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(54) **PROTEINS IMPARTING
BORON-TOLERANCE AND GENES
THEREOF**

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C12N 15/81 (2006.01)

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(58) **Field of Classification Search** None
See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides genes and proteins having
possibilities to generate plants having tolerance against
excessive boron, which can confer a boric acid tolerance to
organisms. 5 types of genes that can confer a boric acid
tolerance to yeast, such as AtPAB2, AtRBP47c', AtRPS20B,
AtMYB13 and AtMYB68, AtRBP45a, AtRBP45b,
AtRBP45c, AtRBP45d, AtRBP47a, AtRBP47b, AtRBP47c,
AtUBP1a, AtUBP1b and AtUBP1c which were found by
expressing several genes of higher plant *Arabidopsis thaliana*
in yeast that is a organism model of eukaryote. Further, a key
to the toxicity mechanism of boric acid exists in the specific
inhibition of splicing, and a gene related to enhancement of
splicing efficiency also confers a boric acid tolerance.

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FIG. 1

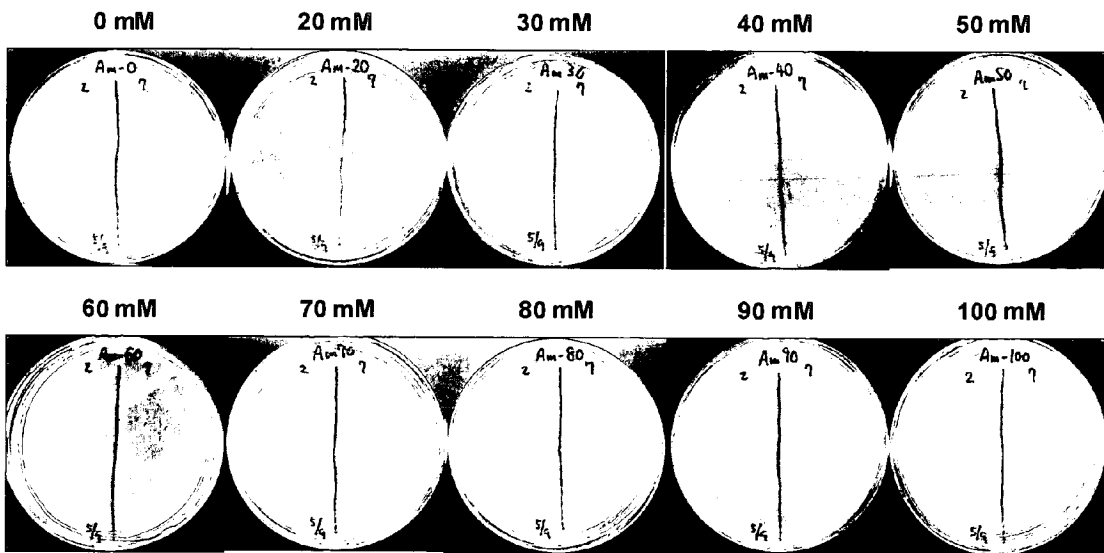


FIG. 2

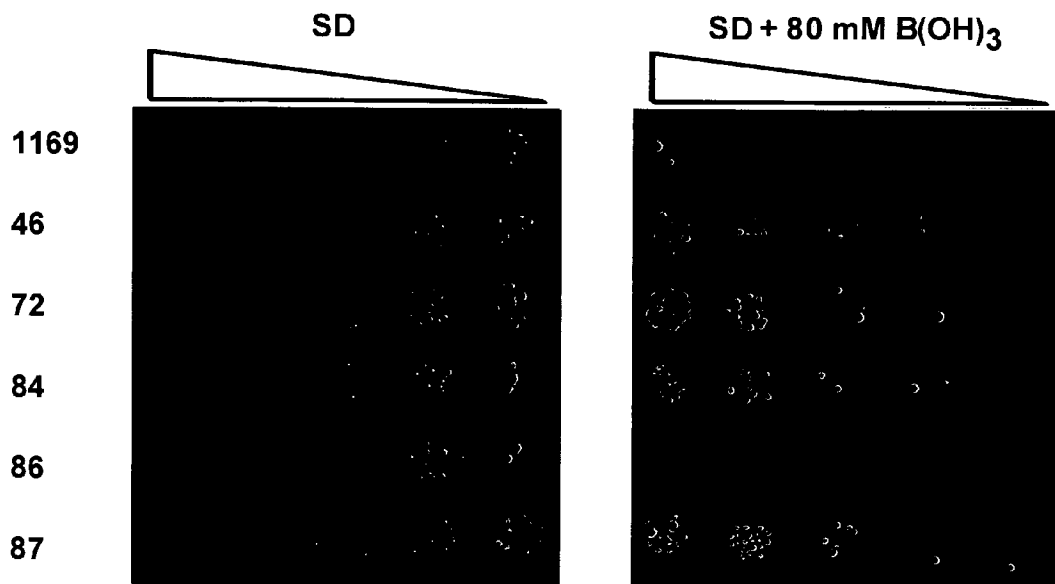


FIG. 3

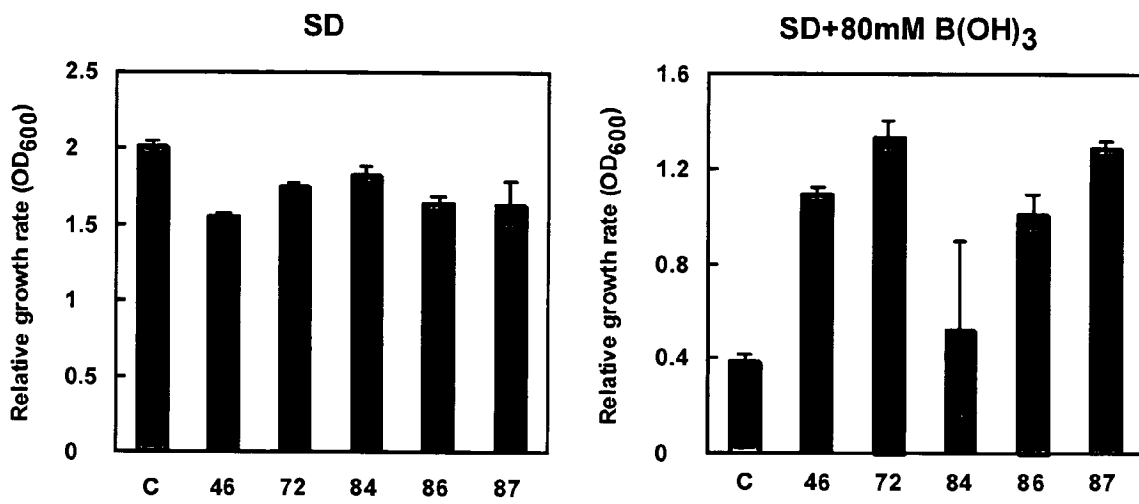


FIG. 4

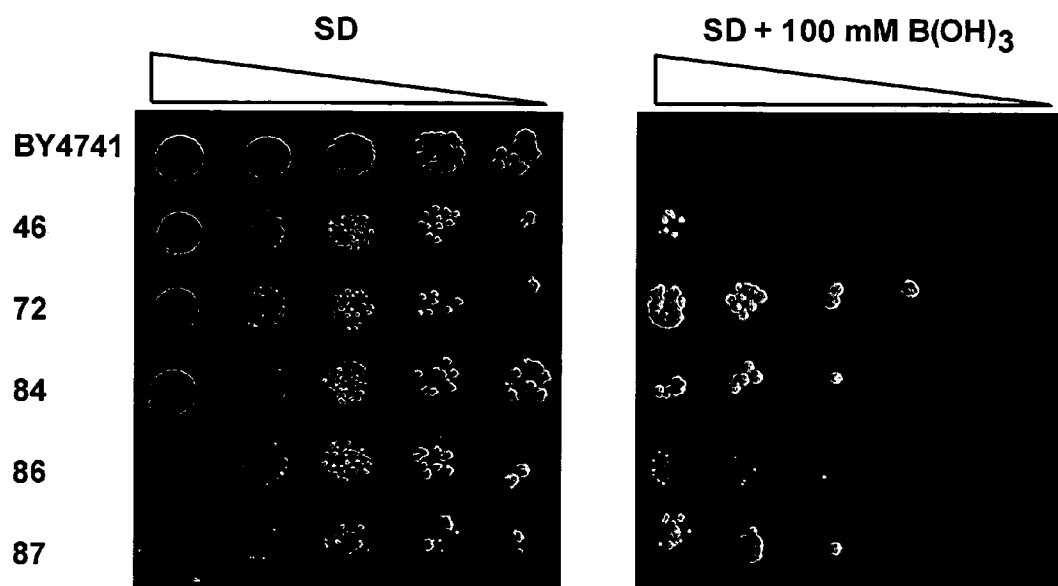


FIG. 5

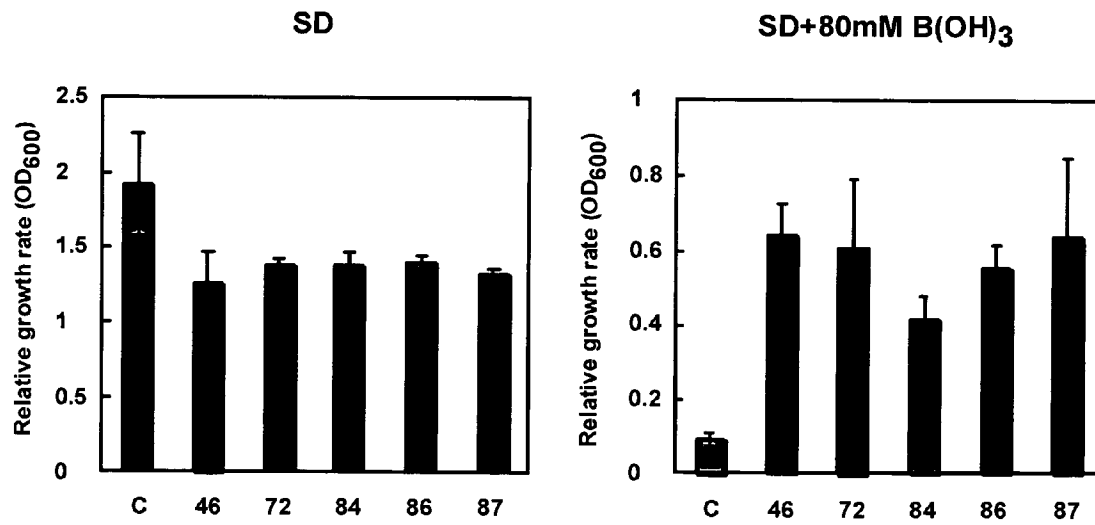


FIG. 6

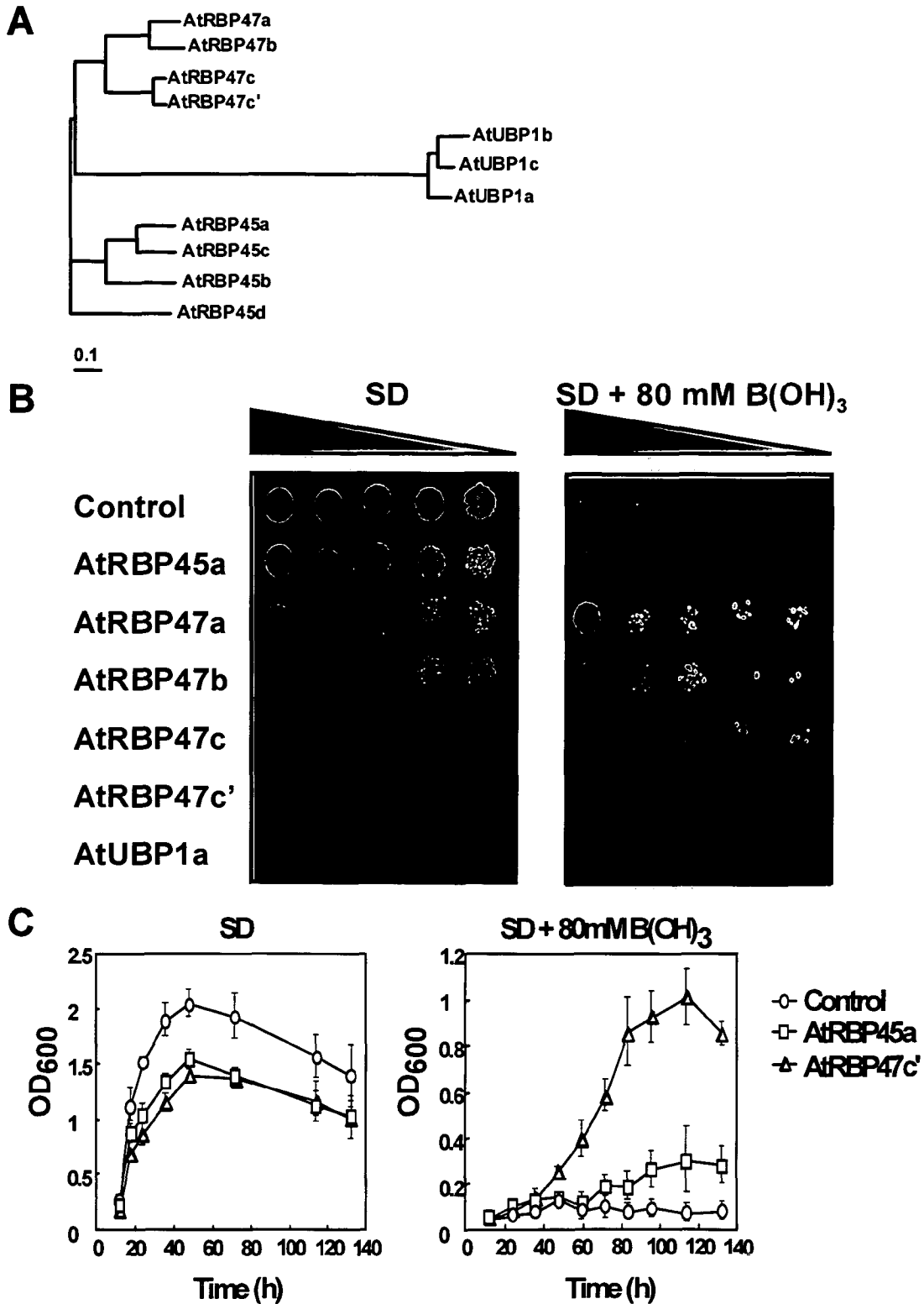


Fig.7

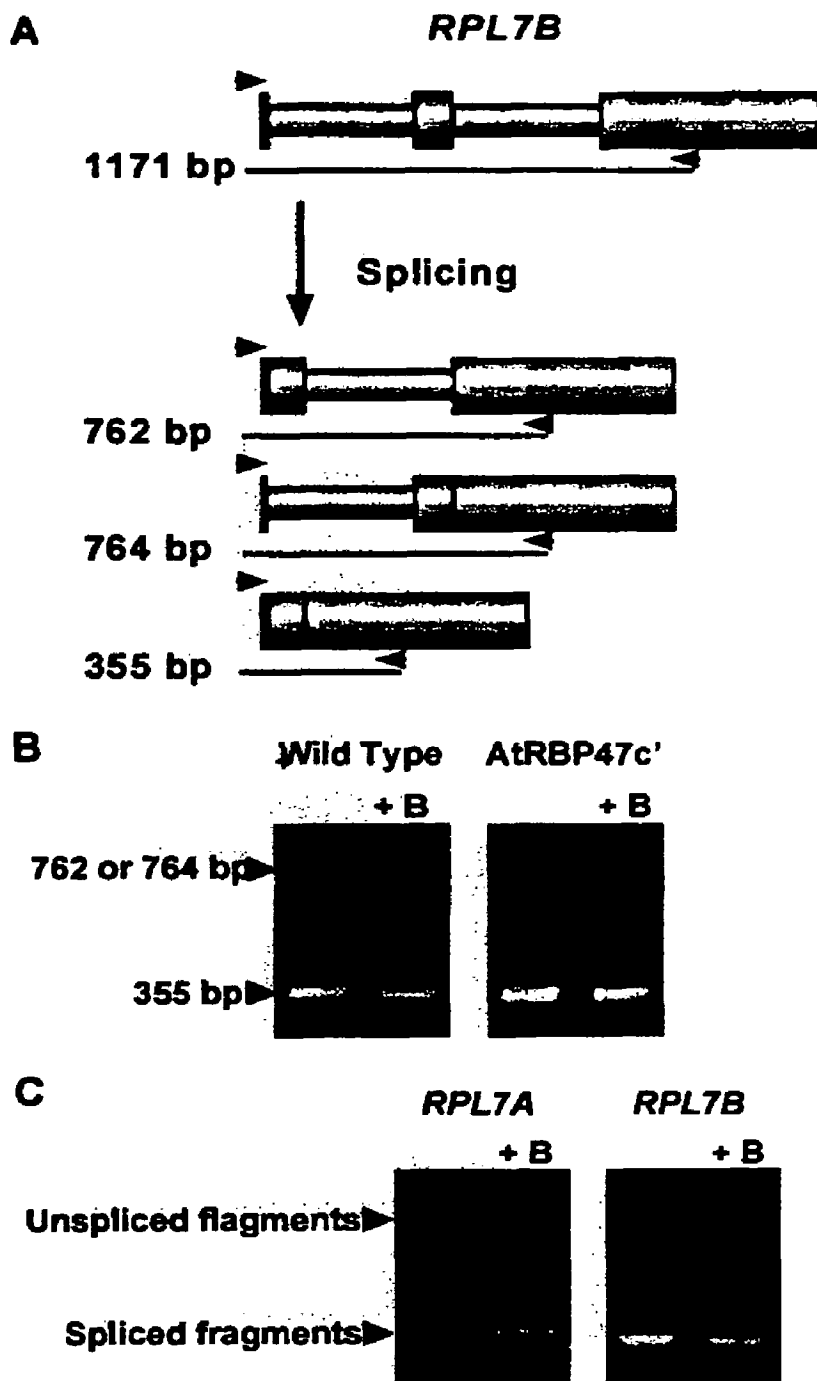


Fig8.

