the agricultural and horticultural agent, the timing for the treatment, the method for the treatment, the site for the treatment, and the object plant to be treated. Also in the case of using the plant growth regulator while diluting the agent with water, the concentration of the agent is generally 0.0001 to 1000 mM and preferably 0.001 to 10 mM, although it is changed depending on the state of the agricultural and horticultural agent, the timing for the treatment, the method for the treatment, the site for the treatment, and the object plant to be treated.

Next, hereinafter given are formulation examples of an agricultural and horticultural agent produced from a substance with the activity of controlling biosynthesis of cytokinin and used as a plant growth regulator. The parts in the following description of the examples denotes the parts by weight.

Formulation example 1

A hydrating agent was obtained by sufficiently pulverizing and mixing 50 parts of a substance with the activity of controlling biosynthesis of cytokinin, 3 parts of calcium ligninsulfonate,

2 parts of sodium laurylsulfate, and 45 parts of synthesized hydrated silicon oxide.

Formulation example 2

5 A hydrating agent was obtained by sufficiently pulverizing and mixing 70 parts of a substance with the activity of controlling biosynthesis of cytokinin, 3 parts of calcium ligninsulfonate, 2 parts of sodium laurylsulfate, and 25 parts of synthesized hydrated silicon oxide.

10

Formulation example 3

 An emulsion agent was obtained by mixing 40 parts of a substance with the activity of controlling biosynthesis of cytokinin, 3 parts of polyoxyethylene sorbitane monoolate, 2
15 parts of CMC (carboxymethyl cellulose), and 52 parts of water and wet-pulverizing the resultant mixture to be 5 μ m or smaller in the particle size.

 The present invention makes it possible to provide a
20 transformed cell co-expressing a cytokinin receptor and a cytokinin biosynthesis enzyme. Further, by using such a transformed cell, it is possible to provide a method for analyzing the activity of controlling biosynthesis of cytokinins.

 Furthermore, by using such an analysis method, it is possible
25 to provide a method for quickly finding substances having the

activity of controlling biosynthesis of cytokinins with small amounts of samples.

EXAMPLES

5 Hereinafter, although the present invention will be described in details with the reference to examples, the present invention is not at all restricted to these examples.

(Example 1) Preparation of a polynucleotide comprising a
10 nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of the polynucleotide (Part 1): production of Arabidopsis cDNA phage library for CRE1 cloning

Seeds of Arabidopsis thaliana ecotype Wassilewskija were
15 sterilized with 70% of ethyl alcohol for 1 minute and were further sterilized with 1.5% of sodium hypochlorite for 10 minutes. The resulting seeds were well washed with sterilized water and then were cultured for 2 weeks in GM culture medium [4.3 g Murashige and Skoog's basal salt mixture, 1% sucrose, 10 ml of 5% MES-KOH
20 (pH 5.7), 0.3% PHYTAGEL™ (gellan gum) (SIGMA)] to obtain 5g of the plant. After the plant was frozen in liquified nitrogen and physically milled with a mortar and a pestle. The resulting milled product was mixed with a mixed solution of 10 ml of an extraction buffer [200 mM Tris-HCl (pH 8.5), 100 mM NaCl, 10
25 mM EDTA, 0.5% SDS, 14 mM β -mercaptoethanol] and 10 g of phenol.

After being mixed by a Vortex mixer, the resulting mixture was mixed further with 10 ml of chloroform and vigorously stirred and subjected to centrifugal separation at 10,000 rotation for 20 minutes. The recovered aqueous layer was mixed with LiCl
5 in the concentration to be 2M of the final concentration, left still at -80°C for 3 hours, thawed and subjected to centrifugal separation at 10,000 rotation for 20 minutes to recover a precipitate. The recovered precipitate was dissolved in 2 ml of TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA] and then further mixed
10 with 0.2 ml of 3 M sodium acetate (pH 5.2) and 5 ml ethanol and subjected to centrifugal separation to recover RNA as a precipitate. Further, the precipitate (RNA) was subjected to treatment with OLIGOTEXTM dT30 super (oligo d(T) latex beads) (Nippon Rosch Co.) to extract RNA integrated with polyA.

15 The production of phage cDNA library from the extracted RNA integrated with polyA was carried out employing ZAP-cDNAR Synthesis Kit (Stratagene Co.) according to the instruction. The potency of the produced phage cDNA library was 500,000 PFU.

20 (Example 2) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of the polynucleotide (Part 1): production of DNA probe of CRE1

 The PCR was carried out employing TAKARA LA TAQTM
25 (thermostable DNA polymerase) (Takara Shuzo Co., Ltd.) and using

a phage solution (about 1,000,000 PFU) of the phage cDNA library produced in the example 1 as a template and a polynucleotide having the nucleotide sequence represented by SEQ ID No: 25 and a polynucleotide having the nucleotide sequence represented by
5 SEQ ID No: 26 as the primers to amplify DNA. The procedure will be described in details below.

A PCR solution was prepared by adding a reaction composition containing dNTP and the like to the phage 1,000,000 pft and respective primer each in 0.2 μ M according to the
10 instruction of the kit and the desired DNA fragment was amplified in PCR conditions: keeping at 94°C for 2 minutes, and further repeating 40 cycles each of which comprised keeping at 94°C for 30 seconds, at 55°C for 30 seconds, and at 68°C for 5 minute. Next, using the amplified DNA fragment as a template, a probe
15 labelled with 32 P was prepared employing Megaprime DNA-labelling system kit (Amersham Pharmacia Co.). Incidentally, reaction solution (25 μ l) was prepared by adding 2.0 MBq of 32 P dCTP to 25 ng of amplified DNA fragment and adding a reaction composition instructed by the kit. The labelling reaction was
20 carried out at 37°C for 10 minutes.

(Example 3) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of
25 the polynucleotide (Part 1): production of phage cDNA clone

holding CRE1 gene

The cloning of desired CRE1 gene was carried out by plaque hybridization using the probe prepared by the example 2. Detailed description will be given below.

5 Using the phage cDNA library produced in the example 1 and according to the instruction of ZAP-cDNAR Synthesis Kit, plaque was produced. DNA was adsorbed on a nitrocellulose filter from the produced plaque and then treated with UV rays to be fixed on the filter. Using the filter prepared in such a manner,
10 hybridized phage cDNA clone was obtained by keeping at 65°C in the presence of 6 x SSC (0.9 M NaCl and 0.09 M trisodium citrate), 5 x Denhard's solution [0.1% (w/v) ficoll 400, 0.1% (w/v) polyvinylpyrrolidone, 0.1% BSA], 0.5% (w/v) SDS and 100 µg/ml degenerated salmon sperm DNA or in DIG EASY Hyb solution
15 (Boeringer-Mannheim Co.) containing 100 µg/ml of degenerated salmon sperm DNA, successively keeping at a room temperature for 15 minutes two times in the presence of 1 x SSC (0.15 M NaCl and 0.015 M trisodium citrate) and 0.5% (w/v) SDS, and further keeping at 68°C for 30 minutes in the presence of 0.1 x SSC (0.015
20 M NaCl and 0.0015 M trisodium citrate) and 0.5% SDS.

(Example 4) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of
25 the polynucleotide (Part 1): cloning of CRE1 cDNA

Using the cDNA of the phage cDNA clone obtained by the example 3 as a template and a polynucleotide having the nucleotide sequence represented by SEQ ID No: 23 and a polynucleotide having the nucleotide sequence represented by SEQ ID No: 24 as primers,
5 DNA having the nucleotide sequence represented by SEQ ID No: 1 was amplified by PCR. Detailed description will be given below.

The PCR was carried out employing Herculanase Enhanced DNA Polymerase (Stratagene Co., Ltd.) in the reaction conditions of keeping at 94°C for 1 minute, and further repeating 25 cycles
10 each of which comprised keeping at 94°C for 30 seconds, at 55°C for 30 seconds, and at 72°C for 4 minute. Incidentally, the PCR solution (50 µl) was prepared by adding a reaction composition containing dNTP and the like to 500 ng of cDNA of the phage cDNA clone and respective primer each in 100 ng according to the
15 instruction of the kit.

The desired DNA fragment was amplified in such a manner.

(Example 5) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a
20 cytokinin receptor and preparation of an expression vector of the polynucleotide (Part 1): construction of CRE1 expression vector

After p415CYC1, a yeast expression vector, [Munberg et al. Gene: 156 119-122 (1995), available from ATCC library (No.
25 873821)] was digested with a restriction enzyme Sma I and then

using T4 DNA ligase, DNA having the nucleotide sequence represented by SEQ ID No: 1 and obtained by the example 4 was ligated downstream of the CYC 1 promoter sequence of the expression vector p415CYC1 as to be integrated to express the
5 desired protein in yeast. The constructed DNA was confirmed to be in the right direction and its nucleotide sequence was confirmed to be the nucleotide sequence represented by SEQ ID No: 1 by an automatic DNA sequencer and then the expression plasmid p415CYC-CRE 1 was obtained.

10

(Example 6) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of the polynucleotide (Part 2): cloning of AHK3 (AAF99730) cDNA

15 Seeds of *Arabidopsis thaliana* ecotype Wassilewskija were sterilized with 70% of ethyl alcohol for 1 minute and further sterilized with 1.5% of sodium hypochlorite for 10 minutes. The resulting seeds were well washed with sterilized water and then cultured for 2 weeks in GM culture medium [4.3 g Murashige and
20 Skoog's basal salt mixture, 1% sucrose, 10 ml of 5% MES-KOH (pH 5.7), 0.3% PHYTAGEL™ (gellan gum) (SIGMA)] to obtain 5g of the plant. After the plant was frozen in liquified nitrogen and physically milled with a mortar and a pestle. The resulting milled product was mixed with a mixed solution of 10 ml of an
25 extraction buffer [200 mM Tris-HCl (pH 8.5), 100 mM NaCl, 10

mM EDTA, 0.5% SDS, 14 mM β -mercaptoethanol] and 10 g of phenol. After being mixed by a Voltex mixer, the resulting mixture was mixed further with 10 ml of chloroform and vigorously stirred and subjected to centrifugal separation at 10,000 rotation for 5 20 minutes. The recovered aqueous layer was mixed with LiCl in the concentration to be 2M of the final concentration, left still at -80°C for 3 hours, thawed and subjected to centrifugal separation at 10,000 rotation for 20 minutes to recover a precipitate. The recovered precipitate was dissolved in 2 ml 10 of TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA] and then further mixed with 0.2 ml of 3 M sodium acetate (pH 5.2) and 5 ml ethanol and subjected to centrifugal separation to recover RNA as a precipitate. Further, 40 μ g of the precipitate (RNA) was mixed with 30 unit of FPLC pureTM DnaseI (RNase free-DNase 15 I) (Amersham-Pharmacia) and 60 unit of Superace (Ambion) to remove mixed genome DNA and the resulting RNA was subjected to the phenol/chloroform treatment and ethanol treatment to purify the RNA. Next, using the purified RNA as a template and oligo (dT) 12-18 (Amersham-Pharmacia) as a primer, RT-PCR was carried out. 20 The RT-PCR was carried out employing Superscript II (GIBCO BRL Co.) at 42°C for 40 minutes. Incidentally the PT-PCR solution was prepared according to the method described in instruction of the Superscript II.

The desired cDNA was amplified in such a manner.

25 Using the amplified cDNA as a template and a polynucleotide

having the nucleotide sequence represented by SEQ ID No: 27 and a polynucleotide having the nucleotide sequence represented by SEQ ID No: 28 as primers, DNA having the nucleotide sequence represented by SEQ ID No: 5 was amplified by PCR. The PCR was
5 carried out using Herculase Enhanced DNA Polymerase (Stratagene Co., Ltd.) in the reaction conditions of keeping at 94°C for 1 minute, and further repeating 41 cycles each of which comprised keeping at 94°C for 30 seconds, at 55°C for 30 seconds, and at 72°C for 4 minute. Incidentally, the PCR solution (50 µl) was
10 prepared by adding a reaction composition containing dNTP and the like to 500 ng of the template DNA and respective primer each in 100 ng according to the instruction of the kit.

The desired DNA fragment was amplified in such a manner.

15 (Example 7) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of the polynucleotide (Part 2): construction of pHM-1

After p415CYC1 [Munberg et al. Gene: 156 119-122 (1995);
20 available from ATCC library (No. 87382)] was digested with the restriction enzyme Spe I and BamH I, synthesized DNA fragments (linkers) having the nucleotide sequence represented by SEQ ID Nos: 29 and 30 were inserted to the expression vector p415CYC1 using T4 DNA ligaseas to newly add the restriction enzyme sites
25 Sac II, Apa I, Nhe I to the plasmid p415CYC1 and construct pHM-1.

(Example 8) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of
5 the polynucleotide (Part 2): construction of AHK3 expression vector

After the pHM-1 was digested with the restriction enzyme Sal I and Sac II, and then using T4 DNA ligase, DNA having the nucleotide sequence represented by SEQ ID No: 5 was introduced
10 downstream of the CYC1 promoter sequence of the expression vector pHM-1 as to be integrated to express the desired protein in yeast. The constructed DNA was confirmed to be right in the direction and its nucleotide sequence was confirmed to be right in the sequence by an automatic DNA sequencer and thus the expression
15 plasmid p415CYC-AHK3 was obtained.

(Example 9) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of
20 the polynucleotide (Part 2): cloning of AHK2 (BAB09274) cDNA

Using the cDNA prepared in the example 6 (the cDNA prepared by reverse transcription of the total RNA) as a template and a polynucleotide having the nucleotide sequence represented by SEQ ID No: 31 and a polynucleotide having the nucleotide sequence
25 represented by SEQ ID No: 32 as primers, DNA having the nucleotide

sequence represented by SEQ ID No: 3 was amplified by PCR. The PCR was carried out employing TAKARA Pfu Turbo denature (Takara Shuzo Co., Ltd.) in the reaction conditions of keeping at 94°C for 1 minute, and further repeating 30 cycles each of which
5 comprised keeping at 94°C for 30 seconds, at 55°C for 30 seconds, and at 72°C for 4 minute. Incidentally, the PCR solution (50 µl) was prepared by adding a reaction composition containing dNTP and the like to 500 ng of the template DNA and respective primer each in 50 ng according to the instruction of the kit.
10 The desired DNA fragment was amplified in such a manner.

(Example 10) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of
15 the polynucleotide (Part 2): cloning of AHK2(BAB09274)ΔcDNA

Using the cDNA prepared in example 6 (the cDNA prepared by reverse transcription of the total RNA) as a template and a polynucleotide having the nucleotide sequence represented by SEQ ID No: 33 and a polynucleotide having the nucleotide sequence
20 represented by SEQ ID No: 34 as primers, PCR was carried out to amplify DNA containing the nucleotide sequence represented by nucleotide numbers 586 to 3531 of SEQ ID No: 3 in which ATG was added to the 5' terminal sites of said nucleotide sequence. The PCR was carried out employing TAKARA Pfu Turbo denature
25 (Takara Shuzo Co., Ltd.) in the reaction conditions of keeping

at 94°C for 1 minute, and further repeating 30 cycles each of which comprised keeping at 94°C for 30 seconds, at 55°C for 30 seconds, and at 72°C for 4 minute. Incidentally, the PCR solution (50 µl) was prepared by adding a reaction composition
5 containing dNTP and the like to 500 ng of the template DNA and respective primer each in 50 ng according to the instruction of the kit. The desired DNA fragment was amplified in such a manner.

10 (Example 11) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin receptor and preparation of an expression vector of the polynucleotide (Part 2): construction of AHK2 and AHK2 Δ expression vectors

15 After pHM-1 was digested with a restriction enzyme Sac II and Nhe I and then using T4 DNA ligase, DNA fragments obtained by the example 9 and the example 10 were respectively ligated downstream to the CYC1 promoter sequence of the expression vector pHM-1 as to be integrated to express the desired protein in yeast.
20 The introduced DNA was confirmed to be right in the direction and its nucleotide sequence was confirmed to be right in the sequence by an automatic DNA sequencer and thus the expression plasmid p415CYC-AHK 2 and p415CYC-AHK2Δ were obtained.

25 (Reference example 1) Production of transformed cells

TM182-CRE1, TM182-AHK2, TM182-AHK2Δ and TM182-AHK3

Transformation of TM182 (*sln1Δ*), *SLN1* genetically
defected strain, [Maeda T et al. Nature: 369 242-245 (1994)]
was carried out using the obtained expression plasmid
5 p415CYC-CRE1 (the example 5), p415CYC-AHK2 (the example 11),
p415CYC-AHK2Δ (the example 11) and p415CYC-AHK3 (the example
8). The transformation was carried out by employing
Polyethylene glycol/lithium acetate (PEG/LiAc)-mediated
transformation method according to the description of VII.
10 Library Transformation & Screening Protocols disclosed in
MATCHAMAKER Two-Hybrid System 3 User Manual p. 22 from CLONTECH
Co. Transformed cells TM182-CRE1, TM182-AHK2, TM182-AHK2Δ and
TM182-AHK3 were produced by selecting transformed cells that
were grown in the DOLU + Gal culture medium based on the fact
15 that leucine auxotrophy disappears in the transformed cells.

(Reference example 2) Response of transformed cells TM182-CRE1,
TM182-AHK2, TM182-AHK2Δ and TM182-AHK3 to cytokinin (Part 1)

A culture solution 10 μl (about 800 clones of yeast) of
20 the transformed cells TM182-CRE1, TM182-AHK2, TM182-AHK2Δ and
TM182-AHK3 produced by the example 12 was spotted on DOLU + Glu
agar media containing 10 μM of trans-zeatin and cultured at 30°C
for 30 hours. After incubation, the growth state of the
transformed cells was observed and photographed by a digital
25 camera.

As a result, each of the transformed cells TM182-CRE1, TM182-AHK2, TM182-AHK2Δ and TM182-AHK3 showed high growth in the case of using the DOLU + Glu agar culture media containing trans-zeatin as compared with that in the case of using the DOLU + Glu agar culture media containing no trans-zeatin. The results showed that the transformed cells responded to cytokinin and that they were possible to be grown in the DOLU + Glu agar culture media. Further the transformed cells were found possible to be grown in the DOLU + Gal agar culture media independently of the existence trans-zeatin.

(Reference example 3) Response of transformed cells TM182-CRE1, TM182-AHK2Δ and TM182-AHK2 to cytokinin (Part 2)

Culture solutions of the transformed cells TM182-CRE1, TM182-AHK2Δ and TM182-AHK2 produced by the reference example 1 were spotted on DOLU + Glu agar media containing cytokinin in a variety of concentrations (1 nM, 10 nM, 100 nM, 1 μM, 10 μM, 100 μM) and cultured at 30°C for 30 hours. After incubation, the growth state of the transformed cells was observed and photographed by a digital camera. The lowest observed supply concentrations of trans-zeatin and cis-zeatin in which the respective transformed cells could be grown were shown in Table 1.

Table 1

cytokinin	TM182-CRE1	TM182-AHK2	TM182-AHK2Δ
trans-zeatin	10μM	1μM	100nM
cis-zeatin	no growth	10μM	1μM

(Reference example 4) Method for searching substance having agonist-activity to cytokinin receptor (Part 1)

5 The transformed cells TM182-AHK2, TM182-AHK2Δ and TM182-AHK3 produced in the reference example 1 were inoculated in 200 ml of DOLU + Gal culture media and pre-cultured at 30°C for 36 hours. The pre-cultured solutions were diluted with DOLU + Glu media as to become 0.100 as the optical density (OD₆₀₀)
 10 and further the resultants were diluted with DOLU + Glu media at a dilution rate of 1/200 to obtain diluted pre-cultured solutions.

An assay plate was prepared by filling respective wells of a 96-well plate with 20 μl of solutions which were prepared
 15 by diluting DMSO solution (10mM) of each examinee substance with DOLU + Glu medium at a dilution rate of 1/100, said solutions containing each examinee substance to be 100 μM at the final concentration. Simultaneously, an assay plate only filled with
 20 20 μl of solutions which were prepared by diluting DMSO solution with DOLU + Glu medium, said solutions containing no examinee substance, was prepared as the blank sections.

The diluted pre-cultured solutions were added in 200 μl

each into the respective wells of both assay plates and cultured at 30°C for 24 hours and then the optical density (OD₆₂₀) of each well was measured by a plate reader. The agonist-activity of the examinee substances to the cytokinin receptor was detected by comparing the optical density measured in the testing sections to which the examinee substances were added with the optical density measured in the blank sections. The optical density of the culture solutions of the transformed cells in the testing sections to which the examinee substances were added were shown in Table 2. Compound B showing the higher optical density measured in the testing sections to which the examinee substances were added than that in the blank sections were selected as agonist-active substance to the cytokinin receptor.

Table 2

Examinee substance	TM182-AHK2	TM182-AHK2Δ	TM182-AHK3
DMSO	0.01	0	0.51
Compound A ^{*1}	0.01	0	0.54
Compound B ^{*2}	0.45	0.89	0.88

^{*1} Absciscic acid (=negative control)

^{*2} 6-Benzyl aminopurine (=positive control)

(Reference example 5) Method for searching substance having agonist-activity to cytokinin receptor (Part 2)

The transformed cells TM182-AHK2, TM182-AHK2Δ and TM182-AHK3 produced in the reference example 1 are inoculated in 200 ml of DOLU + Gal culture media and cultured at 30°C for 30 hours to obtain pre-cultured solutions.

5 The pre-cultured transformed cells (TM182-AHK2, TM182-AHK2Δ and TM182-AHK3) are spot-added in 10 μl to the respective DOLU + Glu agar culture media to which agonist-active substances to the cytokinin receptor selected by the reference example 4 are added while their concentration being changed from
10 10 nM to 100 μM and then are cultured at 30°C for 30 hours. After incubation, the intensity of the agonist-activity of the examinee substances to the cytokinin receptor is detected and confirmed based on the lowest concentration at which transformed cells (TM182-AHK2, TM182-AHK2Δ and TM182-AHK3) are observed to grow.

15

(Example 12) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin biosynthesis enzyme and preparation of an expression vector of the polynucleotide: Cloning of AtIPT4 cDNA)

20 Seeds of *Arabidopsis thaliana* ecotype Wassilewskija were sterilized with 70% ethyl alcohol for 1 minute, and were further sterilized with 1.5% sodium hypochlorite for 10 minutes. These seeds were well washed with sterilized water, and then were cultured for 20 days in GM culture medium (4.3 g Murashige and
25 Skoog's basal salt mixture, 1% sucrose, 10 mL of 5% MES-KOH (pH

5.7), 0.3% PHYTAGELTM (gellangum) (SIGMA)) to obtain 5 g of plants. The plants were frozen in liquified nitrogen, and were then physically milled in a 1.5 mL tube. To the milled product, 100 μ L of 2 $\times$ CTAB (2% (w/v) Cetyl trimethyl ammonium bromide (hereinafter, referred to as "CTAB"), 0.1M Tris-HCl (pH 8.0), 1.4M NaCl, 1% (w/v) PVP) was added. The resulting mixture was left standing at 60°C for 30 minutes, and then 100 μ L of a mixed solution of chloroform and isoamyl alcohol (chloroform: isoamyl alcohol = 24: 1) was added. The resulting mixture was stirred with a Voltex mixer, and was then subjected to centrifugal separation at 15,000 rpm at 20°C for 20 minutes. After centrifugal separation, 75 μ L of the resulting supernatant was placed in a 0.6 mL tube, in which 75 μ L of isoamyl alcohol had been already placed, and the resulting mixture was left standing at 25°C for 20 minutes. Thereafter, the mixture was subjected to centrifugal separation at 15,000 rpm at 20°C for 20 minutes to recover a precipitate. To the recovered precipitate, 300 μ L of 70% ethanol was added, and the resulting mixture was stirred with a Voltex mixer. The mixture was subjected to centrifugal separation at 15,000 rpm at 4°C for 15 minutes to recover a precipitate. The recovered precipitate was left standing at 37°C for 5 minutes, and then 30 μ L of T10E0.2 (10mM Tris-HCl (pH 8.0), 0.2mM EDTA (pH 8.0)) was added to dissolve the precipitate. In this way, a genomic DNA solution was obtained. Next, PCR was carried out using the obtained genomic DNA as a

template and a polynucleotide having the nucleotide sequence represented by SEQ ID No: 35 and a polynucleotide having the nucleotide sequence represented by SEQ ID No: 36 as primers to amplify DNA having the nucleotide sequence represented by SEQ ID No: 11. The PCR was carried out employing Herculase Enhanced DNA Polymerase (Stratagene Co., Ltd.) under the amplifying conditions of keeping at 94°C for 1 minute, repeating 40 cycles each including keeping at 94°C for 30 seconds, at 50°C for 30 seconds, and at 72°C for 60 seconds, and final keeping at 72°C for 1 minute. A PCR solution (50 µL) was prepared by adding a reaction composition such as dNTP and the like to 100 ng of the template DNA and 100 ng of each of the primers according to the instruction of the kit. In this way, a desired DNA fragment was amplified.

15

(Example 13) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin biosynthesis enzyme and preparation of an expression vector of the polynucleotide: construction of AtIPT4 expression vector

After p423ADH, a yeast expression vector, (Munberg et al. Gene: 156 119-122 (1995), available from ATCC library (No. 87371) was digested with a restriction enzyme Sma I and then using T4 DNA ligase, DNA having the nucleotide sequence represented by SEQ ID No: 11 and obtained by the example 12 was ligated downstream

of the ADH promoter sequence of the expression vector p423ADH as to be integrated to express the desired protein in yeast. The constructed DNA was confirmed to be in the right direction and its nucleotide sequence was confirmed to be the nucleotide
5 sequence represented by SEQ ID No: 11 by an automatic DNA sequencer and then the expression plasmid p423ADH-IPT4 was obtained.

In a similar manner, after p423CYC1, a yeast expression vector, (Munberg et al. Gene: 156 119-122 (1995), available from ATCC library (No. 87379) was digested with a restriction enzyme
10 Sma I and then using T4 DNA ligase, DNA having the nucleotide sequence represented by SEQ ID No: 11 and obtained by the example 12 was ligated downstream of the CYC 1 promoter sequence of the expression vector p423CYC1 as to be integrated to express the desired protein in yeast. The constructed DNA was confirmed
15 to be in the right direction and its nucleotide sequence was confirmed to be the nucleotide sequence represented by SEQ ID No: 11 by an automatic DNA sequencer and then the expression plasmid p423CYC1-IPT4 was obtained.

20 (Example 14) Preparation of a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence of a cytokinin biosynthesis enzyme and preparation of an expression vector of the polynucleotide: cloning of AtIPT5 cDNA

In a similar manner to Example 12, PCR was carried out
25 by using the genomic DNA obtained from the plants of Arabidopsis

thaliana ecotype Wassilewskija as a template and a polynucleotide having a nucleotide sequence represented by SEQ ID No: 37 and a polynucleotide having a nucleotide sequence represented by SEQ ID No: 38 as primers to amplify DNA having a nucleotide sequence represented by SEQ ID No: 13. The PCR was carried out employing Herculanase Enhanced DNA Polymerase (Stratagene Co., Ltd.) under the amplifying conditions of keeping at 94°C for 1 minute, repeating 35 cycles each including keeping at 94°C for 30 seconds, at 55°C for 30 seconds, and at 72°C for 90 seconds, and final keeping at 72°C for 1 minute. A PCR solution (50 µL) was prepared by adding a reaction composition such as dNTP and the like to 100 ng of the template DNA and 100 ng of each of the primers according to the introduction of the kit. In this way, a desired DNA fragment was amplified.

15

(Example 16) Production of transformed cells of the present invention: Production of transformed cells TM182-CYC1-CYC1IPT4, TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5, TM182-CYC1-ADHIPT5, TM182-CRE1-CYC1IPT4, TM182-CRE1-ADHIPT4, TM182-CRE1-CYC1IPT5, TM182-CRE1-ADHIPT5, TM182-AHK2-CYC1IPT4, TM182-AHK2-ADHIPT4, TM182-AHK2-CYC1IPT5, and TM182-AHK2-ADHIPT5

Transformation of TM182 (Sln1Δ), *SLN1* genetically defected strain, [Maeda T et al. Nature: 369 242-245 (1994)] was carried out by using any one of the three expression plasmids, commercially available p415CYC1, p415CYC-CRE1 obtained in

Example 5, and p415CYC-AHK2 obtained in Example 11, and any one of the four expression plasmids, p423ADH-IPT4 and p423CYC1-IPT4 obtained in Example 13, and p423ADH-IPT5 and p423CYC1-IPT5 obtained in Example 15 together (that is, by using two expression
5 plasmids at the same time). The transformation was carried out by employing Polyethylene glycol/lithium acetate (PEG/LiAc)-mediated transformation method according to the description of VII. Library Transformation & Screening Protocols disclosed in MATCHMAKER Two-Hybrid System 3 User Manual p. 22
10 from CLONTECH Co. Twelve kinds of transformed cells TM182-CYC1-CYC1IPT4 to which p415CYC1 and p423CYC1-IPT4 are introduced, TM182-CYC1-ADHIPT4 to which p415CYC1 and p423ADH-IPT4 are introduced, TM182-CYC1-CYC1IPT5 to which p415CYC1 and p423CYC1-IPT5 are introduced, TM182-CYC1-ADHIPT5
15 to which p415CYC1 and p423ADH-IPT5 are introduced, TM182-CRE1-CYC1IPT4 to which p415CYC-CRE1 and p423CYC1-IPT4 are introduced, TM182-CRE1-ADHIPT4 to which p415CYC-CRE1 and p423ADH-IPT4 are introduced, TM182-CRE1-CYC1IPT5 to which p415CYC-CRE1 and p423CYC1-IPT5 are introduced,
20 TM182-CRE1-ADHIPT5 to which p415CYC-CRE1 and p423ADH-IPT5 are introduced, TM182-AHK2-CYC1IPT4 to which p415CYC-AHK2 and p423CYC1-IPT4 are introduced, TM182-AHK2-ADHIPT4 to which p415CYC-AHK2 and p423ADH-IPT4 are introduced, TM182-AHK2-CYC1IPT5 to which p415CYC-AHK2 and p423CYC1-IPT5 are
25 introduced, and TM182-AHK2-ADHIPT5 to which p415CYC-AHK2 and

p423ADH-IPT5 are introduced were produced by selecting transformed cells that were grown in DOHLU+Gal culture medium based on the fact that leucine auxotrophy and histidine auxotrophy disappear in the transformed cells.

5

(Example 17) Production of transformed cells of the present invention: production of transformed cells

TM182-AHK2Δ-CYC1IPT4, TM182-AHK2Δ-ADHIPT4,

TM182-AHK2Δ-CYC1IPT5, TM182-AHK2Δ-ADHIPT5,

10 TM182-AHK3-CYC1IPT4, TM182-AHK3-ADHIPT4, TM182-AHK3-CYC1IPT5, and TM182-AHK3-ADHIPT5

Transformation of TM182 (*Sln1*Δ), *SLN1* genetically defected strain, [Maeda T et al. Nature: 369 242-245 (1994)] is carried out by using any one of the two expression plasmids, 15 p415CYC-AHK2Δ obtained in Example 11 and p415CYC-AHK3 obtained in Example 8, and any one of the four expression plasmids, p423ADH-IPT4 and p423CYC1-IPT4 obtained in Example 13, and p423ADH-IPT5 and p423CYC1-IPT5 obtained in Example 15 together (that is, by using two expression plasmids at the same time). 20 The transformation is carried out by employing Polyethylene glycol/lithium acetate (PEG/LiAc)-mediated transformation method according to the description of VII. Library Transformation & Screening Protocols disclosed in MATCHMAKER Two-Hybrid System 3 User Manual p. 22 from CLONTECH Co. Eight 25 kinds of transformed cells TM182-AHK2Δ-CYC1IPT4 to which

p415CYC-AHK2 Δ and p423CYC1-IPT4 are introduced, TM182-AHK2 Δ -ADHIPT4 to which p415CYC-AHK2 Δ and p423ADH-IPT4 are introduced, TM182-AHK2 Δ -CYC1IPT5 to which p415CYC-AHK2 Δ and p423CYC1-IPT5 are introduced, TM182-AHK2 Δ -ADHIPT5 to which
5 p415CYC-AHK2 Δ and p423ADH-IPT5 are introduced, TM182-AHK3-CYC1IPT4 to which p415CYC-AHK3 and p423CYC1-IPT4 are introduced, TM182-AHK3-ADHIPT4 to which p415CYC-AHK3 and p423ADH-IPT4 are introduced, TM182-AHK3-CYC1IPT5 to which p415CYC-AHK3 and p423CYC1-IPT5 are introduced, and
10 TM182-AHK3-ADHIPT5 to which p415CYC-AHK3 and p423ADH-IPT5 are introduced are produced by selecting transformed cells that are grown in DOHLU+Gal culture medium based on the fact that leucine auxotrophy and histidine auxotrophy disappear in the transformed cells.

15

(Example 18) Use of transformed cells of the present invention: culture of transformed cells TM182-CYC1-CYC1IPT4, TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5, TM182-CYC1-ADHIPT5, TM182-CRE1-CYC1IPT4, TM182-CRE1-ADHIPT4, TM182-AHK2-CYC1IPT4,
20 TM182-AHK2-ADHIPT4, TM182-AHK2-CYC1IPT5, and TM182-AHK2-ADHIPT5

10 μ L of the culture solution (containing about 800 clones of yeast) of each of the transformed cells TM182-CYC1-CYC1IPT4, TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5, TM182-CYC1-ADHIPT5,
25 TM182-CRE1-CYC1IPT4, TM182-CRE1-ADHIPT4, TM182-AHK2-CYC1IPT4,

TM182-AHK2-ADHIPT4, TM182-AHK2-CYC1IPT5, and
TM182-AHK2-ADHIPT5 produced in Example 16 was spotted on
DOHLU+Glu agar culture medium, and was cultured at 30°C for 30
hours. After incubation, the growth state of the transformed
5 cells was observed and photographed by a digital camera.

As a result, the transformed cells TM182-CYC1-CYC1IPT4,
TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5, and
TM182-CYC1-ADHIPT5 were not observed to grow, but the transformed
cells TM182-CRE1-CYC1IPT4, TM182-CRE1-ADHIPT4,
10 TM182-AHK2-CYC1IPT4, TM182-AHK2-ADHIPT4, TM182-AHK2-CYC1IPT5,
and TM182-AHK2-ADHIPT5 were observed to grow. Further, in a
case where DOHLU+Glu agar culture medium containing 10 µM
trans-zeatin was used, the transformed cells
TM182-CYC1-CYC1IPT4, TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5,
15 and TM182-CYC1-ADHIPT5 were not observed to grow, but the
transformed cells TM182-CRE1-CYC1IPT4, TM182-CRE1-ADHIPT4,
TM182-AHK2-CYC1IPT4, TM182-AHK2-ADHIPT4, TM182-AHK2-CYC1IPT5,
and TM182-AHK2-ADHIPT5 were observed to grow. This indicates
that the transformed cells TM182-CRE1-CYC1IPT4,
20 TM182-CRE1-ADHIPT4, TM182-AHK2-CYC1IPT4, TM182-AHK2-ADHIPT4,
TM182-AHK2-CYC1IPT5, and TM182-AHK2-ADHIPT5 respond to
cytokinins synthesized by themselves and can grow on DOHLU+Glu
agar culture medium. It was also confirmed that all of the ten
kinds of transformed cells could grow in DOHLU+Gal culture medium
25 regardless of the presence or absence of trans-zeatin.

(Example 19) Use of transformed cells of the present invention:
culture of transformed cells TM182-CYC1-CYC1IPT4,
TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5, TM182-CYC1-ADHIPT5,
5 TM182-CRE1-CYC1IPT4, and TM182-CRE1-ADHIPT5

Each of the transformed cells TM182-CYC1-CYC1IPT4,
TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5, TM182-CYC1-ADHIPT5,
TM182-CRE1-CYC1IPT4, and TM182-CRE1-ADHIPT5 obtained in
Example 16 was inoculated in 200 mL of DOHLU+Gal culture medium,
10 and was pre-cultured at 30°C for 36 hours. The thus obtained
culture solutions were diluted with DOHLU+Glu culture medium
so that OD₆₀₀ became 0.200, and then the diluted ones were further
diluted with DOHLU+Glu culture medium at a dilution rate of 1/200
to obtain diluted pre-cultured solutions.

15 A 96-well assay plate was prepared by filling each well
with 1 µL of a DMSO solution of an examinee substance (Absciscic
acid or 6-Benzyl aminopurine). In this regard, it is to be noted
that the DMSO solution of a examinee substance was prepared so
that the concentration thereof became 200 ppm. Simultaneously,
20 an assay plate was prepared by filling each well with 1 µL of
DMSO as a blank section.

Each of the diluted pre-cultured solutions was added in
a volume of 100 µL per each well of both of the assay plates,
and was cultured at 30°C for 24 hours. After incubation, the
25 turbidity of each well was measured using a plate reader. The

absorbance (OD_{600}) of each of the culture solutions of the transformed cells containing the examinee substance was shown in Table 3. The transformed cells TM182-CYC1-CYC1IPT4, TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5, and
5 TM182-CYC1-ADHIPT5 were not observed to grow whichever examinee substance was added. On the other hand, the transformed cells TM182-CRE1-CYC1IPT4 and TM182-CRE1-ADHIPT5 were observed to grow whichever examinee substance was added. Further, the growth speeds of the transformed cells TM182-CRE1-CYC1IPT4 and
10 TM182-CRE1-ADHIPT5 in the culture medium to which 6-Benzyl aminopurine was added were faster than those in the culture medium to which only DMSO was added. This indicates that the transformed cells TM182-CRE1-CYC1IPT4 and TM182-CRE1-ADHIPT5 respond to cytokinins synthesized by the cells and can grow in DOHLU+Glu
15 culture medium and that the growth speeds of the transformed cells TM182-CRE1-CYC1IPT4 and TM182-CRE1-ADHIPT5 are increased or decreased depending on the amount of cytokinin.

20

25

Table 3

Examinee substance	TM182- CYC1- CYC1- IPT4	TM182- CYC1- ADH- IPT4	TM182- CYC1- CYC1- IPT5	TM182- CYC1- ADH- IPT5	TM182- CRE1- CYC1- IPT4	TM182- CRE1- ADH- IPT5
DMSO	0.042	0.040	0.041	0.043	0.106	0.129
Abscisic acid	0.042	0.040	0.041	0.043	0.102	0.131
6-Benzyl aminopurine	0.042	0.041	0.043	0.043	0.260	0.293

(Example 20) Use of transformed cells of the present invention:

culture of transformed cells TM182-CYC1-CYC1IPT4,

- 5 TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5, TM182-CYC1-ADHIPT5,
TM182-CRE1-ADHIPT4, TM182-AHK2-CYC1IPT4, TM182-AHK2-CYC1IPT5
and TM182-AHK2-ADHIPT5

Each of the transformed cells TM182-CYC1-CYC1IPT4,
TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5, TM182-CYC1-ADHIPT5,
10 TM182-CRE1-ADHIPT4, TM182-AHK2-CYC1IPT4, TM182-AHK2-CYC1IPT5
and TM182-AHK2-ADHIPT5 obtained in Example 16 was inoculated
in 200 mL of DOHLU+Gal culture medium, and was pre-cultured at
30°C for 36 hours. The thus obtained culture solutions were
diluted with DOHLU+Glu culture medium so that OD₆₀₀ became 0.200,
15 and then the diluted ones were further diluted with DOHLU+Glu
culture medium at a dilution rate of 1/200 to obtain diluted

pre-cultured solutions.

A 96-well assay plate was prepared by filling each well with 1 μ L of a DMSO solution of an examinee substance (Absciscic acid or 6-Benzyl aminopurine). In this regard, it is to be noted
5 that the DMSO solution of a examinee substance was prepared so that the concentration thereof became 200 ppm. Simultaneously, an assay plate was prepared by filling each well with 1 μ L of DMSO as a blank section.