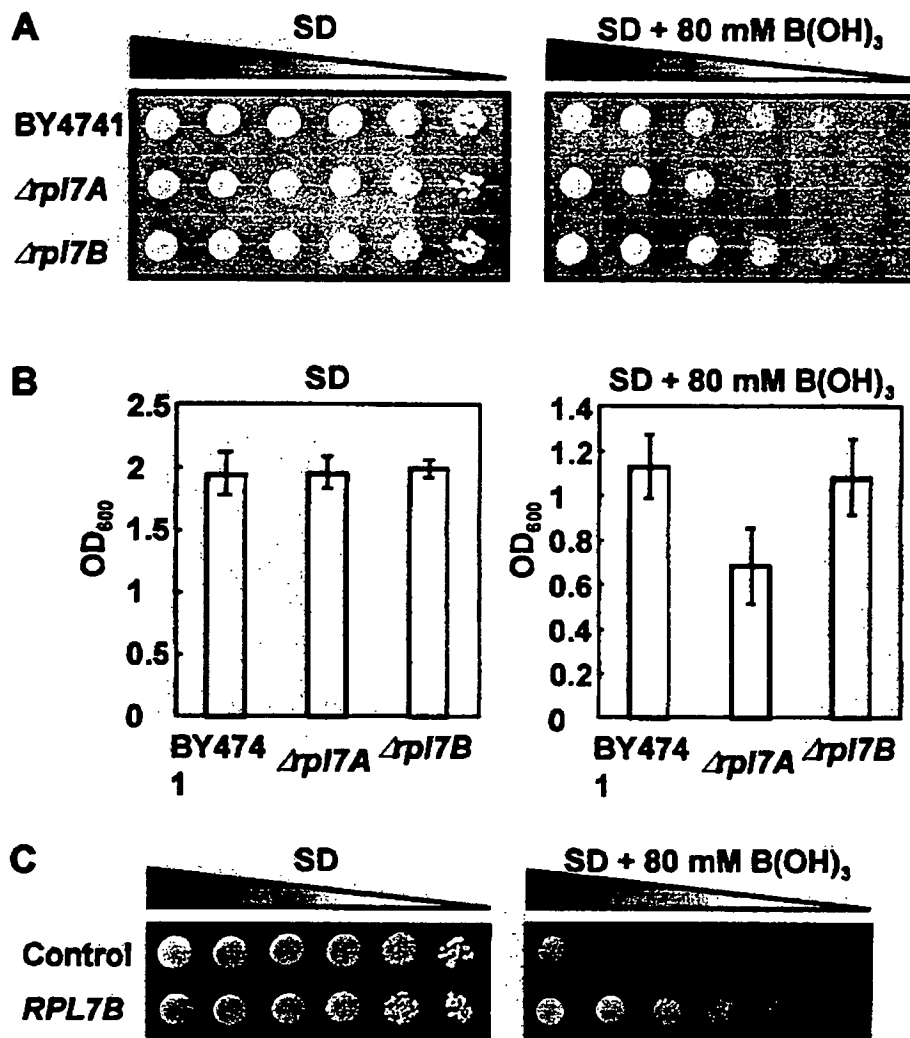


Fig.9