Each of the diluted pre-cultured solutions was added in
10 a volume of 100 μ L per each well of both of the assay plates, and was cultured at 30°C for 24 hours. After incubation, the turbidity of each well was measured using a plate reader. The absorbance (OD_{600}) of each of the culture solutions of the transformed cells containing the examinee substance was shown
15 in Table 4. The transformed cells TM182-CYC1-CYC1IPT4, TM182-CYC1-ADHIPT4, TM182-CYC1-CYC1IPT5 and TM182-CYC1-ADHIPT5 were not observed to grow whichever examinee substance was added. On the other hand, the transformed cells TM182-CRE1-ADHIPT4, TM182-AHK2-CYC1IPT4, TM182-AHK2-CYC1IPT5
20 and TM182-AHK2-ADHIPT5 were observed to grow whichever examinee substance was added. Further, the growth speeds of the transformed cells TM182-CRE1-ADHIPT4, TM182-AHK2-CYC1IPT4, TM182-AHK2-CYC1IPT5 and TM182-AHK2-ADHIPT5 in the culture medium to which 6-Benzyl aminopurine was added were faster than
25 those in the culture medium to which only DMSO was added. This

indicates that the transformed cells TM182-CRE1-ADHIPT4,
 TM182-AHK2-CYC1IPT4, TM182-AHK2-CYC1IPT5 and
 TM182-AHK2-ADHIPT5 respond to cytokinins synthesized by the
 cells and can grow in DOHLU+Glu culture medium and that the growth
 5 speeds of the transformed cells TM182-CRE1-ADHIPT4,
 TM182-AHK2-CYC1IPT4, TM182-AHK2-CYC1IPT5 and
 TM182-AHK2-ADHIPT5 are increased or decreased depending on the
 amount of cytokinin.

10 Table 4

Examinee substance	TM182- CYC1- CYC1- IPT4	TM182- CYC1- ADH- IPT4	TM182- CYC1- CYC1- IPT5	TM182- CYC1- ADH- IPT5	TM182- CRE1- ADH- IPT4	TM182- AHK2- CYC1- IPT4	TM182- AHK2- CYC1- IPT5	TM182- AHK2- ADH- IPT5
DMSO	0.035	0.035	0.035	0.035	0.044	0.199	0.071	0.301
Abscisic acid	0.035	0.035	0.036	0.035	0.045	0.194	0.069	0.297
6-Benzyl amino- purine	0.034	0.034	0.035	0.035	0.058	0.339	0.430	0.427

(Example 21) Use of transformed cells of the present invention:
 method for searching substances having activity of controlling
 cytokinin biosynthesis

15 The transformed cell TM182-CRE1-ADHIPT5 obtained in
 Example 16 is inoculated in 200 mL of DOHLU+Gal culture medium,
 and is then pre-cultured at 30°C for 36 hours. The culture

solution is diluted with DOHLU+Glu culture medium so that OD₆₀₀ become 0.200. The diluted one is further diluted with DOHLU+Glu culture medium at a dilution ratio of 1/200 to obtain a diluted pre-cultured solution. Similarly, TM182 (*sln1* Δ), a *SLN1* 5 genetically defected strain in which *PTP2* Tyrosine phosphatase gene is introduced, is inoculated in 200 mL of DOLU+Gal culture medium, and is then pre-cultured at 30°C for 36 hours. The culture solution is diluted with DOLU+Gal culture medium so that OD₆₀₀ become 0.100. The diluted one is further diluted with DOLU+Gal 10 culture medium at a dilution ratio of 1/200 to obtain a diluted pre-cultured solution. Further, the transformed cell TM182-CRE1 obtained in Reference Example 1 is inoculated in 200 mL of DOLU+Gal culture medium, and is then pre-cultured at 30°C for 36 hours. The culture solution is diluted with DOLU+Glu 15 culture medium so that OD₆₀₀ become 0.100. The diluted one is further diluted with DOLU+Glu culture medium at a dilution ratio of 1/200 to obtain a diluted pre-cultured solution. A diluted pre-cultured solution of transformed cell TM182-CRE1 to which 6-Benzyl aminopurine (cytokinin) is added is also prepared so 20 that the concentration of the 6-Benzyl aminopurine become 2 ppm.

A 96-well assay plate is prepared by filling each well with 1 μL of a DMSO solution of a examinee substance. In this regard, it is to be noted that the DMSO solution of a examinee substance is prepared so that the concentration thereof become 25 200 ppm. Simultaneously, an assay plate is prepared by filling

each well with 1 μ L of DMSO as a blank section.

Each of the diluted pre-cultured solutions is added in a volume of 100 μ L per each well of both of the assay plates, and is cultured at 30°C for 24 hours. After incubation, the
5 turbidity of each well is measured using a plate reader. In a case where it is confirmed that the growth speed of the transformed cell TM182-CRE1-ADHIPT5 in a well containing a examinee substance is increased or decreased as compared with that in a well of the blank section to which only DMSO is added,
10 the examinee substance contained in the well can be selected as a substance having the activity of controlling cytokinin biosynthesis. Incidentally, in a case where, in a well containing the examinee substance which is confirmed to increase or decrease the growth speed of the transformed cell
15 TM182-CRE1-ADHIPT5 as compared with that in the blank section to which only DMSO is added, the growth speed of the transformed cell TM182-CRE1 is not increased or decreased as compared with that in the blank section to which only DMSO is added, the examinee substance can be selected as a substance that has the activity
20 of controlling cytokinin biosynthesis and that may not directly affect signal transduction system from cytokinin receptor. Further, in a case where, in a well containing the examinee substance which is confirmed to increase or decrease the growth speed of the transformed cell TM182-CRE1-ADHIPT5 as compared
25 with that in the blank section to which only DMSO is added, the

growth speed of the transformed cell TM182 (*sln1* Δ) is not increased or decreased as compared with that in the blank section to which only DMSO is added, the examinee substance can be selected as a substance that has the activity of controlling cytokinin biosynthesis and that may not affect somewhere not involved in biosynthesis of cytokinin nor signal transduction from cytokinin receptor.

Hereinafter, the medium compositions to be used in the present invention will be described:

(a) DOLU + Glu culture medium

Bacto-yeast nitrogen base without amino acids	6.7g
Glucose	20g
Drop-out mix (x)	2.0g
15 Distilled water	1000ml

(b) DOLU + Gal culture medium

Bacto-yeast nitrogen base without amino acids	6.7g
Galactose	20g
20 Drop-out mix (x)	2.0g
Distilled water	1000ml

(x) Drop-out mix: Drop-out mix is a combination of the following ingredients.

25

Adenine	0.5g	Lysine	2.0g
Alanine	2.0g	Methionine	2.0g
Arginine	2.0g	para-Aminobenzoic acid	0.2g
Asparagine	2.0g	Phenylalanine	2.0g
Aspartic acid	2.0g	Proline	2.0g
Cysteine	2.0g	Serine	2.0g
Glutamine	2.0g	Threonine	2.0g
Glutamic acid	2.0g	Tryptophan	2.0g
Glycine	2.0g	Tyrosine	2.0g
Histidine	2.0g	Valine	2.0g
Inositol	2.0g	Isoleucine	2.0g

(c) DOLU + Glu agar culture medium

A solid culture medium prepared by adding 2% (W/V) of agar into the culture medium (a)

5

(d) DOLU + Gal agar culture medium

A solid culture medium prepared by adding 2% (W/V) of agar into the culture medium (c)

10 (e) DOHLU + Glu culture medium

Bacto-yeast nitrogen base without amino acids	6.7g
Glucose	20g
Drop-out mix (y)	2.0g

Distilled water 1000ml

(f) DOHLU + Gal culture medium

Bacto-yeast nitrogen base without amino acids 6.7g

5 Galactose 20g

Drop-out mix (y) 2.0g

Distilled water 1000ml

(y) Drop-out mix: Drop-out mix is a combination of the following
10 ingredients.

Adenine	0.5g	Lysine	2.0g
Alanine	2.0g	Methionine	2.0g
Arginine	2.0g	para-Aminobenzoic acid	0.2g
Asparagine	2.0g	Phenylalanine	2.0g
Aspartic acid	2.0g	Proline	2.0g
Cysteine	2.0g	Serine	2.0g
Glutamine	2.0g	Threonine	2.0g
Glutamic acid	2.0g	Tryptophan	2.0g
Glycine	2.0g	Tyrosine	2.0g
Inositol	2.0g	Valine	2.0g
Isoleucine	2.0g		

(g) DOHLU + Glu agar culture medium

A solid culture medium prepared by adding 2% (W/V) of agar

into the culture medium (e)

(h) DOHLU + Gal agar culture medium

A solid culture medium prepared by adding 2% (W/V) of agar
5 into the culture medium (f)

Free text in Sequence Listing

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Designed oligonucleotide primer for PCR

10 SEQ ID No: 24

Designed oligonucleotide primer for PCR

SEQ ID No: 25

Designed oligonucleotide primer for PCR

SEQ ID No: 26

15 Designed oligonucleotide primer for PCR

SEQ ID No: 27

Designed oligonucleotide primer for PCR

SEQ ID No: 28

Designed oligonucleotide primer for PCR

20 SEQ ID No: 29

Designed oligonucleotide primer for PCR

SEQ ID No: 30

Designed oligonucleotide primer for PCR

SEQ ID No: 31

25 Designed oligonucleotide primer for PCR

SEQ ID No: 32

Designed oligonucleotide primer for PCR

SEQ ID No: 33

Designed oligonucleotide primer for PCR

5 SEQ ID No: 34

Designed oligonucleotide primer for PCR

SEQ ID No: 35

Designed oligonucleotide primer for PCR

SEQ ID No: 36

10 Designed oligonucleotide primer for PCR

SEQ ID No: 37

Designed oligonucleotide primer for PCR

SEQ ID No: 38

Designed oligonucleotide primer for PCR

WHAT IS CLAIMED IS:

1. A cell transformed with
a polynucleotide comprising a nucleotide sequence encoding an
amino acid sequence of a cytokinin receptor and
5 a polynucleotide comprising a nucleotide sequence encoding an
amino acid sequence of a cytokinin biosynthesis enzyme,
wherein said transformed cell is produced by introducing said
polynucleotides into a host cell that is defective in one or
more histidine kinases.

10

2. The transformed cell according to claim 1, wherein
said cytokinin receptor is selected from the group consisting
of:

(a) a cytokinin receptor having the amino acid sequence
15 represented by SEQ ID No: 2;

(b) a cytokinin receptor having the amino acid sequence
represented by SEQ ID No: 4;

(c) a cytokinin receptor having the amino acid sequence
represented by SEQ ID No: 6;

20 (d) a partially transmembrane region-deleted type
cytokinin receptor;

(e) a cytokinin receptor having the amino acid sequence
represented by amino acids 196 to 1176 of SEQ ID No: 4;

(f) a cytokinin receptor having the amino acid sequence
25 represented by amino acids 50 to 1176 of SEQ ID No: 4;

(g) a cytokinin receptor having the amino acid sequence represented by amino acids 32 to 1036 of SEQ ID No: 6;

(h) a chimera-type cytokinin receptor comprising an extracellular region of the cytokinin receptor, transmembrane
5 regions of the cytokinin receptor, a histidine kinase region of the cytokinin receptor, and a receiver region of the histidine kinase, wherein said extracellular region, said transmembrane regions and said histidine kinase region are homogeneous to one another and the receiver region is heterogeneous thereto;

10 (i) a cytokinin receptor having the amino acid sequence of (a), (b), (c), (e), (f), or (g) with deletion, substitution, or addition of one or a plurality of amino acids; and

(j) a cytokinin receptor having an amino acid sequence encoded by a nucleotide sequence of a polynucleotide, wherein
15 said polynucleotide hybridizes under a stringent condition to a polynucleotide that has a nucleotide sequence complementary to a nucleotide sequence encoding the amino acid sequence represented by SEQ ID Nos: 2, 4, or 6.

20 3. The transformed cell according to claim 1, wherein said cytokinin biosynthesis enzyme is an isopentenyltransferase.

4. The transformed cell according to claim 1, wherein said cytokinin biosynthesis enzyme is selected from the group
25 consisting of:

(a) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 8;

(b) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 10;

5 (c) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 12;

(d) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 14;

(e) a cytokinin biosynthesis enzyme having the amino acid
10 sequence represented by SEQ ID No: 16;

(f) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 18;

(g) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID No: 20;

15 (h) a cytokinin biosynthesis enzyme having the amino acid sequence represented by SEQ ID Nos: 8, 10, 12, 14, 16, 18, or 20 with deletion, substitution, or addition of one or a plurality of amino acids; and

(i) a cytokinin biosynthesis enzyme having an amino acid
20 sequence encoded by a nucleotide sequence of a polynucleotide, wherein said polynucleotide hybridizes under a stringent condition to a polynucleotide that has a nucleotide sequence complementary to a nucleotide sequence encoding the amino acid sequence represented by SEQ ID Nos: 8, 10, 12, 14, 16, 18, or
25 20.

5. The transformed cell according to claim 1, wherein said cell is yeast.

6. The transformed cell according to claim 1, wherein
5 said cell is budding yeast.

SEQUENCE LISTING

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<120> TRANSFORMED CELL CO-EXPRESSING CYTOKININ RECEPTOR AND CYTOKININ
BIOSYNTHESIS ENZYME

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420

425

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440

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650

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660

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705

710

715

720

agc cgg cct cat att gga agc acg ttc tgg ttc acg gct gtt tta gag 2208
Ser Arg Pro His Ile Gly Ser Thr Phe Trp Phe Thr Ala Val Leu Glu

725

730

735

aaa tgc gat aaa tgc agt gcg att aac cat atg aag aaa cct aat gtg 2256
Lys Cys Asp Lys Cys Ser Ala Ile Asn His Met Lys Lys Pro Asn Val

740

745

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gaa cac ttg cct tct act ttt aaa gga atg aaa gct ata gtt gtt gat 2304

Glu His Leu Pro Ser Thr Phe Lys Gly Met Lys Ala Ile Val Val Asp	
755	760
765	
gct aag cct gtt aga gct gct gtg act aga tac cat atg aaa aga ctc	2352
Ala Lys Pro Val Arg Ala Ala Val Thr Arg Tyr His Met Lys Arg Leu	
770	775
780	
gga atc aat gtt gat gtc gtg aca agt ctc aaa acc gct gtt gtt gca	2400
Gly Ile Asn Val Asp Val Val Thr Ser Leu Lys Thr Ala Val Val Ala	
785	790
795	800
gct gct gcg ttt gaa aga aac ggt tct cct ctc cca aca aaa ccg caa	2448
Ala Ala Ala Phe Glu Arg Asn Gly Ser Pro Leu Pro Thr Lys Pro Gln	
805	810
815	
ctt gat atg atc tta gta gag aaa gat tca tgg att tca act gaa gat	2496
Leu Asp Met Ile Leu Val Glu Lys Asp Ser Trp Ile Ser Thr Glu Asp	
820	825
830	
aat gac tca gag att cgt tta ttg aat tca aga acc aac gga aac gtt	2544
Asn Asp Ser Glu Ile Arg Leu Leu Asn Ser Arg Thr Asn Gly Asn Val	
835	840
845	
cat cac aag tct ccg aaa cta gct cta ttc gca aca aac atc aca aat	2592
His His Lys Ser Pro Lys Leu Ala Leu Phe Ala Thr Asn Ile Thr Asn	
850	855
860	
tcg gag ttc gac aga gct aaa tcc gca gga ttt gca gat acg gta ata	2640
Ser Glu Phe Asp Arg Ala Lys Ser Ala Gly Phe Ala Asp Thr Val Ile	
865	870
875	880

atg aaa ccg tta aga gca agc atg att ggg gcg tgt ctg caa caa gtt 2688
Met Lys Pro Leu Arg Ala Ser Met Ile Gly Ala Cys Leu Gln Gln Val

885

890

895

ctc gag ctg aga aaa aca aga caa caa cat cca gaa gga tca tca ccc 2736
Leu Glu Leu Arg Lys Thr Arg Gln Gln His Pro Glu Gly Ser Ser Pro

900

905

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gca act ctc aag agc ttg ctt aca ggg aag aag att ctt gtg gtt gat 2784
Ala Thr Leu Lys Ser Leu Leu Thr Gly Lys Lys Ile Leu Val Val Asp

915

920

925

gat aat ata gtt aac agg aga gta gct gca gga gct ctc aag aaa ttt 2832
Asp Asn Ile Val Asn Arg Arg Val Ala Ala Gly Ala Leu Lys Lys Phe

930

935

940

gga gca gaa gtg gtt tgt gca gag agt ggt caa gtt gct ttg ggt ttg 2880
Gly Ala Glu Val Val Cys Ala Glu Ser Gly Gln Val Ala Leu Gly Leu

945

950

955

960

ctt cag att cca cac act ttc gat gct tgc ttc atg gat att caa atg 2928
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965

970

975

cca cag atg gac gga ttt gaa gca act cgt cag ata aga atg atg gag 2976
Pro Gln Met Asp Gly Phe Glu Ala Thr Arg Gln Ile Arg Met Met Glu

980

985

990

aag gaa gct aaa gag aag acg aat ctc gaa tgg cat tta ccg att cta 3024

Lys Glu Ala Lys Glu Lys Thr Asn Leu Glu Trp His Leu Pro Ile Leu

995

1000

1005

gcg atg act gcg gat gtg ata cac gcg acc tac gag gaa tgt ctg aaa 3072

Ala Met Thr Ala Asp Val Ile His Ala Thr Tyr Glu Glu Cys Leu Lys

1010

1015

1020

agt ggg atg gat ggt tac gtc tcc aaa cct ttt gaa gaa gag aat ctc 3120

Ser Gly Met Asp Gly Tyr Val Ser Lys Pro Phe Glu Glu Glu Asn Leu

1025

1030

1035

1040

tat aaa tcc gtt gcc aaa tca ttc aaa cct aat cct atc tca cct tcg 3168

Tyr Lys Ser Val Ala Lys Ser Phe Lys Pro Asn Pro Ile Ser Pro Ser

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1050

1055

tcg taa

3174

Ser

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25

30

Asp Phe Tyr Gln Leu Gly Gly Gly Gly Ala Leu Asn Ser Ser Glu Lys

35	40	45
Pro Arg Lys Ile Asp Phe Trp Arg Ser Gly Leu Met Gly Phe Ala Lys		
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Met Gln Gln Gln Gln Gln Leu Gln His Ser Val Ala Val Lys Met Asn		
65	70	75
Asn Asn Asn Asn Asn Asp Leu Met Gly Asn Lys Lys Gly Ser Thr Phe		
85	90	95
Ile Gln Glu His Arg Ala Leu Leu Pro Lys Ala Leu Ile Leu Trp Ile		
100	105	110
Ile Ile Val Gly Phe Ile Ser Ser Gly Ile Tyr Gln Trp Met Asp Asp		
115	120	125
Ala Asn Lys Ile Arg Arg Glu Glu Val Leu Val Ser Met Cys Asp Gln		
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Arg Ala Arg Met Leu Gln Asp Gln Phe Ser Val Ser Val Asn His Val		
145	150	155
His Ala Leu Ala Ile Leu Val Ser Thr Phe His Tyr His Lys Asn Pro		
165	170	175
Ser Ala Ile Asp Gln Glu Thr Phe Ala Glu Tyr Thr Ala Arg Thr Ala		
180	185	190
Phe Glu Arg Pro Leu Leu Ser Gly Val Ala Tyr Ala Glu Lys Val Val		
195	200	205
Asn Phe Glu Arg Glu Met Phe Glu Arg Gln His Asn Trp Val Ile Lys		
210	215	220
Thr Met Asp Arg Gly Glu Pro Ser Pro Val Arg Asp Glu Tyr Ala Pro		
225	230	235
Val Ile Phe Ser Gln Asp Ser Val Ser Tyr Leu Glu Ser Leu Asp Met		
245	250	255
Met Ser Gly Glu Glu Asp Arg Glu Asn Ile Leu Arg Ala Arg Glu Thr		
260	265	270
Gly Lys Ala Val Leu Thr Ser Pro Phe Arg Leu Leu Glu Thr His His		

275	280	285
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290	295	300
Asn Pro Thr Val Glu Glu Arg Ile Ala Ala Thr Ala Gly Tyr Leu Gly		
305	310	315
Gly Ala Phe Asp Val Glu Ser Leu Val Glu Asn Leu Leu Gly Gln Leu		
325	330	335
Ala Gly Asn Gln Ala Ile Val Val His Val Tyr Asp Ile Thr Asn Ala		
340	345	350
Ser Asp Pro Leu Val Met Tyr Gly Asn Gln Asp Glu Glu Ala Asp Arg		
355	360	365
Ser Leu Ser His Glu Ser Lys Leu Asp Phe Gly Asp Pro Phe Arg Lys		
370	375	380
His Lys Met Ile Cys Arg Tyr His Gln Lys Ala Pro Ile Pro Leu Asn		
385	390	395
Val Leu Thr Thr Val Pro Leu Phe Phe Ala Ile Gly Phe Leu Val Gly		
405	410	415
Tyr Ile Leu Tyr Gly Ala Ala Met His Ile Val Lys Val Glu Asp Asp		
420	425	430
Phe His Glu Met Gln Glu Leu Lys Val Arg Ala Glu Ala Ala Asp Val		
435	440	445
Ala Lys Ser Gln Phe Leu Ala Thr Val Ser His Glu Ile Arg Thr Pro		
450	455	460
Met Asn Gly Ile Leu Gly Met Leu Ala Met Leu Leu Asp Thr Glu Leu		
465	470	475
Ser Ser Thr Gln Arg Asp Tyr Ala Gln Thr Ala Gln Val Cys Gly Lys		
485	490	495
Ala Leu Ile Ala Leu Ile Asn Glu Val Leu Asp Arg Ala Lys Ile Glu		
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Ala Gly Lys Leu Glu Leu Glu Ser Val Pro Phe Asp Ile Arg Ser Ile		

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Ile Glu Leu Ala Val	Phe Val Ser Asp Lys Val Pro Glu Ile Val Lys	
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Gly Asp Ser Gly Arg	Phe Arg Gln Ile Ile Ile Asn Leu Val Gly Asn	
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Ser Val Lys Phe Thr	Glu Lys Gly His Ile Phe Val Lys Val His Leu	
	580	585
Ala Glu Gln Ser Lys Asp	Glu Ser Glu Pro Lys Asn Ala Leu Asn Gly	
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Gly Val Ser Glu Glu Met	Ile Val Val Ser Lys Gln Ser Ser Tyr Asn	
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Thr Leu Ser Gly Tyr	Glu Ala Ala Asp Gly Arg Asn Ser Trp Asp Ser	
625	630	635
Phe Lys His Leu Val	Ser Glu Glu Gln Ser Leu Ser Glu Phe Asp Ile	
	645	650
Ser Ser Asn Val Arg	Leu Met Val Ser Ile Glu Asp Thr Gly Ile Gly	
	660	665
Ile Pro Leu Val Ala	Gln Gly Arg Val Phe Met Pro Phe Met Gln Ala	
	675	680
Asp Ser Ser Thr Ser	Arg Asn Tyr Gly Gly Thr Gly Ile Gly Leu Ser	
	690	695
Ile Ser Lys Cys Leu	Val Glu Leu Met Arg Gly Gln Ile Asn Phe Ile	
705	710	715
Ser Arg Pro His Ile	Gly Ser Thr Phe Trp Phe Thr Ala Val Leu Glu	
	725	730
Lys Cys Asp Lys Cys	Ser Ala Ile Asn His Met Lys Lys Pro Asn Val	
	740	745
Glu His Leu Pro Ser	Thr Phe Lys Gly Met Lys Ala Ile Val Val Asp	

755	760	765
Ala Lys Pro Val Arg	Ala Ala Val Thr Arg Tyr His Met Lys Arg Leu	
770	775	780
Gly Ile Asn Val Asp Val Val Thr Ser Leu Lys Thr Ala Val Val Ala		
785	790	795
Ala Ala Ala Phe Glu Arg Asn Gly Ser Pro Leu Pro Thr Lys Pro Gln		
805	810	815
Leu Asp Met Ile Leu Val Glu Lys Asp Ser Trp Ile Ser Thr Glu Asp		
820	825	830
Asn Asp Ser Glu Ile Arg Leu Leu Asn Ser Arg Thr Asn Gly Asn Val		
835	840	845
His His Lys Ser Pro Lys Leu Ala Leu Phe Ala Thr Asn Ile Thr Asn		
850	855	860
Ser Glu Phe Asp Arg Ala Lys Ser Ala Gly Phe Ala Asp Thr Val Ile		
865	870	875
Met Lys Pro Leu Arg Ala Ser Met Ile Gly Ala Cys Leu Gln Gln Val		
885	890	895
Leu Glu Leu Arg Lys Thr Arg Gln Gln His Pro Glu Gly Ser Ser Pro		
900	905	910
Ala Thr Leu Lys Ser Leu Leu Thr Gly Lys Lys Ile Leu Val Val Asp		
915	920	925
Asp Asn Ile Val Asn Arg Arg Val Ala Ala Gly Ala Leu Lys Lys Phe		
930	935	940
Gly Ala Glu Val Val Cys Ala Glu Ser Gly Gln Val Ala Leu Gly Leu		
945	950	955
Leu Gln Ile Pro His Thr Phe Asp Ala Cys Phe Met Asp Ile Gln Met		
965	970	975
Pro Gln Met Asp Gly Phe Glu Ala Thr Arg Gln Ile Arg Met Met Glu		
980	985	990
Lys Glu Ala Lys Glu Lys Thr Asn Leu Glu Trp His Leu Pro Ile Leu		

995	1000	1005
Ala Met Thr Ala Asp Val Ile His Ala Thr Tyr Glu Glu Cys Leu Lys		
1010	1015	1020
Ser Gly Met Asp Gly Tyr Val Ser Lys Pro Phe Glu Glu Glu Asn Leu		
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Tyr Lys Ser Val Ala Lys Ser Phe Lys Pro Asn Pro Ile Ser Pro Ser		
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Ser		

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1	5	10	15
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aag tcg tcg agc agt gac aag aaa tgg cta aag aag cct ctc ttc ttc 96

Lys Ser Ser Ser Ser Asp Lys Lys Trp Leu Lys Lys Pro Leu Phe Phe

20	25	30
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ctg att ttg tgt ggc tct ttg gta att gtt ttg gtt atg ttc tta cgg 144

Leu Ile Leu Cys Gly Ser Leu Val Ile Val Leu Val Met Phe Leu Arg	
35 40 45	
tta ggt aga agt cag aag gag gag aca gat tct tgt aat gga gaa gag	192
Leu Gly Arg Ser Gln Lys Glu Glu Thr Asp Ser Cys Asn Gly Glu Glu	
50 55 60	
aaa gtg ttg tat aga cat caa aat gtc aca aga agt gag att cat gat	240
Lys Val Leu Tyr Arg His Gln Asn Val Thr Arg Ser Glu Ile His Asp	
65 70 75 80	
ttg gtc tct ttg ttc tct gat tca gat cag gta aca tcc ttt gaa tgt	288
Leu Val Ser Leu Phe Ser Asp Ser Asp Gln Val Thr Ser Phe Glu Cys	
85 90 95	
cat aag gaa tca agc cct gga atg tgg aca aac tat ggt att aca tgt	336
His Lys Glu Ser Ser Pro Gly Met Trp Thr Asn Tyr Gly Ile Thr Cys	
100 105 110	
tcc ctg agt gtg cgt tct gat aaa caa gag act aga ggg ctt ccc tgg	384
Ser Leu Ser Val Arg Ser Asp Lys Gln Glu Thr Arg Gly Leu Pro Trp	
115 120 125	
aat ctt ggc tta gga cat tct atc tca tca aca tct tgt atg tgt ggt	432
Asn Leu Gly Leu Gly His Ser Ile Ser Ser Thr Ser Cys Met Cys Gly	
130 135 140	
aat ctt gaa ccg att tta cag caa cct gaa aac ctt gag gaa gaa aac	480
Asn Leu Glu Pro Ile Leu Gln Gln Pro Glu Asn Leu Glu Glu Glu Asn	
145 150 155 160	

cat gaa gaa ggg ctg gag cag ggt ttg tca tcg tat tta aga aat gca	528
His Glu Glu Gly Leu Glu Gln Gly Leu Ser Ser Tyr Leu Arg Asn Ala	
165 170 175	
tggtgg tgg tgt cta atc ctt ggt gtg tta gtg tgc cat aag att tat gta	576
Trp Trp Cys Leu Ile Leu Gly Val Leu Val Cys His Lys Ile Tyr Val	
180 185 190	
tct cat tct aaa gca cga ggt gag agg aaa gag aaa gta cat ctg caa	624
Ser His Ser Lys Ala Arg Gly Glu Arg Lys Glu Lys Val His Leu Gln	
195 200 205	
gag gct tta gct cca aag aag cag caa caa cgt gct cag act tct tct	672
Glu Ala Leu Ala Pro Lys Lys Gln Gln Gln Arg Ala Gln Thr Ser Ser	
210 215 220	
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Arg Gly Ala Gly Arg Trp Arg Lys Asn Ile Leu Leu Leu Gly Ile Leu	
225 230 235 240	
gga gga gtt tcc ttc tct gtt tgg tgg ttt tgg gac act aat gag gag	768
Gly Gly Val Ser Phe Ser Val Trp Trp Phe Trp Asp Thr Asn Glu Glu	
245 250 255	
atc ata atg aaa agg agg gag act ttg gca aac atg tgt gac gaa cga	816
Ile Ile Met Lys Arg Arg Glu Thr Leu Ala Asn Met Cys Asp Glu Arg	
260 265 270	
gca cgt gtt tta caa gat cag ttc aat gtt agc ttg aac cat gtt cat	864

Ala Arg Val Leu Gln Asp Gln Phe Asn Val Ser Leu Asn His Val His	
275 280 285	
gcc ttg tct att ctt gta tct aca ttt cat cat ggt aaa atc cca tct	912
Ala Leu Ser Ile Leu Val Ser Thr Phe His His Gly Lys Ile Pro Ser	
290 295 300	
gcc att gat cag aga aca ttt gaa gaa tat act gag aga aca aac ttt	960
Ala Ile Asp Gln Arg Thr Phe Glu Glu Tyr Thr Glu Arg Thr Asn Phe	
305 310 315 320	
gag agg cca ctt act agt ggt gta gcg tat gct ttg aaa gtc cca cac	1008
Glu Arg Pro Leu Thr Ser Gly Val Ala Tyr Ala Leu Lys Val Pro His	
325 330 335	
tca gaa aga gag aaa ttt gaa aag gag cat gga tgg gca ata aag aaa	1056
Ser Glu Arg Glu Lys Phe Glu Lys Glu His Gly Trp Ala Ile Lys Lys	
340 345 350	
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Met Glu Thr Glu Asp Gln Thr Val Val Gln Asp Cys Val Pro Glu Asn	
355 360 365	
ttt gat ccc gca ccg att caa gac gaa tac gcg cca gtt ata ttt gct	1152
Phe Asp Pro Ala Pro Ile Gln Asp Glu Tyr Ala Pro Val Ile Phe Ala	
370 375 380	
caa gaa act gtt tcc cat att gta tcg gtc gac atg atg tct gga gaa	1200
Gln Glu Thr Val Ser His Ile Val Ser Val Asp Met Met Ser Gly Glu	
385 390 395 400	

gaa gac cgt gaa aac atc tta cgg gca agg gca tca gga aaa gga gtg 1248
 Glu Asp Arg Glu Asn Ile Leu Arg Ala Arg Ala Ser Gly Lys Gly Val

405 410 415

tta aca tct cca ttt aag ctt ctt aag tca aat cat ctt ggt gtt gtg 1296
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420 425 430

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435 440 445

gaa cag cgt gtt gaa gca act att ggg tac ctt ggt gca tca tat gat 1392
 Glu Gln Arg Val Glu Ala Thr Ile Gly Tyr Leu Gly Ala Ser Tyr Asp

450 455 460

atg cca tcg ctg gtg gag aaa ctt ctt cac caa ctt gcc agc aaa cag 1440
 Met Pro Ser Leu Val Glu Lys Leu Leu His Gln Leu Ala Ser Lys Gln

465 470 475 480

aca att gct gtg gat gtt tac gac aca act aac act tca ggt cta ata 1488
 Thr Ile Ala Val Asp Val Tyr Asp Thr Thr Asn Thr Ser Gly Leu Ile

485 490 495

aaa atg tat ggc tca gaa att ggg gat ata agt gag cag cat ata agt 1536
 Lys Met Tyr Gly Ser Glu Ile Gly Asp Ile Ser Glu Gln His Ile Ser

500 505 510

agc ctt gat ttt ggt gat cca tca agg aac cat gag atg cat tgc agg 1584

Ser Leu Asp Phe Gly Asp Pro Ser Arg Asn His Glu Met His Cys Arg	
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Phe Lys His Lys Leu Pro Ile Pro Trp Thr Ala Ile Thr Pro Ser Ile	
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Leu Val Leu Val Ile Thr Phe Leu Val Gly Tyr Ile Leu Tyr Glu Ala	
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555	560
atc aac cga att gcg aca gtt gaa gag gat tgt cag aag atg agg gaa	1728
Ile Asn Arg Ile Ala Thr Val Glu Glu Asp Cys Gln Lys Met Arg Glu	
565	570
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ctc aaa gct cgt gct gag gcc gct gac att gca aag tca cag ttc cta	1776
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580	585
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595	600
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Tyr Ala Gln Thr Ala His Gly Ser Gly Lys Asp Leu Thr Ser Leu Ile	
625	630
635	640

aat gag gtt ctt gat cag gca aag att gaa tcc gga agg ctc gag ctt 1968
 Asn Glu Val Leu Asp Gln Ala Lys Ile Glu Ser Gly Arg Leu Glu Leu

645

650

655

gaa aat gtg cct ttt gat atg cgt ttt att ctt gat aat gtt tca tct 2016
 Glu Asn Val Pro Phe Asp Met Arg Phe Ile Leu Asp Asn Val Ser Ser

660

665

670

ctc ctc tct ggc aag gca aat gaa aaa gga att gag ttg gcc gtt tat 2064
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675

680

685

gtt tct agt caa gtt cct gat gtt gta gtc ggt gat ccg agt cgg ttc 2112
 Val Ser Ser Gln Val Pro Asp Val Val Val Gly Asp Pro Ser Arg Phe

690

695

700

cgg cag atc att aca aac ctg gtt gga aac tca atc aaa ttc aca cag 2160
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705

710

715

720

gaa agg gga cac ata ttt atc tca gtg cac ctt gca gat gag gta aag 2208
 Glu Arg Gly His Ile Phe Ile Ser Val His Leu Ala Asp Glu Val Lys

725

730

735

gag cct ctt act att gaa gac gca gtg cta aaa cag cga cta gct tta 2256
 Glu Pro Leu Thr Ile Glu Asp Ala Val Leu Lys Gln Arg Leu Ala Leu

740

745

750

gga tgc agc gag tcc ggt gag aca gtt agc ggg ttt cct gcg gta aat 2304

Gly Cys Ser Glu Ser Gly Glu Thr Val Ser Gly Phe Pro Ala Val Asn	
755	760
765	
gca tgg gga agc tgg aag aat ttc aag aca tgt tac agt act gag agt	2352
Ala Trp Gly Ser Trp Lys Asn Phe Lys Thr Cys Tyr Ser Thr Glu Ser	
770	775
780	
cag aat tct gat caa atc aaa ttg cta gtt aca gtg gag gac act gga	2400
Gln Asn Ser Asp Gln Ile Lys Leu Leu Val Thr Val Glu Asp Thr Gly	
785	790
795	800
gtt ggc ata cct gtg gat gca caa ggc cga atc ttc aca cct ttt atg	2448
Val Gly Ile Pro Val Asp Ala Gln Gly Arg Ile Phe Thr Pro Phe Met	
805	810
815	
caa gcc gac agt tcc aca tcg cgg act tat ggt gga act ggc ata ggt	2496
Gln Ala Asp Ser Ser Thr Ser Arg Thr Tyr Gly Gly Thr Gly Ile Gly	
820	825
830	
ttg agt ata agc aaa cgt ttg gtt gaa ctc atg caa gga gag atg ggg	2544
Leu Ser Ile Ser Lys Arg Leu Val Glu Leu Met Gln Gly Glu Met Gly	
835	840
845	
ttt gtg agt gag ccc ggg ata ggc agt act ttt tca ttt act gga gtt	2592
Phe Val Ser Glu Pro Gly Ile Gly Ser Thr Phe Ser Phe Thr Gly Val	
850	855
860	
ttc ggg aaa gca gaa aca aat acg tcg att act aag ctg gaa cga ttc	2640
Phe Gly Lys Ala Glu Thr Asn Thr Ser Ile Thr Lys Leu Glu Arg Phe	
865	870
875	880

gat cta gct att cag gag ttt aca gga ttg aga gca tta gtt att gat 2688
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885 890 895

aac aga aac att aga gca gag gtc acc agg tac gaa ctt cgg aga ctg 2736
 Asn Arg Asn Ile Arg Ala Glu Val Thr Arg Tyr Glu Leu Arg Arg Leu

900 905 910

gga ata tct gca gac att gtt tca agt ctg aga atg gca tgc act tgt 2784
 Gly Ile Ser Ala Asp Ile Val Ser Ser Leu Arg Met Ala Cys Thr Cys

915 920 925

tgt atc agc aaa tta gaa aat ttg gct atg att cta ata gac aaa gac 2832
 Cys Ile Ser Lys Leu Glu Asn Leu Ala Met Ile Leu Ile Asp Lys Asp

930 935 940

gcc tgg aac aag gaa gaa ttt tca gta ctt gac gag ttg ttt acc cga 2880
 Ala Trp Asn Lys Glu Glu Phe Ser Val Leu Asp Glu Leu Phe Thr Arg

945 950 955 960

agc aaa gta acc ttt aca aga gtc cca aag att ttt ctt ttg gca act 2928
 Ser Lys Val Thr Phe Thr Arg Val Pro Lys Ile Phe Leu Leu Ala Thr

965 970 975

tct gca act ctt act gag cgc agt gag atg aag tct act ggt ctc atc 2976
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980 985 990

gat gag gtg gtg ata aag cct ctt cgg atg agt gtc tta ata tgt tgc 3024

Asp Glu Val Val Ile Lys Pro Leu Arg Met Ser Val Leu Ile Cys Cys	
995 1000 1005	
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cga aga aat ctt gga cac ttg cta aga gaa aaa cag att ctg gtt gtg	3120
Arg Arg Asn Leu Gly His Leu Leu Arg Glu Lys Gln Ile Leu Val Val	
1025 1030 1035 1040	
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Asp Asp Asn Leu Val Asn Arg Arg Val Ala Glu Gly Ala Leu Lys Lys	
1045 1050 1055	
tat gga gct att gtt aca tgc gtt gag agt ggc aaa gct gca ttg gca	3216
Tyr Gly Ala Ile Val Thr Cys Val Glu Ser Gly Lys Ala Ala Leu Ala	
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Met Pro Glu Met Asp Gly Phe Glu Ala Thr Arg Arg Val Arg Glu Leu	
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Glu Arg Glu Ile Asn Lys Lys Ile Ala Ser Gly Glu Val Ser Ala Glu	
1105 1110 1115 1120	

atg ttc tgt aaa ttt agt agt tgg cac gtc ccg ata tta gca atg aca 3408
Met Phe Cys Lys Phe Ser Ser Trp His Val Pro Ile Leu Ala Met Thr

1125

1130

1135

gca gat gtt att cag gct act cat gaa gaa tgc atg aaa tgt gga atg 3456
Ala Asp Val Ile Gln Ala Thr His Glu Glu Cys Met Lys Cys Gly Met

1140

1145

1150

gat ggt tat gta tca aaa ccg ttt gaa gag gaa gtg ctc tac aca gcg 3504
Asp Gly Tyr Val Ser Lys Pro Phe Glu Glu Glu Val Leu Tyr Thr Ala

1155

1160

1165

gta gca aga ttc ttt gaa cct tgt taa 3531
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1175

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Lys Ser Ser Ser Ser Asp Lys Lys Trp Leu Lys Lys Pro Leu Phe Phe