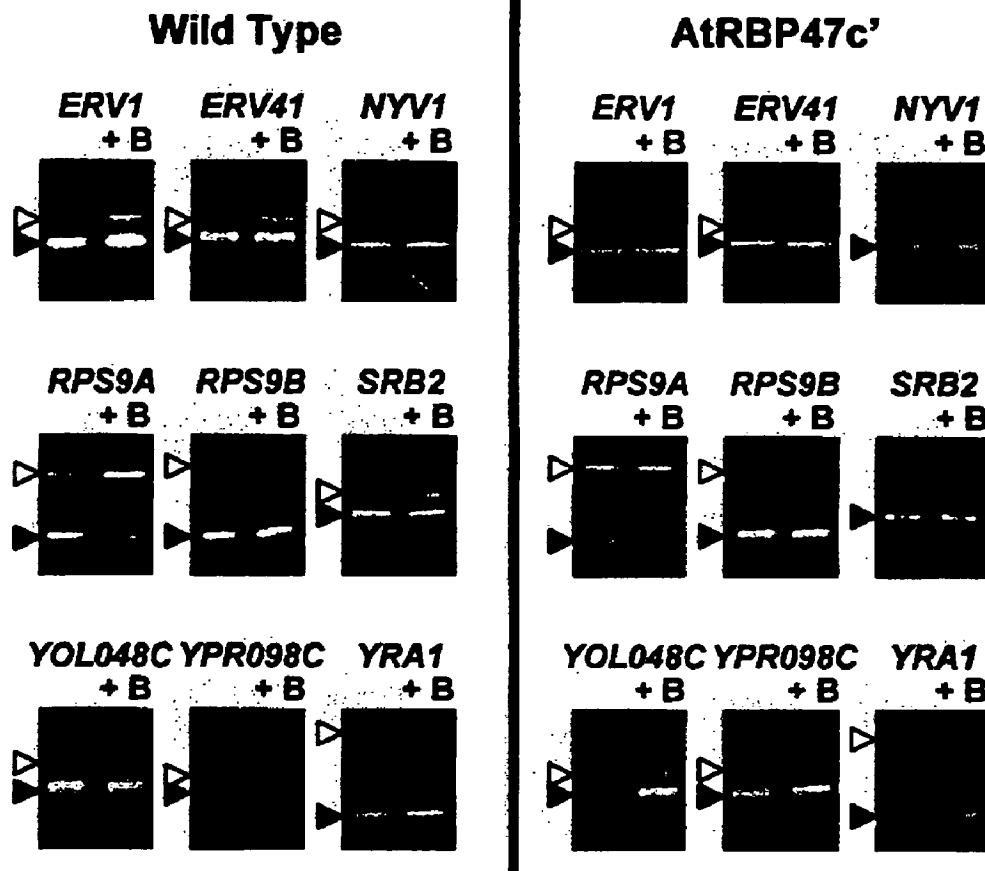
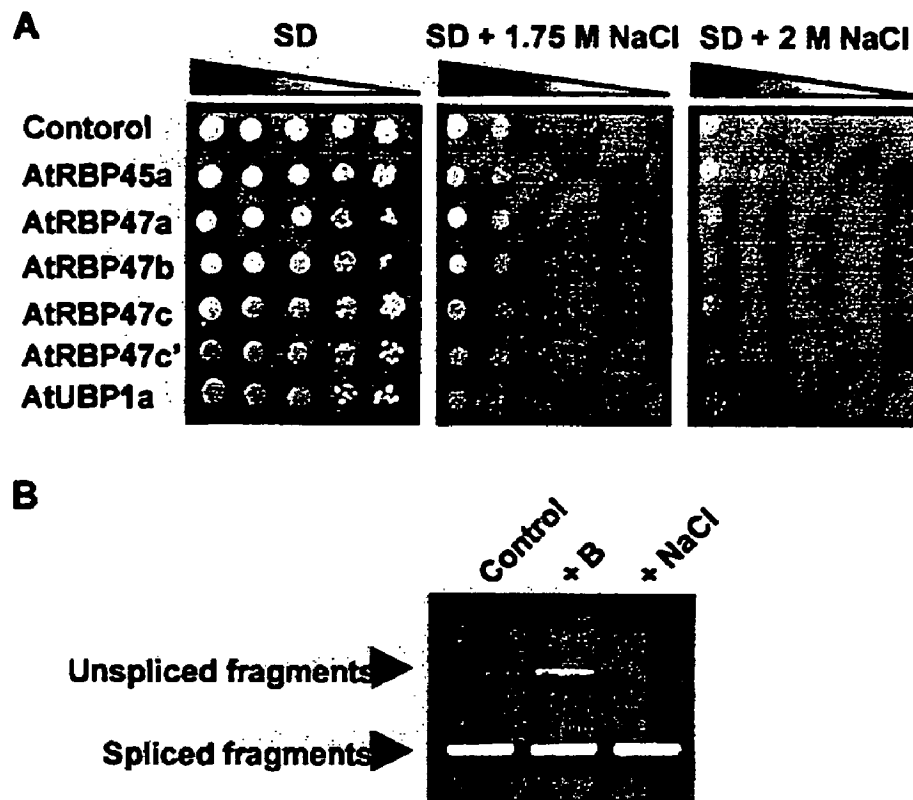