20

25

30

Leu Ile Leu Cys Gly Ser Leu Val Ile Val Leu Val Met Phe Leu Arg

35

40

45

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

Ala Leu Ser Ile Leu Val Ser Thr Phe His His Gly Lys Ile Pro Ser			
290	295	300	
Ala Ile Asp Gln Arg Thr Phe Glu Glu Tyr Thr Glu Arg Thr Asn Phe			
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Glu Arg Pro Leu Thr Ser Gly Val Ala Tyr Ala Leu Lys Val Pro His			
325	330	335	
Ser Glu Arg Glu Lys Phe Glu Lys Glu His Gly Trp Ala Ile Lys Lys			
340	345	350	
Met Glu Thr Glu Asp Gln Thr Val Val Gln Asp Cys Val Pro Glu Asn			
355	360	365	
Phe Asp Pro Ala Pro Ile Gln Asp Glu Tyr Ala Pro Val Ile Phe Ala			
370	375	380	
Gln Glu Thr Val Ser His Ile Val Ser Val Asp Met Met Ser Gly Glu			
385	390	395	400
Glu Asp Arg Glu Asn Ile Leu Arg Ala Arg Ala Ser Gly Lys Gly Val			
405	410	415	
Leu Thr Ser Pro Phe Lys Leu Leu Lys Ser Asn His Leu Gly Val Val			
420	425	430	
Leu Thr Phe Ala Val Tyr Asp Thr Ser Leu Pro Pro Asp Ala Thr Glu			
435	440	445	
Glu Gln Arg Val Glu Ala Thr Ile Gly Tyr Leu Gly Ala Ser Tyr Asp			
450	455	460	
Met Pro Ser Leu Val Glu Lys Leu Leu His Gln Leu Ala Ser Lys Gln			
465	470	475	480
Thr Ile Ala Val Asp Val Tyr Asp Thr Thr Asn Thr Ser Gly Leu Ile			
485	490	495	
Lys Met Tyr Gly Ser Glu Ile Gly Asp Ile Ser Glu Gln His Ile Ser			
500	505	510	
Ser Leu Asp Phe Gly Asp Pro Ser Arg Asn His Glu Met His Cys Arg			
515	520	525	

Phe	Lys	His	Lys	Leu	Pro	Ile	Pro	Trp	Thr	Ala	Ile	Thr	Pro	Ser	Ile
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Leu	Val	Leu	Val	Ile	Thr	Phe	Leu	Val	Gly	Tyr	Ile	Leu	Tyr	Glu	Ala
545							550								560
Ile	Asn	Arg	Ile	Ala	Thr	Val	Glu	Glu	Asp	Cys	Gln	Lys	Met	Arg	Glu
							565								575
Leu	Lys	Ala	Arg	Ala	Glu	Ala	Ala	Asp	Ile	Ala	Lys	Ser	Gln	Phe	Leu
							580								590
Ala	Thr	Val	Ser	His	Glu	Ile	Arg	Thr	Pro	Met	Asn	Gly	Val	Leu	Gly
							595								605
Met	Leu	Lys	Met	Leu	Met	Asp	Thr	Asp	Leu	Asp	Ala	Lys	Gln	Met	Asp
							610								620
Tyr	Ala	Gln	Thr	Ala	His	Gly	Ser	Gly	Lys	Asp	Leu	Thr	Ser	Leu	Ile
625							630								640
Asn	Glu	Val	Leu	Asp	Gln	Ala	Lys	Ile	Glu	Ser	Gly	Arg	Leu	Glu	Leu
							645								655
Glu	Asn	Val	Pro	Phe	Asp	Met	Arg	Phe	Ile	Leu	Asp	Asn	Val	Ser	Ser
							660								670
Leu	Leu	Ser	Gly	Lys	Ala	Asn	Glu	Lys	Gly	Ile	Glu	Leu	Ala	Val	Tyr
							675								685
Val	Ser	Ser	Gln	Val	Pro	Asp	Val	Val	Val	Gly	Asp	Pro	Ser	Arg	Phe
							690								700
Arg	Gln	Ile	Ile	Thr	Asn	Leu	Val	Gly	Asn	Ser	Ile	Lys	Phe	Thr	Gln
705							710								720
Glu	Arg	Gly	His	Ile	Phe	Ile	Ser	Val	His	Leu	Ala	Asp	Glu	Val	Lys
							725								735
Glu	Pro	Leu	Thr	Ile	Glu	Asp	Ala	Val	Leu	Lys	Gln	Arg	Leu	Ala	Leu
							740								750
Gly	Cys	Ser	Glu	Ser	Gly	Glu	Thr	Val	Ser	Gly	Phe	Pro	Ala	Val	Asn
							755								765

Ala Trp Gly Ser Trp Lys Asn Phe Lys Thr Cys Tyr Ser Thr Glu Ser			
770	775	780	
Gln Asn Ser Asp Gln Ile Lys Leu Leu Val Thr Val Glu Asp Thr Gly			
785	790	795	800
Val Gly Ile Pro Val Asp Ala Gln Gly Arg Ile Phe Thr Pro Phe Met			
805	810	815	
Gln Ala Asp Ser Ser Thr Ser Arg Thr Tyr Gly Gly Thr Gly Ile Gly			
820	825	830	
Leu Ser Ile Ser Lys Arg Leu Val Glu Leu Met Gln Gly Glu Met Gly			
835	840	845	
Phe Val Ser Glu Pro Gly Ile Gly Ser Thr Phe Ser Phe Thr Gly Val			
850	855	860	
Phe Gly Lys Ala Glu Thr Asn Thr Ser Ile Thr Lys Leu Glu Arg Phe			
865	870	875	880
Asp Leu Ala Ile Gln Glu Phe Thr Gly Leu Arg Ala Leu Val Ile Asp			
885	890	895	
Asn Arg Asn Ile Arg Ala Glu Val Thr Arg Tyr Glu Leu Arg Arg Leu			
900	905	910	
Gly Ile Ser Ala Asp Ile Val Ser Ser Leu Arg Met Ala Cys Thr Cys			
915	920	925	
Cys Ile Ser Lys Leu Glu Asn Leu Ala Met Ile Leu Ile Asp Lys Asp			
930	935	940	
Ala Trp Asn Lys Glu Glu Phe Ser Val Leu Asp Glu Leu Phe Thr Arg			
945	950	955	960
Ser Lys Val Thr Phe Thr Arg Val Pro Lys Ile Phe Leu Leu Ala Thr			
965	970	975	
Ser Ala Thr Leu Thr Glu Arg Ser Glu Met Lys Ser Thr Gly Leu Ile			
980	985	990	
Asp Glu Val Val Ile Lys Pro Leu Arg Met Ser Val Leu Ile Cys Cys			
995	1000	1005	

Leu Gln Glu Thr Leu Val Asn Gly Lys Lys Arg Gln Pro Asn Arg Gln
 1010 1015 1020
 Arg Arg Asn Leu Gly His Leu Leu Arg Glu Lys Gln Ile Leu Val Val
 1025 1030 1035 1040
 Asp Asp Asn Leu Val Asn Arg Arg Val Ala Glu Gly Ala Leu Lys Lys
 1045 1050 1055
 Tyr Gly Ala Ile Val Thr Cys Val Glu Ser Gly Lys Ala Ala Leu Ala
 1060 1065 1070
 Met Leu Lys Pro Pro His Asn Phe Asp Ala Cys Phe Met Asp Leu Gln
 1075 1080 1085
 Met Pro Glu Met Asp Gly Phe Glu Ala Thr Arg Arg Val Arg Glu Leu
 1090 1095 1100
 Glu Arg Glu Ile Asn Lys Lys Ile Ala Ser Gly Glu Val Ser Ala Glu
 1105 1110 1115 1120
 Met Phe Cys Lys Phe Ser Ser Trp His Val Pro Ile Leu Ala Met Thr
 1125 1130 1135
 Ala Asp Val Ile Gln Ala Thr His Glu Glu Cys Met Lys Cys Gly Met
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ttc tgg atg cta tgc tgc tgg ttt gtt tct tgg ttc gtt gat aat ggg 96

Phe Trp Met Leu Cys Cys Trp Phe Val Ser Trp Phe Val Asp Asn Gly

20 25 30

atc gag gac aag tct ggt ctt tta gtt ggc tct gtc ggt gat ctt gag 144

Ile Glu Asp Lys Ser Gly Leu Leu Val Gly Ser Val Gly Asp Leu Glu

35 40 45

aag act aag atg act acg ttg aag aag aag aac aag atg tgg ttc tgg 192

Lys Thr Lys Met Thr Thr Leu Lys Lys Lys Asn Lys Met Trp Phe Trp

50 55 60

aat aag atc tct agc agc gga ctc aag atc ccg agt ttc tct tat cag 240

Asn Lys Ile Ser Ser Ser Gly Leu Lys Ile Pro Ser Phe Ser Tyr Gln

65 70 75 80

ttt ctt ggc tct gtt aaa ttc aac aag gcg tgg tgg agg aag ctt gtg 288

Phe Leu Gly Ser Val Lys Phe Asn Lys Ala Trp Trp Arg Lys Leu Val

85 90 95

gtg gtt tgg gtt gtc ttc tgg gtc ttg gtc tct att tgg acg ttt tgg 336

Val Val Trp Val Val Phe Trp Val Leu Val Ser Ile Trp Thr Phe Trp

100	105	110	
tac ttt agc tcg caa gct atg gag aag agg aaa gag acg cta gct agt			384
Tyr Phe Ser Ser Gln Ala Met Glu Lys Arg Lys Glu Thr Leu Ala Ser			
115	120	125	
atg tgt gat gag aga gct cgt atg ctg cag gat cag ttc aac gtt agc			432
Met Cys Asp Glu Arg Ala Arg Met Leu Gln Asp Gln Phe Asn Val Ser			
130	135	140	
atg aat cat gtt caa gcc atg tct atc ttg atc tca acc ttc cac cat			480
Met Asn His Val Gln Ala Met Ser Ile Leu Ile Ser Thr Phe His His			
145	150	155	160
ggc aag att cct tct gct atc gat cag aga aca ttc tca gag tac act			528
Gly Lys Ile Pro Ser Ala Ile Asp Gln Arg Thr Phe Ser Glu Tyr Thr			
165	170	175	
gat aga act tcc ttt gag agg cct ctt act agc ggg gta gct tat gct			576
Asp Arg Thr Ser Phe Glu Arg Pro Leu Thr Ser Gly Val Ala Tyr Ala			
180	185	190	
atg agg gtg ctc cat tca gag agg gaa gag ttc gag agg caa caa ggt			624
Met Arg Val Leu His Ser Glu Arg Glu Glu Phe Glu Arg Gln Gln Gly			
195	200	205	
tgg act att agg aag atg tat tct ctt gaa caa aac cca gtt cac aag			672
Trp Thr Ile Arg Lys Met Tyr Ser Leu Glu Gln Asn Pro Val His Lys			
210	215	220	

340	345	350	
acc aat cac tct caa ccg att agc atg tat ggt aca aat gtg tcg gct			1104
Thr Asn His Ser Gln Pro Ile Ser Met Tyr Gly Thr Asn Val Ser Ala			
355	360	365	
gat ggg ttg gaa cgt gtt agt cca cta atc ttt ggc gat cca ttg aga			1152
Asp Gly Leu Glu Arg Val Ser Pro Leu Ile Phe Gly Asp Pro Leu Arg			
370	375	380	
aag cat gag atg cgt tgc aga ttt aag cag aaa cca cca tgg cca gtg			1200
Lys His Glu Met Arg Cys Arg Phe Lys Gln Lys Pro Pro Trp Pro Val			
385	390	395	400
cta tca atg gtg aca tca ttc ggt atc ctt gtg att gcg tta ctt gtt			1248
Leu Ser Met Val Thr Ser Phe Gly Ile Leu Val Ile Ala Leu Leu Val			
405	410	415	
gca cat ata atc cac gca acc gtt agt cga ata cac aaa gtt gaa gaa			1296
Ala His Ile Ile His Ala Thr Val Ser Arg Ile His Lys Val Glu Glu			
420	425	430	
gat tgt gat aaa atg aag cag ctc aag aaa aag gct gaa gca gca gat			1344
Asp Cys Asp Lys Met Lys Gln Leu Lys Lys Lys Ala Glu Ala Ala Asp			
435	440	445	
gtt gca aag tca cag ttc ctt gcc act gtt tca cat gaa atc aga act			1392
Val Ala Lys Ser Gln Phe Leu Ala Thr Val Ser His Glu Ile Arg Thr			
450	455	460	

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 465 470 475 480

tta gat gtt acg caa cag gat tat gtt agg acc gca cag gca agt gga 1488
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 485 490 495

aaa gct tta gtc tcg cta ata aat gag gtt ttg gac caa gca aag att 1536
 Lys Ala Leu Val Ser Leu Ile Asn Glu Val Leu Asp Gln Ala Lys Ile
 500 505 510

gaa tct gga aag ctt gaa ctt gag gag gtg cgg ttt gat ttg aga gga 1584
 Glu Ser Gly Lys Leu Glu Leu Glu Glu Val Arg Phe Asp Leu Arg Gly
 515 520 525

ata tta gat gat gtc ctg tca ctc ttc tct agc aag tcc caa caa aag 1632
 Ile Leu Asp Asp Val Leu Ser Leu Phe Ser Ser Lys Ser Gln Gln Lys
 530 535 540

ggg gtg gag ttg gca gta tac ata tct gat cgt gtt cca gat atg tta 1680
 Gly Val Glu Leu Ala Val Tyr Ile Ser Asp Arg Val Pro Asp Met Leu
 545 550 555 560

att ggt gat cct ggg agg ttt cga caa ata ctc aca aat ctt atg ggt 1728
 Ile Gly Asp Pro Gly Arg Phe Arg Gln Ile Leu Thr Asn Leu Met Gly
 565 570 575

aat tcc att aag ttc act gag aaa gga cac atc ttt gta act gtt cat 1776
 Asn Ser Ile Lys Phe Thr Glu Lys Gly His Ile Phe Val Thr Val His

580	585	590	
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Leu Val Asp Glu Leu Phe Glu Ser Ile Asp Gly Glu Thr Ala Ser Ser			
595	600	605	
ccg gaa agt aca ctg agt ggg ctt cca gtt gca gac cgg cag agg agc	1872		
Pro Glu Ser Thr Leu Ser Gly Leu Pro Val Ala Asp Arg Gln Arg Ser			
610	615	620	
tgg gaa aac ttt aaa gct ttc agc tcc aac ggg cat cgg agc ttt gaa	1920		
Trp Glu Asn Phe Lys Ala Phe Ser Ser Asn Gly His Arg Ser Phe Glu			
625	630	635	640
cca tct ccc cct gat ata aac cta atc gtc tca gtt gag gat act ggc	1968		
Pro Ser Pro Pro Asp Ile Asn Leu Ile Val Ser Val Glu Asp Thr Gly			
645	650	655	
gta ggg atc cct gta gaa gcg cag tcc cgt att ttt acg cct ttc atg	2016		
Val Gly Ile Pro Val Glu Ala Gln Ser Arg Ile Phe Thr Pro Phe Met			
660	665	670	
caa gtc gga cca tcc ata tcc agg acg cat gga ggc aca gga att gga	2064		
Gln Val Gly Pro Ser Ile Ser Arg Thr His Gly Gly Thr Gly Ile Gly			
675	680	685	
ctt agc ata agc aaa tgt cta gtt gga ctg atg aag gga gaa att gga	2112		
Leu Ser Ile Ser Lys Cys Leu Val Gly Leu Met Lys Gly Glu Ile Gly			
690	695	700	

ttc tcg agt act ccc aag gtt ggg tcc aca ttc aca ttt act gct gta	2160
Phe Ser Ser Thr Pro Lys Val Gly Ser Thr Phe Thr Phe Thr Ala Val	
705 710 715 720	
ttt tcc aat ggg atg caa cca gct gaa aga aag aat gac aac aac cag	2208
Phe Ser Asn Gly Met Gln Pro Ala Glu Arg Lys Asn Asp Asn Asn Gln	
725 730 735	
ccc ata ttc tcg gaa ttc cgg ggc atg aaa gct gtg gtt gtg gac cat	2256
Pro Ile Phe Ser Glu Phe Arg Gly Met Lys Ala Val Val Val Asp His	
740 745 750	
agg cct gca agg gca aaa gtc tcg tgg tac cat ttt cag cgt ctt gga	2304
Arg Pro Ala Arg Ala Lys Val Ser Trp Tyr His Phe Gln Arg Leu Gly	
755 760 765	
att cga gtc gaa gta gtt cca cgt gtt gaa cag gct cta cat tat ctg	2352
Ile Arg Val Glu Val Val Pro Arg Val Glu Gln Ala Leu His Tyr Leu	
770 775 780	
aag att ggt act acc act gtg aat atg ata ctc ata gag caa gaa ata	2400
Lys Ile Gly Thr Thr Thr Val Asn Met Ile Leu Ile Glu Gln Glu Ile	
785 790 795 800	
tgg aat agg gaa gca gat gat ttc att aaa aag cta cag aaa gac cct	2448
Trp Asn Arg Glu Ala Asp Asp Phe Ile Lys Lys Leu Gln Lys Asp Pro	
805 810 815	
ctt ttc ctt tct cct aag ttg att ttg tta gca aac tca gta gaa tcg	2496
Leu Phe Leu Ser Pro Lys Leu Ile Leu Leu Ala Asn Ser Val Glu Ser	

820	825	830	
tca ata tca gag gct tta tgc acc ggt ata gat cct cca ata gtg ata			2544
Ser Ile Ser Glu Ala Leu Cys Thr Gly Ile Asp Pro Pro Ile Val Ile			
835	840	845	
gtg aaa cca ttg agg gcg agt atg cta gca gca act ttg cag agg gga			2592
Val Lys Pro Leu Arg Ala Ser Met Leu Ala Ala Thr Leu Gln Arg Gly			
850	855	860	
ttg ggt att gga atc aga gaa cca cct caa cac aag gga cct cct gct			2640
Leu Gly Ile Gly Ile Arg Glu Pro Pro Gln His Lys Gly Pro Pro Ala			
865	870	875	880
ttg att ctc agg aat ctt ctc ctt ggt aga aaa att tta atc gtg gat			2688
Leu Ile Leu Arg Asn Leu Leu Leu Gly Arg Lys Ile Leu Ile Val Asp			
885	890	895	
gat aac aac gta aac ctc aga gtg gca gcg gga gct ctg aaa aag tac			2736
Asp Asn Asn Val Asn Leu Arg Val Ala Ala Gly Ala Leu Lys Lys Tyr			
900	905	910	
gga gct gat gtg gtc tgc gct gag agt ggg ata aag gca atc tca ttg			2784
Gly Ala Asp Val Val Cys Ala Glu Ser Gly Ile Lys Ala Ile Ser Leu			
915	920	925	
ctt aag cca cct cac gag ttt gat gct tgc ttc atg gac att cag atg			2832
Leu Lys Pro Pro His Glu Phe Asp Ala Cys Phe Met Asp Ile Gln Met			
930	935	940	

cca gaa atg gat gga ttt gaa gct aca agg aga ata cga gat atg gaa 2880
 Pro Glu Met Asp Gly Phe Glu Ala Thr Arg Arg Ile Arg Asp Met Glu
 945 950 955 960

gag gag atg aac aag aga ata aag aat ggg gag gct ttg ata gta gag 2928
 Glu Glu Met Asn Lys Arg Ile Lys Asn Gly Glu Ala Leu Ile Val Glu
 965 970 975

aac ggt aac aaa aca agc tgg cat ctt ccg gta tta gca atg acg gca 2976
 Asn Gly Asn Lys Thr Ser Trp His Leu Pro Val Leu Ala Met Thr Ala
 980 985 990

gat gtg atc caa gca acg cat gag gaa tgt ctg aag tgt gga atg gat 3024
 Asp Val Ile Gln Ala Thr His Glu Glu Cys Leu Lys Cys Gly Met Asp
 995 1000 1005

ggg tat gta tca aaa cca ttt gaa gca gag cag ctg tac agg gaa gtt 3072
 Gly Tyr Val Ser Lys Pro Phe Glu Ala Glu Gln Leu Tyr Arg Glu Val
 1010 1015 1020

tct cgc ttt ttc aat tcg cct tca gat aca gaa tca taa 3111
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Ile Glu Asp Lys Ser Gly Leu Leu Val Gly Ser Val Gly Asp Leu Glu
35 40 45
Lys Thr Lys Met Thr Thr Leu Lys Lys Lys Asn Lys Met Trp Phe Trp
50 55 60
Asn Lys Ile Ser Ser Ser Gly Leu Lys Ile Pro Ser Phe Ser Tyr Gln
65 70 75 80
Phe Leu Gly Ser Val Lys Phe Asn Lys Ala Trp Trp Arg Lys Leu Val
85 90 95
Val Val Trp Val Val Phe Trp Val Leu Val Ser Ile Trp Thr Phe Trp
100 105 110
Tyr Phe Ser Ser Gln Ala Met Glu Lys Arg Lys Glu Thr Leu Ala Ser
115 120 125
Met Cys Asp Glu Arg Ala Arg Met Leu Gln Asp Gln Phe Asn Val Ser
130 135 140
Met Asn His Val Gln Ala Met Ser Ile Leu Ile Ser Thr Phe His His
145 150 155 160
Gly Lys Ile Pro Ser Ala Ile Asp Gln Arg Thr Phe Ser Glu Tyr Thr
165 170 175
Asp Arg Thr Ser Phe Glu Arg Pro Leu Thr Ser Gly Val Ala Tyr Ala
180 185 190
Met Arg Val Leu His Ser Glu Arg Glu Glu Phe Glu Arg Gln Gln Gly
195 200 205
Trp Thr Ile Arg Lys Met Tyr Ser Leu Glu Gln Asn Pro Val His Lys
210 215 220
Asp Asp Tyr Asp Leu Glu Ala Leu Glu Pro Ser Pro Val Gln Glu Glu

225	230	235	240
Tyr Ala Pro Val Ile Phe Ala Gln Asp Thr Val Ser His Val Val Ser			
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Leu Asp Met Leu Ser Gly Lys Glu Asp Arg Glu Asn Val Leu Arg Ala			
	260	265	270
Arg Ser Ser Gly Lys Gly Val Leu Thr Ala Pro Phe Pro Leu Ile Lys			
	275	280	285
Thr Asn Arg Leu Gly Val Ile Leu Thr Phe Ala Val Tyr Lys Arg Asp			
	290	295	300
Leu Pro Ser Asn Ala Thr Pro Lys Glu Arg Ile Glu Ala Thr Asn Gly			
305	310	315	320
Tyr Leu Gly Gly Val Phe Asp Ile Glu Ser Leu Val Glu Asn Leu Leu			
	325	330	335
Gln Gln Leu Ala Ser Lys Gln Thr Ile Leu Val Asn Val Tyr Asp Ile			
	340	345	350
Thr Asn His Ser Gln Pro Ile Ser Met Tyr Gly Thr Asn Val Ser Ala			
	355	360	365
Asp Gly Leu Glu Arg Val Ser Pro Leu Ile Phe Gly Asp Pro Leu Arg			
	370	375	380
Lys His Glu Met Arg Cys Arg Phe Lys Gln Lys Pro Pro Trp Pro Val			
385	390	395	400
Leu Ser Met Val Thr Ser Phe Gly Ile Leu Val Ile Ala Leu Leu Val			
	405	410	415
Ala His Ile Ile His Ala Thr Val Ser Arg Ile His Lys Val Glu Glu			
	420	425	430
Asp Cys Asp Lys Met Lys Gln Leu Lys Lys Lys Ala Glu Ala Ala Asp			
	435	440	445
Val Ala Lys Ser Gln Phe Leu Ala Thr Val Ser His Glu Ile Arg Thr			
	450	455	460
Pro Met Asn Gly Val Leu Gly Met Leu His Met Leu Met Asp Thr Glu			

465	470	475	480
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Lys Ala Leu Val Ser Leu Ile Asn Glu Val Leu Asp Gln Ala Lys Ile			
	500	505	510
Glu Ser Gly Lys Leu Glu Leu Glu Glu Val Arg Phe Asp Leu Arg Gly			
	515	520	525
Ile Leu Asp Asp Val Leu Ser Leu Phe Ser Ser Lys Ser Gln Gln Lys			
	530	535	540
Gly Val Glu Leu Ala Val Tyr Ile Ser Asp Arg Val Pro Asp Met Leu			
545	550	555	560
Ile Gly Asp Pro Gly Arg Phe Arg Gln Ile Leu Thr Asn Leu Met Gly			
	565	570	575
Asn Ser Ile Lys Phe Thr Glu Lys Gly His Ile Phe Val Thr Val His			
	580	585	590
Leu Val Asp Glu Leu Phe Glu Ser Ile Asp Gly Glu Thr Ala Ser Ser			
	595	600	605
Pro Glu Ser Thr Leu Ser Gly Leu Pro Val Ala Asp Arg Gln Arg Ser			
	610	615	620
Trp Glu Asn Phe Lys Ala Phe Ser Ser Asn Gly His Arg Ser Phe Glu			
625	630	635	640
Pro Ser Pro Pro Asp Ile Asn Leu Ile Val Ser Val Glu Asp Thr Gly			
	645	650	655
Val Gly Ile Pro Val Glu Ala Gln Ser Arg Ile Phe Thr Pro Phe Met			
	660	665	670
Gln Val Gly Pro Ser Ile Ser Arg Thr His Gly Gly Thr Gly Ile Gly			
	675	680	685
Leu Ser Ile Ser Lys Cys Leu Val Gly Leu Met Lys Gly Glu Ile Gly			
	690	695	700
Phe Ser Ser Thr Pro Lys Val Gly Ser Thr Phe Thr Phe Thr Ala Val			

705	710	715	720
Phe Ser Asn Gly Met Gln Pro Ala Glu Arg Lys Asn Asp Asn Asn Gln			
	725	730	735
Pro Ile Phe Ser Glu Phe Arg Gly Met Lys Ala Val Val Val Asp His			
	740	745	750
Arg Pro Ala Arg Ala Lys Val Ser Trp Tyr His Phe Gln Arg Leu Gly			
	755	760	765
Ile Arg Val Glu Val Val Pro Arg Val Glu Gln Ala Leu His Tyr Leu			
	770	775	780
Lys Ile Gly Thr Thr Thr Val Asn Met Ile Leu Ile Glu Gln Glu Ile			
785	790	795	800
Trp Asn Arg Glu Ala Asp Asp Phe Ile Lys Lys Leu Gln Lys Asp Pro			
	805	810	815
Leu Phe Leu Ser Pro Lys Leu Ile Leu Leu Ala Asn Ser Val Glu Ser			
	820	825	830
Ser Ile Ser Glu Ala Leu Cys Thr Gly Ile Asp Pro Pro Ile Val Ile			
	835	840	845
Val Lys Pro Leu Arg Ala Ser Met Leu Ala Ala Thr Leu Gln Arg Gly			
	850	855	860
Leu Gly Ile Gly Ile Arg Glu Pro Pro Gln His Lys Gly Pro Pro Ala			
865	870	875	880
Leu Ile Leu Arg Asn Leu Leu Leu Gly Arg Lys Ile Leu Ile Val Asp			
	885	890	895
Asp Asn Asn Val Asn Leu Arg Val Ala Ala Gly Ala Leu Lys Lys Tyr			
	900	905	910
Gly Ala Asp Val Val Cys Ala Glu Ser Gly Ile Lys Ala Ile Ser Leu			
	915	920	925
Leu Lys Pro Pro His Glu Phe Asp Ala Cys Phe Met Asp Ile Gln Met			
	930	935	940
Pro Glu Met Asp Gly Phe Glu Ala Thr Arg Arg Ile Arg Asp Met Glu			

945	950	955	960
Glu Glu Met Asn Lys Arg Ile Lys Asn Gly Glu Ala Leu Ile Val Glu			
	965	970	975
Asn Gly Asn Lys Thr Ser Trp His Leu Pro Val Leu Ala Met Thr Ala			
	980	985	990
Asp Val Ile Gln Ala Thr His Glu Glu Cys Leu Lys Cys Gly Met Asp			
	995	1000	1005
Gly Tyr Val Ser Lys Pro Phe Glu Ala Glu Gln Leu Tyr Arg Glu Val			
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Ser Arg Phe Phe Asn Ser Pro Ser Asp Thr Glu Ser			
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<211> 1074

<212> DNA

<213> Arabidopsis thaliana

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Met Thr Glu Leu Asn Phe His Leu Leu Pro Ile Ile Ser Asp Arg Phe	

1	5	10	15
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acg acg acg acg aca aca tca ccg tcg ttc tcg tca cat tct tct tct	96
Thr Thr Thr Thr Thr Thr Ser Pro Ser Phe Ser Ser His Ser Ser Ser	

20	25	30
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tct tct tct ctt ctc tct ttc acc aaa cga aga cga aaa cac caa cct 144
 Ser Ser Ser Leu Leu Ser Phe Thr Lys Arg Arg Arg Lys His Gln Pro

35

40

45

tta gta tca tcc ata cgc atg gaa cag tca cgg tca cgg aat cgg aaa 192
 Leu Val Ser Ser Ile Arg Met Glu Gln Ser Arg Ser Arg Asn Arg Lys

50

55

60

gac aaa gtc gtc gtc att tta gga gca acc ggc gcc gga aaa tca aga 240
 Asp Lys Val Val Val Ile Leu Gly Ala Thr Gly Ala Gly Lys Ser Arg

65

70

75

80

ctt tcc gtc gat ctc gct act cgt ttc cct tca gag atc ata aac tcc 288
 Leu Ser Val Asp Leu Ala Thr Arg Phe Pro Ser Glu Ile Ile Asn Ser

85

90

95

gat aaa atc caa gtc tac gaa gga tta gag atc aca acg aat cag att 336
 Asp Lys Ile Gln Val Tyr Glu Gly Leu Glu Ile Thr Thr Asn Gln Ile

100

105

110

acg tta caa gac cgt cgc ggc gtt cct cac cat ctc ctc ggc gtc atc 384
 Thr Leu Gln Asp Arg Arg Gly Val Pro His His Leu Leu Gly Val Ile

115

120

125

aac ccc gaa cac ggc gaa cta acc gcc gga gag ttt cgc tcc gcc gct 432
 Asn Pro Glu His Gly Glu Leu Thr Ala Gly Glu Phe Arg Ser Ala Ala

130

135

140

tca aac gtc gtc aaa gag ata act tct cgt caa aag gtt ccg att atc 480

Ser	Asn	Val	Val	Lys	Glu	Ile	Thr	Ser	Arg	Gln	Lys	Val	Pro	Ile	Ile	
145					150					155					160	
gcc	ggt	gga	tct	aac	tct	ttc	gtc	cac	gca	ctc	tta	gct	caa	cga	ttc	528
Ala	Gly	Gly	Ser	Asn	Ser	Phe	Val	His	Ala	Leu	Leu	Ala	Gln	Arg	Phe	
				165					170					175		
gac	cca	aag	ttc	gat	cct	ttt	tca	tcc	ggg	tcg	tgt	tta	atc	agc	tcc	576
Asp	Pro	Lys	Phe	Asp	Pro	Phe	Ser	Ser	Gly	Ser	Cys	Leu	Ile	Ser	Ser	
			180					185					190			
gat	ttg	cgt	tac	gag	tgt	tgt	ttc	atc	tgg	gtc	gat	gta	tcg	gag	act	624
Asp	Leu	Arg	Tyr	Glu	Cys	Cys	Phe	Ile	Trp	Val	Asp	Val	Ser	Glu	Thr	
			195					200					205			
gtt	ctc	tac	gag	tat	ctt	ctc	aga	aga	gtc	gac	gaa	atg	atg	gat	tca	672
Val	Leu	Tyr	Glu	Tyr	Leu	Leu	Arg	Arg	Val	Asp	Glu	Met	Met	Asp	Ser	
			210					215					220			
ggt	atg	ttc	gaa	gag	ctg	tct	aga	ttc	tac	gac	ccg	gtt	aaa	tcc	ggt	720
Gly	Met	Phe	Glu	Glu	Leu	Ser	Arg	Phe	Tyr	Asp	Pro	Val	Lys	Ser	Gly	
225					230					235					240	
tta	gaa	acc	cgg	ttt	ggg	att	agg	aaa	gct	ata	ggt	gta	ccg	gag	ttt	768
Leu	Glu	Thr	Arg	Phe	Gly	Ile	Arg	Lys	Ala	Ile	Gly	Val	Pro	Glu	Phe	
				245					250					255		
gac	ggt	tac	ttc	aaa	gag	tat	cca	ccg	gag	aag	aag	atg	ata	aag	tgg	816
Asp	Gly	Tyr	Phe	Lys	Glu	Tyr	Pro	Pro	Glu	Lys	Lys	Met	Ile	Lys	Trp	
				260					265					270		

gcc ggt gga tct aac tct ttc gtc cac gca ctc tta gct caa cga ttc 528
Ala Gly Gly Ser Asn Ser Phe Val His Ala Leu Leu Ala Gln Arg Phe
165 170 175

gac cca aag ttc gat cct ttt tca tcc ggg tcg tgt tta atc agc tcc 576
Asp Pro Lys Phe Asp Pro Phe Ser Ser Gly Ser Cys Leu Ile Ser Ser
180 185 190

gat ttg cgt tac gag tgt tgt ttc atc tgg gtc gat gta tcg gag act 624
Asp Leu Arg Tyr Glu Cys Cys Phe Ile Trp Val Asp Val Ser Glu Thr
195 200 205

ggt ctc tac gag tat ctt ctc aga aga gtc gac gaa atg atg gat tca 672
Val Leu Tyr Glu Tyr Leu Leu Arg Arg Val Asp Glu Met Met Asp Ser
210 215 220

ggg atg ttc gaa gag ctg tct aga ttc tac gac ccg gtt aaa tcc ggt 720
Gly Met Phe Glu Glu Leu Ser Arg Phe Tyr Asp Pro Val Lys Ser Gly
225 230 235 240

tta gaa acc cgg ttt ggg att agg aaa gct ata ggt gta ccg gag ttt 768
Leu Glu Thr Arg Phe Gly Ile Arg Lys Ala Ile Gly Val Pro Glu Phe
245 250 255

gac ggt tac ttc aaa gag tat cca ccg gag aag aag atg ata aag tgg 816
Asp Gly Tyr Phe Lys Glu Tyr Pro Pro Glu Lys Lys Met Ile Lys Trp
260 265 270

gac gct tta aga aaa gcg gcg tac gat aag gcg gtt gat gat atc aaa 864
 Asp Ala Leu Arg Lys Ala Ala Tyr Asp Lys Ala Val Asp Asp Ile Lys

275

280

285

agg aac acg tgg acg tta gcg aag aga caa gtg aag aag att gag atg 912
 Arg Asn Thr Trp Thr Leu Ala Lys Arg Gln Val Lys Lys Ile Glu Met

290

295

300

cta aaa gac gct ggt tgg gaa ata gaa aga gtt gat gca acg gcg tcg 960
 Leu Lys Asp Ala Gly Trp Glu Ile Glu Arg Val Asp Ala Thr Ala Ser

305

310

315

320

ttt aaa gca gtg atg atg aag agt tcg tcg gag aag aag tgg aga gag 1008
 Phe Lys Ala Val Met Met Lys Ser Ser Ser Glu Lys Lys Trp Arg Glu

325

330

335

aat tgg gaa gag caa gtg ttg gag cca agc gta aag att gtg aag cgg 1056
 Asn Trp Glu Glu Gln Val Leu Glu Pro Ser Val Lys Ile Val Lys Arg

340

345

350

cat ttg gtg caa aat tag 1074
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355

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

[illegible]

atg atc atg aag ata tct atg gct atg tgc aaa caa cca ttg cct cct	48
Met Ile Met Lys Ile Ser Met Ala Met Cys Lys Gln Pro Leu Pro Pro	
1 5 10 15	
tcg ccg act tta gac ttc cct cca gcg aga ttt ggt ccc aat atg cta	96
Ser Pro Thr Leu Asp Phe Pro Pro Ala Arg Phe Gly Pro Asn Met Leu	
20 25 30	
act cta aac cca tac ggt cca aag gac aaa gtt gtg gtc atc atg ggt	144
Thr Leu Asn Pro Tyr Gly Pro Lys Asp Lys Val Val Val Ile Met Gly	
35 40 45	
gct acc ggg aca ggc aag tca cga ctc tcc gtg gat ata gcc aca cgt	192
Ala Thr Gly Thr Gly Lys Ser Arg Leu Ser Val Asp Ile Ala Thr Arg	
50 55 60	
ttt cgg gct gag atc ata aac tca gac aag ata caa gtc cac caa ggt	240
Phe Arg Ala Glu Ile Ile Asn Ser Asp Lys Ile Gln Val His Gln Gly	
65 70 75 80	
cta gac att gta acc aac aag atc acg agc gag gag agc tgc ggg gta	288
Leu Asp Ile Val Thr Asn Lys Ile Thr Ser Glu Glu Ser Cys Gly Val	
85 90 95	
ccg cac cat ctc ctc ggc gtc ttg ccg cct gaa gcc gac tta acc gcc	336
Pro His His Leu Leu Gly Val Leu Pro Pro Glu Ala Asp Leu Thr Ala	
100 105 110	
gcg aat tac tgt cac atg gcg aat ctc tcc att gaa tcc gtc cta aac	384
Ala Asn Tyr Cys His Met Ala Asn Leu Ser Ile Glu Ser Val Leu Asn	

115	120	125	
cgt gga aag ctt cca atc atc gtt gga ggt tcc aac tct tac gtg gag			432
Arg Gly Lys Leu Pro Ile Ile Val Gly Gly Ser Asn Ser Tyr Val Glu			
130	135	140	
gct cta gtg gat gac aaa gaa aac aag ttc agg tcg aga tac gac tgt			480
Ala Leu Val Asp Asp Lys Glu Asn Lys Phe Arg Ser Arg Tyr Asp Cys			
145	150	155	160
tgt ttt cta tgg gtg gac gtg gca ctt ccc gtt ttg cac ggg ttc gtg			528
Cys Phe Leu Trp Val Asp Val Ala Leu Pro Val Leu His Gly Phe Val			
	165	170	175
tct gag aga gtt gac aag atg gtg gag agt gga atg gtt gag gaa gtc			576
Ser Glu Arg Val Asp Lys Met Val Glu Ser Gly Met Val Glu Glu Val			
	180	185	190
aga gaa ttt ttc gac ttt tcg aac tct gat tac tca aga ggg atc aag			624
Arg Glu Phe Phe Asp Phe Ser Asn Ser Asp Tyr Ser Arg Gly Ile Lys			
	195	200	205
aaa gca atc gga ttt ccg gag ttt gac agg ttt ttc agg aac gag cag			672
Lys Ala Ile Gly Phe Pro Glu Phe Asp Arg Phe Phe Arg Asn Glu Gln			
	210	215	220
ttc ttg aat gtg gaa gac aga gaa gaa ctg tta agt aaa gtg ttg gaa			720
Phe Leu Asn Val Glu Asp Arg Glu Glu Leu Leu Ser Lys Val Leu Glu			
225	230	235	240

gaa ata aag agg aat aca ttt gag tta gct tgt agg cag aga gaa aag 768
 Glu Ile Lys Arg Asn Thr Phe Glu Leu Ala Cys Arg Gln Arg Glu Lys

245

250

255

atc gaa cgg ttg aga aaa gtg aag aag tgg tct att cag aga gtg gat 816
 Ile Glu Arg Leu Arg Lys Val Lys Lys Trp Ser Ile Gln Arg Val Asp

260

265

270

gcg act cca gtc ttt aca aag cga agg tcc aag atg gat gct aac gtg 864
 Ala Thr Pro Val Phe Thr Lys Arg Arg Ser Lys Met Asp Ala Asn Val

275

280

285

gcc tgg gag agg ctc gtg gct gga cca agc aca gat act gtg tcg cgg 912
 Ala Trp Glu Arg Leu Val Ala Gly Pro Ser Thr Asp Thr Val Ser Arg

290

295

300

ttt ctg ctg gac att gcc agc cga cga ccg ctc gtg gaa gct tca aca 960
 Phe Leu Leu Asp Ile Ala Ser Arg Arg Pro Leu Val Glu Ala Ser Thr

305

310

315

320

gcg gtt gcg gcc gcc atg gaa cgc gag ttg tcg cgg tgt cta gtg gcg 1008
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335

tga 1011

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<212> PRT

<213> Arabidopsis thaliana

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Ser Pro Thr Leu Asp Phe Pro Pro Ala Arg Phe Gly Pro Asn Met Leu

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Thr Leu Asn Pro Tyr Gly Pro Lys Asp Lys Val Val Val Ile Met Gly

35 40 45

Ala Thr Gly Thr Gly Lys Ser Arg Leu Ser Val Asp Ile Ala Thr Arg

50 55 60

Phe Arg Ala Glu Ile Ile Asn Ser Asp Lys Ile Gln Val His Gln Gly

65 70 75 80

Leu Asp Ile Val Thr Asn Lys Ile Thr Ser Glu Glu Ser Cys Gly Val

85 90 95

Pro His His Leu Leu Gly Val Leu Pro Pro Glu Ala Asp Leu Thr Ala

100 105 110

Ala Asn Tyr Cys His Met Ala Asn Leu Ser Ile Glu Ser Val Leu Asn

115 120 125

Arg Gly Lys Leu Pro Ile Ile Val Gly Gly Ser Asn Ser Tyr Val Glu

130 135 140

Ala Leu Val Asp Asp Lys Glu Asn Lys Phe Arg Ser Arg Tyr Asp Cys

145 150 155 160

Cys Phe Leu Trp Val Asp Val Ala Leu Pro Val Leu His Gly Phe Val

165 170 175

Ser Glu Arg Val Asp Lys Met Val Glu Ser Gly Met Val Glu Glu Val

180 185 190

Arg Glu Phe Phe Asp Phe Ser Asn Ser Asp Tyr Ser Arg Gly Ile Lys

195	200	205
Lys Ala Ile Gly Phe Pro Glu Phe Asp Arg Phe Phe Arg Asn Glu Gln		
210	215	220
Phe Leu Asn Val Glu Asp Arg Glu Glu Leu Leu Ser Lys Val Leu Glu		
225	230	235
Glu Ile Lys Arg Asn Thr Phe Glu Leu Ala Cys Arg Gln Arg Glu Lys		
245	250	255
Ile Glu Arg Leu Arg Lys Val Lys Lys Trp Ser Ile Gln Arg Val Asp		
260	265	270
Ala Thr Pro Val Phe Thr Lys Arg Arg Ser Lys Met Asp Ala Asn Val		
275	280	285
Ala Trp Glu Arg Leu Val Ala Gly Pro Ser Thr Asp Thr Val Ser Arg		
290	295	300
Phe Leu Leu Asp Ile Ala Ser Arg Arg Pro Leu Val Glu Ala Ser Thr		
305	310	315
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<211> 957

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<222> (1)..(957)

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ggc	aag	tca	tca	ctc	tct	gtt	gat	ctc	gct	tta	cat	ttt	aaa	gcc	gag	96	
Gly	Lys	Ser	Ser	Leu	Ser	Val	Asp	Leu	Ala	Leu	His	Phe	Lys	Ala	Glu		
				20				25					30				
atc	atc	aac	tct	gac	aaa	atg	cag	ttc	tac	gat	ggc	ttg	aag	atc	acc	144	
Ile	Ile	Asn	Ser	Asp	Lys	Met	Gln	Phe	Tyr	Asp	Gly	Leu	Lys	Ile	Thr		
				35			40					45					
acg	aat	caa	tcg	acc	att	gaa	gat	cga	cgt	gga	gtg	cca	cat	cac	ott	192	
Thr	Asn	Gln	Ser	Thr	Ile	Glu	Asp	Arg	Arg	Gly	Val	Pro	His	His	Leu		
				50			55				60						
ctc	ggg	gaa	cta	aac	ccg	gag	gct	gga	gaa	gtc	aca	gcg	gca	gaa	ttt	240	
Leu	Gly	Glu	Leu	Asn	Pro	Glu	Ala	Gly	Glu	Val	Thr	Ala	Ala	Glu	Phe		
65					70					75				80			
cgc	gtt	atg	gcg	gct	gaa	gcc	atc	tcc	gag	att	act	caa	cgt	aaa	aag	288	
Arg	Val	Met	Ala	Ala	Glu	Ala	Ile	Ser	Glu	Ile	Thr	Gln	Arg	Lys	Lys		
								85		90				95			
ctc	cca	atc	ctt	gcc	ggg	gga	tcc	aac	tca	tac	att	cat	gct	ctc	ott	336	
Leu	Pro	Ile	Leu	Ala	Gly	Gly	Ser	Asn	Ser	Tyr	Ile	His	Ala	Leu	Leu		
				100				105					110				
gca	aaa	tct	tat	gac	cct	gaa	aac	tat	ccg	ttt	tct	gat	cac	aag	ggc	384	
Ala	Lys	Ser	Tyr	Asp	Pro	Glu	Asn	Tyr	Pro	Phe	Ser	Asp	His	Lys	Gly		
				115				120					125				

tca atc tgc tcc gag ttg aaa tat gat tgt tgt ttc att tgg ata gat 432
 Ser Ile Cys Ser Glu Leu Lys Tyr Asp Cys Cys Phe Ile Trp Ile Asp

130

135

140

gtg gat cag tct gtg tta ttc gag tat ctt tct tta cgt ttg gat ctt 480
 Val Asp Gln Ser Val Leu Phe Glu Tyr Leu Ser Leu Arg Leu Asp Leu

145

150

155

160

atg atg aag tca ggt atg ttc gag gag atc gct gag ttc cac cgc tct 528
 Met Met Lys Ser Gly Met Phe Glu Glu Ile Ala Glu Phe His Arg Ser

165

170

175

aag aag gcc ccg aaa gag cca ttg ggg ata tgg aag gct ata gga gtg 576
 Lys Lys Ala Pro Lys Glu Pro Leu Gly Ile Trp Lys Ala Ile Gly Val

180

185

190

caa gag ttt gat gac tac ctc aaa atg tac aag tgg gac aat gac atg 624
 Gln Glu Phe Asp Asp Tyr Leu Lys Met Tyr Lys Trp Asp Asn Asp Met

195

200

205

gat aaa tgg gac cct atg aga aag gag gct tat gag aag gcg gtg aga 672
 Asp Lys Trp Asp Pro Met Arg Lys Glu Ala Tyr Glu Lys Ala Val Arg

210

215

220

gcc atc aaa gaa aac aca ttt cag ctc aca aag gat caa atc acg aag 720
 Ala Ile Lys Glu Asn Thr Phe Gln Leu Thr Lys Asp Gln Ile Thr Lys

225

230

235

240

atc aac aag ctg aga aat gcc ggg tgg gac ata aag aag gtg gat gct 768

Ile Asn Lys Leu Arg Asn Ala Gly Trp Asp Ile Lys Lys Val Asp Ala	
245 250 255	
aca gca tcg ttt cga gag gca att agg gca gcc aag gaa ggc gaa ggt	816
Thr Ala Ser Phe Arg Glu Ala Ile Arg Ala Ala Lys Glu Gly Glu Gly	
260 265 270	
gta gcc gag atg cag aga aag ata tgg aac aag gaa gtg ttg gaa ccg	864
Val Ala Glu Met Gln Arg Lys Ile Trp Asn Lys Glu Val Leu Glu Pro	
275 280 285	
tgt gtg aag att gtc agg agc cac ttg gac caa ccg atc aac tat tat	912
Cys Val Lys Ile Val Arg Ser His Leu Asp Gln Pro Ile Asn Tyr Tyr	
290 295 300	
tat tat tac ttt tat tta cta aaa aga ttt tta agt ctt aac tag	957
Tyr Tyr Tyr Phe Tyr Leu Leu Lys Arg Phe Leu Ser Leu Asn	
305 310 315	

<210> 12

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<212> PRT

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Gly Lys Ser Ser Leu Ser Val Asp Leu Ala Leu His Phe Lys Ala Glu	
20 25 30	

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

Val Ala Glu Met Gln Arg Lys Ile Trp Asn Lys Glu Val Leu Glu Pro

275

280

285

Cys Val Lys Ile Val Arg Ser His Leu Asp Gln Pro Ile Asn Tyr Tyr

290

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300

Tyr Tyr Tyr Phe Tyr Leu Leu Lys Arg Phe Leu Ser Leu Asn

305

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315

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<222> (1)..(993)

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5

10

15

ttg aac ttc caa gga aac atg gtg gac gtt ccg ttt ttc cgg cga aaa 96

Leu Asn Phe Gln Gly Asn Met Val Asp Val Pro Phe Phe Arg Arg Lys

20

25

30

gac aag gtt gtt ttc gtc atg gga gcc acc gga acc ggc aaa tct cgt 144

Asp Lys Val Val Phe Val Met Gly Ala Thr Gly Thr Gly Lys Ser Arg

35

40

45

cta gcc att gac cta gcc act cgt ttt ccg gcg gag att gta aac tcc 192
 Leu Ala Ile Asp Leu Ala Thr Arg Phe Pro Ala Glu Ile Val Asn Ser

50

55

60

gac aag atc cag gtc tat aaa ggt cta gac att gtg act aac aaa gtc 240
 Asp Lys Ile Gln Val Tyr Lys Gly Leu Asp Ile Val Thr Asn Lys Val

65

70

75

80

act cct gag gaa agc ctt ggc gtt cct cac cac ctt ctc ggc acc gtc 288
 Thr Pro Glu Glu Ser Leu Gly Val Pro His His Leu Leu Gly Thr Val

85

90

95

cac gac act tac gaa gat ttc acg gcg gag gat ttt cag cgt gaa gca 336
 His Asp Thr Tyr Glu Asp Phe Thr Ala Glu Asp Phe Gln Arg Glu Ala

100

105

110

atc agg gcc gtc gag tca atc gtc cag aga gac cgt gtc ccg atc ata 384
 Ile Arg Ala Val Glu Ser Ile Val Gln Arg Asp Arg Val Pro Ile Ile

115

120

125

gcc ggt ggt tcc aat tct tac atc gag gct ctg gtc aac gat tgc gtt 432
 Ala Gly Gly Ser Asn Ser Tyr Ile Glu Ala Leu Val Asn Asp Cys Val

130

135

140

gac ttc cgg tta agg tat aat tgt tgc ttc ttg tgg gtc gac gtc tct 480
 Asp Phe Arg Leu Arg Tyr Asn Cys Cys Phe Leu Trp Val Asp Val Ser

145

150

155

160

aga ccg gtt tta cac tcg ttt gtc tcg gag cga gtt gat aag atg gtt 528
 Arg Pro Val Leu His Ser Phe Val Ser Glu Arg Val Asp Lys Met Val

165	170	175	
gat atg ggt ctc gtc gac gag gtt cgc cgc atc ttc gat ccg tct tcg			576
Asp Met Gly Leu Val Asp Glu Val Arg Arg Ile Phe Asp Pro Ser Ser			
180	185	190	
tcg gat tac tcc gct gga att cgc cga gcc att gga gtt cca gag ctc			624
Ser Asp Tyr Ser Ala Gly Ile Arg Arg Ala Ile Gly Val Pro Glu Leu			
195	200	205	
gac gaa ttt ctc cgt tcg gag atg cgg aat tat ccg gcg gag acg acg			672
Asp Glu Phe Leu Arg Ser Glu Met Arg Asn Tyr Pro Ala Glu Thr Thr			
210	215	220	
gag aga ctt ctt gaa acg gcg atc gag aag att aaa gag aac act tgt			720
Glu Arg Leu Leu Glu Thr Ala Ile Glu Lys Ile Lys Glu Asn Thr Cys			
225	230	235	240
ttg ctt gcg tgt aga caa ttg cag aag att caa agg ctt tac aag cag			768
Leu Leu Ala Cys Arg Gln Leu Gln Lys Ile Gln Arg Leu Tyr Lys Gln			
245	250	255	
tgg aag tgg aac atg cac cgt gtc gac gcg acg gag gtt ttt ctc cga			816
Trp Lys Trp Asn Met His Arg Val Asp Ala Thr Glu Val Phe Leu Arg			
260	265	270	
cga gga gaa gaa gct gat gag gct tgg gat aac tca gtg gct cat ccg			864
Arg Gly Glu Glu Ala Asp Glu Ala Trp Asp Asn Ser Val Ala His Pro			
275	280	285	

agc gca ctc gcc gtc gaa aag ttc ctt agt tac agc gat gac cac cat 912
 Ser Ala Leu Ala Val Glu Lys Phe Leu Ser Tyr Ser Asp Asp His His
 290 295 300

ttg gaa ggc gcc aat att ctc cta ccg gag atc tct gcc gtt ccg cct 960
 Leu Glu Gly Ala Asn Ile Leu Leu Pro Glu Ile Ser Ala Val Pro Pro
 305 310 315 320

ctt cca gcc gcc gtg gcg gcg att tcc cgg tga 993
 Leu Pro Ala Ala Val Ala Ala Ile Ser Arg
 325 330

<210> 14

<211> 330

<212> PRT

<213> Arabidopsis thaliana

<400> 14

Met Lys Pro Cys Met Thr Ala Leu Arg Gln Val Ile Gln Pro Leu Ser
 1 5 10 15
 Leu Asn Phe Gln Gly Asn Met Val Asp Val Pro Phe Phe Arg Arg Lys
 20 25 30
 Asp Lys Val Val Phe Val Met Gly Ala Thr Gly Thr Gly Lys Ser Arg
 35 40 45
 Leu Ala Ile Asp Leu Ala Thr Arg Phe Pro Ala Glu Ile Val Asn Ser
 50 55 60
 Asp Lys Ile Gln Val Tyr Lys Gly Leu Asp Ile Val Thr Asn Lys Val
 65 70 75 80
 Thr Pro Glu Glu Ser Leu Gly Val Pro His His Leu Leu Gly Thr Val

85					90					95						
His	Asp	Thr	Tyr	Glu	Asp	Phe	Thr	Ala	Glu	Asp	Phe	Gln	Arg	Glu	Ala	
100					105					110						
Ile	Arg	Ala	Val	Glu	Ser	Ile	Val	Gln	Arg	Asp	Arg	Val	Pro	Ile	Ile	
115					120					125						
Ala	Gly	Gly	Ser	Asn	Ser	Tyr	Ile	Glu	Ala	Leu	Val	Asn	Asp	Cys	Val	
130					135					140						
Asp	Phe	Arg	Leu	Arg	Tyr	Asn	Cys	Cys	Phe	Leu	Trp	Val	Asp	Val	Ser	
145					150					155					160	
Arg	Pro	Val	Leu	His	Ser	Phe	Val	Ser	Glu	Arg	Val	Asp	Lys	Met	Val	
165					170					175						
Asp	Met	Gly	Leu	Val	Asp	Glu	Val	Arg	Arg	Ile	Phe	Asp	Pro	Ser	Ser	
180					185					190						
Ser	Asp	Tyr	Ser	Ala	Gly	Ile	Arg	Arg	Ala	Ile	Gly	Val	Pro	Glu	Leu	
195					200					205						
Asp	Glu	Phe	Leu	Arg	Ser	Glu	Met	Arg	Asn	Tyr	Pro	Ala	Glu	Thr	Thr	
210					215					220						
Glu	Arg	Leu	Leu	Glu	Thr	Ala	Ile	Glu	Lys	Ile	Lys	Glu	Asn	Thr	Cys	
225					230					235					240	
Leu	Leu	Ala	Cys	Arg	Gln	Leu	Gln	Lys	Ile	Gln	Arg	Leu	Tyr	Lys	Gln	
245					250					255						
Trp	Lys	Trp	Asn	Met	His	Arg	Val	Asp	Ala	Thr	Glu	Val	Phe	Leu	Arg	
260					265					270						
Arg	Gly	Glu	Glu	Ala	Asp	Glu	Ala	Trp	Asp	Asn	Ser	Val	Ala	His	Pro	
275					280					285						
Ser	Ala	Leu	Ala	Val	Glu	Lys	Phe	Leu	Ser	Tyr	Ser	Asp	Asp	His	His	
290					295					300						
Leu	Glu	Gly	Ala	Asn	Ile	Leu	Leu	Pro	Glu	Ile	Ser	Ala	Val	Pro	Pro	
305					310					315					320	
Leu	Pro	Ala	Ala	Val	Ala	Ala	Ile	Ser	Arg							

<210> 15

<211> 1029

<212> DNA

<213> *Arabidopsis thaliana*

<220>

<221> CDS

<222> (1)..(1029)

<400> 15

atg caa caa ctc atg acc ttg tta tca cca cca ctc tct cat tct tct 48

Met Gln Gln Leu Met Thr Leu Leu Ser Pro Pro Leu Ser His Ser Ser

1

5

10

15

ctc ctt ccc acc gtc act acc aaa ttc ggg tca cca cga tta gtc act 96

Leu Leu Pro Thr Val Thr Thr Lys Phe Gly Ser Pro Arg Leu Val Thr

20

25

30

acg tgc atg ggc cat gca ggg cgt aaa aat atc aag gat aag gtg gtt 144

Thr Cys Met Gly His Ala Gly Arg Lys Asn Ile Lys Asp Lys Val Val

35

40

45

ctc atc aca ggt aca aca ggc aca ggc aag tca cgc ctc tca gtc gat 192

Leu Ile Thr Gly Thr Thr Gly Thr Gly Lys Ser Arg Leu Ser Val Asp

50

55

60

ctt gcc acc cgt ttt ttt ccc gcc gag atc ata aac tcg gac aaa atg 240

Leu	Ala	Thr	Arg	Phe	Phe	Pro	Ala	Glu	Ile	Ile	Asn	Ser	Asp	Lys	Met		
65					70					75					80		
caa	atc	tac	aag	gga	ttc	gag	att	gtc	aca	aat	cta	atc	cca	ctg	cat	288	
Gln	Ile	Tyr	Lys	Gly	Phe	Glu	Ile	Val	Thr	Asn	Leu	Ile	Pro	Leu	His		
				85					90					95			
gag	caa	gga	gga	gtc	ccg	cac	cat	ctt	cta	ggt	cag	ttc	cac	cca	caa	336	
Glu	Gln	Gly	Gly	Val	Pro	His	His	Leu	Leu	Gly	Gln	Phe	His	Pro	Gln		
			100					105					110				
gac	ggt	gaa	ctc	acc	cct	gca	gag	ttc	cgt	tct	ttg	gcg	aca	ctg	tcc	384	
Asp	Gly	Glu	Leu	Thr	Pro	Ala	Glu	Phe	Arg	Ser	Leu	Ala	Thr	Leu	Ser		
	115						120					125					
atc	tct	aaa	cta	att	tct	agc	aag	aaa	ctc	ccg	att	gta	ggt	ggt	gga	432	
Ile	Ser	Lys	Leu	Ile	Ser	Ser	Lys	Lys	Leu	Pro	Ile	Val	Val	Gly	Gly		
	130					135					140						
tcc	aac	tcc	ttc	aat	cac	gct	cta	ctc	gcc	gag	cgt	ttt	gac	ccg	gat	480	
Ser	Asn	Ser	Phe	Asn	His	Ala	Leu	Leu	Ala	Glu	Arg	Phe	Asp	Pro	Asp		
145				150						155					160		
att	gat	cca	ttc	tct	ccc	gga	tcg	agt	ctt	tca	acg	atc	tgc	tct	gac	528	
Ile	Asp	Pro	Phe	Ser	Pro	Gly	Ser	Ser	Leu	Ser	Thr	Ile	Cys	Ser	Asp		
			165					170					175				
cta	agg	tac	aaa	tgt	tgc	atc	tta	tgg	gtt	gat	gtt	tta	gag	ccg	gtt	576	
Leu	Arg	Tyr	Lys	Cys	Cys	Ile	Leu	Trp	Val	Asp	Val	Leu	Glu	Pro	Val		
	180							185					190				

ctg ttc caa cac ttg tgc aat cgt gtc gac caa atg atc gag tcg gga 624
 Leu Phe Gln His Leu Cys Asn Arg Val Asp Gln Met Ile Glu Ser Gly

195

200

205

ttg gtc gag cag ctt gcc gaa ttg tac gac cct gtt gta gat tcg ggt 672
 Leu Val Glu Gln Leu Ala Glu Leu Tyr Asp Pro Val Val Asp Ser Gly

210

215

220

cga cga cta ggg gtt cgg aag acg ata gga gta gag gag ttc gac cga 720
 Arg Arg Leu Gly Val Arg Lys Thr Ile Gly Val Glu Glu Phe Asp Arg

225

230

235

240

tac ttt aga gta tac cct aag gag atg gac aag gga att tgg gac tta 768
 Tyr Phe Arg Val Tyr Pro Lys Glu Met Asp Lys Gly Ile Trp Asp Leu

245

250

255

gcg aga aag gcg gcg tac gag gag aca gtg aag ggg atg aaa gag agg 816
 Ala Arg Lys Ala Ala Tyr Glu Glu Thr Val Lys Gly Met Lys Glu Arg

260

265

270

aca tgt cgg ttg gtg aag aag cag aaa gag aag atc atg aag ctg ata 864
 Thr Cys Arg Leu Val Lys Lys Gln Lys Glu Lys Ile Met Lys Leu Ile

275

280

285

aga ggt ggt tgg gag att aag agg ctt gac gct acg gcg gca att atg 912
 Arg Gly Gly Trp Glu Ile Lys Arg Leu Asp Ala Thr Ala Ala Ile Met

290

295

300

gct gag ctg aat caa agt acg gca aag gga gaa gga aag aat ggg aga 960

Ala Glu Leu Asn Gln Ser Thr Ala Lys Gly Glu Gly Lys Asn Gly Arg
 305 310 315 320

gag att tgg gaa aaa cac att gtg gat gaa agt gtc gag att gtc aag 1008
 Glu Ile Trp Glu Lys His Ile Val Asp Glu Ser Val Glu Ile Val Lys
 325 330 335

aag ttt ttg ttg gaa gtt tag 1029
 Lys Phe Leu Leu Glu Val
 340

<210> 16

<211> 342

<212> PRT

<213> Arabidopsis thaliana

<400> 16

Met Gln Gln Leu Met Thr Leu Leu Ser Pro Pro Leu Ser His Ser Ser
 1 5 10 15
 Leu Leu Pro Thr Val Thr Thr Lys Phe Gly Ser Pro Arg Leu Val Thr
 20 25 30
 Thr Cys Met Gly His Ala Gly Arg Lys Asn Ile Lys Asp Lys Val Val
 35 40 45
 Leu Ile Thr Gly Thr Thr Gly Thr Gly Lys Ser Arg Leu Ser Val Asp
 50 55 60
 Leu Ala Thr Arg Phe Phe Pro Ala Glu Ile Ile Asn Ser Asp Lys Met
 65 70 75 80
 Gln Ile Tyr Lys Gly Phe Glu Ile Val Thr Asn Leu Ile Pro Leu His
 85 90 95

Lys Phe Leu Leu Glu Val

340

<210> 17

<211> 990

<212> DNA

<213> *Arabidopsis thaliana*

<220>

<221> CDS

<222> (1)..(990)

<400> 17

atg aag ttc tca atc tca tca ctg aag cag gta caa cca atc ttg tgc 48

Met Lys Phe Ser Ile Ser Ser Leu Lys Gln Val Gln Pro Ile Leu Cys

1 5 10 15

ttc aag aac aag cta tct aag gtc aac gtc aac tct ttt ctc cat ccc 96

Phe Lys Asn Lys Leu Ser Lys Val Asn Val Asn Ser Phe Leu His Pro

20 25 30

aaa gaa aaa gtc atc ttt gtg atg gga gct acc gga tcg ggt aag tct 144

Lys Glu Lys Val Ile Phe Val Met Gly Ala Thr Gly Ser Gly Lys Ser

35 40 45

cgt ctc gcc atc gac cta gca act cgt ttt caa gga gag atc ata aac 192

Arg Leu Ala Ile Asp Leu Ala Thr Arg Phe Gln Gly Glu Ile Ile Asn

50 55 60

tcc gac aag att caa ctt tac aag ggc cta gac gtc cta aca aac aaa 240

Ser Asp Lys Ile Gln Leu Tyr Lys Gly Leu Asp Val Leu Thr Asn Lys

65 70 75 80

gtc acc cct aaa gaa tgc cga ggc gtg cct cac cac ttg ctt gga gta 288

Val Thr Pro Lys Glu Cys Arg Gly Val Pro His His Leu Leu Gly Val

85 90 95

ttc gac tcc gaa gcc gga aac cta acg gcc acc cag tat agc cgc ctt 336

Phe Asp Ser Glu Ala Gly Asn Leu Thr Ala Thr Gln Tyr Ser Arg Leu

100 105 110

gcg tca caa gca atc tcg aaa ctc tca gcg aac aac aag ctt ccc ata 384

Ala Ser Gln Ala Ile Ser Lys Leu Ser Ala Asn Asn Lys Leu Pro Ile

115 120 125

gta gcc ggt gga tca aac tct tac atc gaa gca ctt gtt aat cat tcc 432

Val Ala Gly Gly Ser Asn Ser Tyr Ile Glu Ala Leu Val Asn His Ser

130 135 140

tcg ggg ttt tta tta aac aac tac gat tgt tgt ttc att tgg gtc gac 480

Ser Gly Phe Leu Leu Asn Asn Tyr Asp Cys Cys Phe Ile Trp Val Asp

145 150 155 160

gtt tcc tta ccc gta ctt aac tcc ttt gtc tca aaa cgt gtc gac cgc 528

Val Ser Leu Pro Val Leu Asn Ser Phe Val Ser Lys Arg Val Asp Arg

165 170 175

atg atg gaa gca gga tta ctc gaa gaa gta aga gaa gtg ttc aat cca 576

Met Met Glu Ala Gly Leu Leu Glu Glu Val Arg Glu Val Phe Asn Pro

180	185	190	
aaa gcg aat tac tcc gta ggg ata cga cga gct atc gga gtc ccc gag			624
Lys Ala Asn Tyr Ser Val Gly Ile Arg Arg Ala Ile Gly Val Pro Glu			
195	200	205	
ctc cat gaa tat tta cgt aac gaa tct cta gtg gac cgt gcc aca aaa			672
Leu His Glu Tyr Leu Arg Asn Glu Ser Leu Val Asp Arg Ala Thr Lys			
210	215	220	
agt aaa atg ctt gac gta gcc gtt aaa aat atc aaa aag aac act gag			720
Ser Lys Met Leu Asp Val Ala Val Lys Asn Ile Lys Lys Asn Thr Glu			
225	230	235	240
att tta gct tgt cga cag tta aaa aag att caa cgg ctt cac aag aag			768
Ile Leu Ala Cys Arg Gln Leu Lys Lys Ile Gln Arg Leu His Lys Lys			
245	250	255	
tgg aag atg tct atg cat cgt gtt gac gcc act gag gtg ttc ttg aaa			816
Trp Lys Met Ser Met His Arg Val Asp Ala Thr Glu Val Phe Leu Lys			
260	265	270	
cgc aac gta gaa gaa caa gac gag gct tgg gag aat ctt gta gcg aga			864
Arg Asn Val Glu Glu Gln Asp Glu Ala Trp Glu Asn Leu Val Ala Arg			
275	280	285	
cca agc gag aga atc gtc gat aag ttt tat aat aat aat aac caa ctg			912
Pro Ser Glu Arg Ile Val Asp Lys Phe Tyr Asn Asn Asn Asn Gln Leu			
290	295	300	

aaa aat gat gat gtt gag cac tgt ttg gcg gca tct tac ggc gga gga 960
 Lys Asn Asp Asp Val Glu His Cys Leu Ala Ala Ser Tyr Gly Gly Gly
 305 310 315 320

agt gga agt aga gcc cac aat atg ata tga 990
 Ser Gly Ser Arg Ala His Asn Met Ile
 325

<210> 18

<211> 329

<212> PRT

<213> Arabidopsis thaliana

<400> 18

Met Lys Phe Ser Ile Ser Ser Leu Lys Gln Val Gln Pro Ile Leu Cys
 1 5 10 15
 Phe Lys Asn Lys Leu Ser Lys Val Asn Val Asn Ser Phe Leu His Pro
 20 25 30
 Lys Glu Lys Val Ile Phe Val Met Gly Ala Thr Gly Ser Gly Lys Ser
 35 40 45
 Arg Leu Ala Ile Asp Leu Ala Thr Arg Phe Gln Gly Glu Ile Ile Asn
 50 55 60
 Ser Asp Lys Ile Gln Leu Tyr Lys Gly Leu Asp Val Leu Thr Asn Lys
 65 70 75 80
 Val Thr Pro Lys Glu Cys Arg Gly Val Pro His His Leu Leu Gly Val
 85 90 95
 Phe Asp Ser Glu Ala Gly Asn Leu Thr Ala Thr Gln Tyr Ser Arg Leu
 100 105 110
 Ala Ser Gln Ala Ile Ser Lys Leu Ser Ala Asn Asn Lys Leu Pro Ile

115	120	125
Val Ala Gly Gly Ser Asn Ser Tyr Ile Glu Ala Leu Val Asn His Ser		
130	135	140
Ser Gly Phe Leu Leu Asn Asn Tyr Asp Cys Cys Phe Ile Trp Val Asp		
145	150	155
Val Ser Leu Pro Val Leu Asn Ser Phe Val Ser Lys Arg Val Asp Arg		
165	170	175
Met Met Glu Ala Gly Leu Leu Glu Glu Val Arg Glu Val Phe Asn Pro		
180	185	190
Lys Ala Asn Tyr Ser Val Gly Ile Arg Arg Ala Ile Gly Val Pro Glu		
195	200	205
Leu His Glu Tyr Leu Arg Asn Glu Ser Leu Val Asp Arg Ala Thr Lys		
210	215	220
Ser Lys Met Leu Asp Val Ala Val Lys Asn Ile Lys Lys Asn Thr Glu		
225	230	235
Ile Leu Ala Cys Arg Gln Leu Lys Lys Ile Gln Arg Leu His Lys Lys		
245	250	255
Trp Lys Met Ser Met His Arg Val Asp Ala Thr Glu Val Phe Leu Lys		
260	265	270
Arg Asn Val Glu Glu Gln Asp Glu Ala Trp Glu Asn Leu Val Ala Arg		
275	280	285
Pro Ser Glu Arg Ile Val Asp Lys Phe Tyr Asn Asn Asn Asn Gln Leu		
290	295	300
Lys Asn Asp Asp Val Glu His Cys Leu Ala Ala Ser Tyr Gly Gly Gly		
305	310	315
Ser Gly Ser Arg Ala His Asn Met Ile		
325		

<210> 19

<211> 993

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> CDS

<222> (1)..(993)

<400> 19

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Met Gln Asn Leu Thr Ser Thr Phe Val Ser Pro Ser Met Ile Pro Ile

1 5 10 15

act tct ccg cgg ctg cga ctg cca cca cca cga tca gta gtt ccc atg 96

Thr Ser Pro Arg Leu Arg Leu Pro Pro Pro Arg Ser Val Val Pro Met

20 25 30

act acc gtt tgc atg gaa caa tca tac aag caa aaa gtg gtt gtg atc 144

Thr Thr Val Cys Met Glu Gln Ser Tyr Lys Gln Lys Val Val Val Ile

35 40 45

atg gga gcc acc gga tca ggc aag tca tgc ctc tca atc gat cta gca 192

Met Gly Ala Thr Gly Ser Gly Lys Ser Cys Leu Ser Ile Asp Leu Ala

50 55 60

act cgt ttc tct ggc gag atc gtc aat tcc gac aag att caa ttc tac 240

Thr Arg Phe Ser Gly Glu Ile Val Asn Ser Asp Lys Ile Gln Phe Tyr

65 70 75 80

gat gga ttg aag gtc act acg aat caa atg agc atc ctt gag aga tgt 288

Asp Gly Leu Lys Val Thr Thr Asn Gln Met Ser Ile Leu Glu Arg Cys	
85 90 95	
gga gtc cct cac cat ctc ctt ggt gag ctc cct ccg gat gat agc gaa	336
Gly Val Pro His His Leu Leu Gly Glu Leu Pro Pro Asp Asp Ser Glu	
100 105 110	
cta act acc tcc gaa ttc cgc tct ttg gcg tcg cgg tcc atc tcc gag	384
Leu Thr Thr Ser Glu Phe Arg Ser Leu Ala Ser Arg Ser Ile Ser Glu	
115 120 125	
att act gct cgt gga aac ctc ccg att ata gct ggt gga tca aac tcc	432
Ile Thr Ala Arg Gly Asn Leu Pro Ile Ile Ala Gly Gly Ser Asn Ser	
130 135 140	
ttc att cat gct ctc ctt gtc gac cgt ttt gac ccc aaa acc tat cca	480
Phe Ile His Ala Leu Leu Val Asp Arg Phe Asp Pro Lys Thr Tyr Pro	
145 150 155 160	
ttc tct tct gag aca tcc atc tct tcc ggc ttg agg tac gag tgt tgc	528
Phe Ser Ser Glu Thr Ser Ile Ser Ser Gly Leu Arg Tyr Glu Cys Cys	
165 170 175	
ttc ctt tgg gtg gat gtc tca gtg tcg gtc ctg ttc gag tac ctc tcg	576
Phe Leu Trp Val Asp Val Ser Val Ser Val Leu Phe Glu Tyr Leu Ser	
180 185 190	
aaa cgt gtc gac cag atg atg gag tca ggg atg ttc gag gag cta gcc	624
Lys Arg Val Asp Gln Met Met Glu Ser Gly Met Phe Glu Glu Leu Ala	
195 200 205	

ggt ttc tac gac ccg aga tat tcc ggg tcc gca atc cga gcc cac ggg	672
Gly Phe Tyr Asp Pro Arg Tyr Ser Gly Ser Ala Ile Arg Ala His Gly	
210 215 220	
att cac aag acc ata gga ata ccc gag ttc gac cgg tac ttc agc tta	720
Ile His Lys Thr Ile Gly Ile Pro Glu Phe Asp Arg Tyr Phe Ser Leu	
225 230 235 240	
tac ccg cct gag aga aag cag aag atg tcc gaa tgg gac caa gca aga	768
Tyr Pro Pro Glu Arg Lys Gln Lys Met Ser Glu Trp Asp Gln Ala Arg	
245 250 255	
aag ggg gcg tat gac gaa gct gtc caa gag atc aaa gag aac aca tgg	816
Lys Gly Ala Tyr Asp Glu Ala Val Gln Glu Ile Lys Glu Asn Thr Trp	
260 265 270	
agg ctt gcg aag aag cag att gag agg atc atg aag ctg aaa agc agc	864
Arg Leu Ala Lys Lys Gln Ile Glu Arg Ile Met Lys Leu Lys Ser Ser	
275 280 285	
gga tgg gac att cag agg ttg gac gct acg ccg tca ttt gga aga tcg	912
Gly Trp Asp Ile Gln Arg Leu Asp Ala Thr Pro Ser Phe Gly Arg Ser	
290 295 300	
tca aga gag att tgg gac aat act gtt ttg gat gaa agc atc aag gtt	960
Ser Arg Glu Ile Trp Asp Asn Thr Val Leu Asp Glu Ser Ile Lys Val	
305 310 315 320	
gtg aaa cgc ttc ttg gtg aaa gac aaa gtg tga	993

Val Lys Arg Phe Leu Val Lys Asp Lys Val

325

330

<210> 20

<211> 330

<212> PRT

<213> Arabidopsis thaliana

<400> 20

Met Gln Asn Leu Thr Ser Thr Phe Val Ser Pro Ser Met Ile Pro Ile

1

5

10

15

Thr Ser Pro Arg Leu Arg Leu Pro Pro Pro Arg Ser Val Val Pro Met

20

25

30

Thr Thr Val Cys Met Glu Gln Ser Tyr Lys Gln Lys Val Val Val Ile

35

40

45

Met Gly Ala Thr Gly Ser Gly Lys Ser Cys Leu Ser Ile Asp Leu Ala

50

55

60

Thr Arg Phe Ser Gly Glu Ile Val Asn Ser Asp Lys Ile Gln Phe Tyr

65

70

75

80

Asp Gly Leu Lys Val Thr Thr Asn Gln Met Ser Ile Leu Glu Arg Cys

85

90

95

Gly Val Pro His His Leu Leu Gly Glu Leu Pro Pro Asp Asp Ser Glu

100

105

110

Leu Thr Thr Ser Glu Phe Arg Ser Leu Ala Ser Arg Ser Ile Ser Glu

115

120

125

Ile Thr Ala Arg Gly Asn Leu Pro Ile Ile Ala Gly Gly Ser Asn Ser

130

135

140

Phe Ile His Ala Leu Leu Val Asp Arg Phe Asp Pro Lys Thr Tyr Pro

145

150

155

160

Phe Ser Ser Glu Thr Ser Ile Ser Ser Gly Leu Arg Tyr Glu Cys Cys			
	165	170	175
Phe Leu Trp Val Asp Val Ser Val Ser Val Leu Phe Glu Tyr Leu Ser			
	180	185	190
Lys Arg Val Asp Gln Met Met Glu Ser Gly Met Phe Glu Glu Leu Ala			
	195	200	205
Gly Phe Tyr Asp Pro Arg Tyr Ser Gly Ser Ala Ile Arg Ala His Gly			
	210	215	220
Ile His Lys Thr Ile Gly Ile Pro Glu Phe Asp Arg Tyr Phe Ser Leu			
	225	230	235
Tyr Pro Pro Glu Arg Lys Gln Lys Met Ser Glu Trp Asp Gln Ala Arg			
	245	250	255
Lys Gly Ala Tyr Asp Glu Ala Val Gln Glu Ile Lys Glu Asn Thr Trp			
	260	265	270
Arg Leu Ala Lys Lys Gln Ile Glu Arg Ile Met Lys Leu Lys Ser Ser			
	275	280	285
Gly Trp Asp Ile Gln Arg Leu Asp Ala Thr Pro Ser Phe Gly Arg Ser			
	290	295	300
Ser Arg Glu Ile Trp Asp Asn Thr Val Leu Asp Glu Ser Ile Lys Val			
	305	310	315
Val Lys Arg Phe Leu Val Lys Asp Lys Val			
	325	330	

<210> 21

<211> 125

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 21

Glu Thr Ser Val Lys Ile Leu Val Val Glu Asp Asn His Val Asn Gln
1 5 10 15

Glu Val Ile Lys Arg Met Leu Asn Leu Glu Gly Ile Glu Asn Ile Glu
20 25 30

Leu Ala Cys Asp Gly Gln Glu Ala Phe Asp Lys Val Lys Glu Leu Thr
35 40 45

Ser Lys Gly Glu Asn Tyr Asn Met Ile Phe Met Asp Val Gln Met Pro
50 55 60

Lys Val Asp Gly Leu Leu Ser Thr Lys Met Ile Arg Arg Asp Leu Gly
65 70 75 80

Tyr Thr Ser Pro Ile Val Ala Leu Thr Ala Phe Ala Asp Asp Ser Asn
85 90 95

Ile Lys Glu Cys Leu Glu Ser Gly Met Asn Gly Phe Leu Ser Lys Pro
100 105 110

Ile Lys Arg Pro Lys Leu Lys Thr Ile Leu Thr Glu Phe
115 120 125

<210> 22

<211> 118

<212> PRT

<213> Escherichia coli

<400> 22

Asn Asp Asp Met Met Ile Leu Val Val Asp Asp His Pro Ile Asn Arg

1 5 10 15

Arg Leu Leu Ala Asp Gln Leu Gly Ser Leu Gly Tyr Gln Cys Lys Thr

20 25 30

Ala Asn Asp Gly Val Asp Ala Leu Asn Val Leu Ser Lys Asn His Ile

35 40 45

Asp Ile Val Leu Ser Asp Val Asn Met Pro Asn Met Asp Gly Tyr Arg

50 55 60

Leu Thr Gln Arg Ile Arg Gln Leu Gly Leu Thr Leu Pro Val Ile Gly

65 70 75 80

Val Thr Ala Asn Ala Leu Ala Glu Glu Lys Gln Arg Cys Leu Glu Ser

85 90 95

Gly Met Asp Ser Cys Leu Ser Lys Pro Val Thr Leu Asp Val Ile Lys

100 105 110

Gln Ser Leu Thr Leu Tyr

115

<210> 23

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 23

accatgaact gggcactcaa caatcatcaa g

31

<210> 24

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 24

ggattacgac gaaggtgaga taggattagg

30

<210> 25

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 25

tcagatatga actgggcact caac

24

<210> 26

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 26

ctcaatgctt ttgttccttg actc

24

<210> 27

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 27

tccccgcgga aaatgttctt acggttaggt ag

32

<210> 28

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 28

tcggtcgact tatgattctg tatctgaagg cga

33

<210> 29

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 29

gatccagct agctagggcc ctaccgcggg ga

32

<210> 30

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 30

ctagtccccg cggtagggcc ctagctagct gg

32

<210> 31

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 31

tccccgcgga aaatgtctat aacttgtag c

31

<210> 32

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide

primer for PCR

<400> 32

ctagctagct taacaagggtt caaagaatct tgc

33

<210> 33

<211> 33

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 33

tccccgcgga aaatgaaagc acgaggtgag agg

33

<210> 34

<211> 32

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 34

ctagctagct taacaagggtt caaagaattt gc

32

<210> 35

<211> 30

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 35

aaaatgaagt gtaatgacaa aatggttgtg

30

<210> 36

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 36

gacatttggtt ttgcggtgat attagtc

27

<210> 37

<211> 34

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 37

ttatacatat gaagccatgc atgacggctc taag

34

<210> 38

<211> 28

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Designed oligonucleotide primer
for PCR

<400> 38

cgggatcctc accgggaaat cgccgcca

28