Fig. 10



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**PROTEINS IMPARTING
BORON-TOLERANCE AND GENES
THEREOF**

INCORPORATION BY REFERENCE

This application is a continuation-in-part application of international patent application Serial No. PCT/JP2005/004553 filed Mar. 15, 2005, which published as international publication No. WO 2005/087928 on Sep. 22, 2005, which claims priority to Japanese patent application Serial No. JP 2004-073324 filed Mar. 4, 2004.

The foregoing applications, and all documents cited therein or during their prosecution (“appln cited documents”) and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein (“herein cited documents”), and all documents cited or referenced in herein cited documents, together with any manufacturer’s instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

FIELD OF THE INVENTION

The present invention relates to a protein conferring a boric acid tolerance in *Arabidopsis thaliana* and a gene thereof, a recombinant vector containing the gene, a transformant introduced with the recombinant vector, and a screening method of a gene conferring a boric acid tolerance.

BACKGROUND OF THE INVENTION

Boron is one of the essential trace elements for higher plants (e.g., see nonpatent document 1). As boron also has toxicity, by over ingesting it, plant growth is inhibited and animal dies of acute intoxication. Boron exists in uncharged molecule state in soil solution. Therefore, boron eluviates with relative ease and boron deficiency is easily developed in agricultural crops. Lowering of yield point and quality in agriculture caused by boron deficiency is reported in 130 varieties in 80 or more countries worldwide including Japan (e.g., see nonpatent document 2). Boron is also known to have a restricted range of optimal concentration compared with other elements, and has little difference between the concentrations at which deficiency symptoms develop and excess symptoms develop. Therefore, the quantity adjustment of boron fertilizer application in agriculture is considered to be difficult. Especially, when boron is fertilized excessively, removal of the boron is difficult and crop production in the agricultural land would be affected. Further, as boron is contained in tap water, damages caused-by excessive boron often become a problem in drylands when irrigated agriculture is performed. In addition to agricultural lands over-fertilized with boron in this way, land areas with high concentration of boron are found worldwide. Countries having such areas have an important agenda for taking measures against damages caused by excessive boron in agricultural policy. Further, as boron is also present in agents for treating metal surface and bleaches, wastewater from factories using these agents and bleaches contains boron in appreciable quantities. Although lethal dose of boron for human is 15-20 mg, it is known that various disorders involving digestive organs and nervous systems are developed with less than the lethal dose of boron. At present, the amount of boron contained in wastewater from factories is becoming an issue.

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Recently, a role of boron in plants has been elucidated. It was elucidated that boron bridges pectic polysaccharides in cell walls (e.g., see nonpatent document 3), and showed that the crossbridges are essential for plant growth (e.g., see nonpatent document 4). This is the first knowledge regarding the physiological function of boron at a molecular level in plants. On the other hand, many unclear points remains to be elucidated in the boron transportation mechanism in plants. It was thought for a long time that boron enters into cells by passive diffusion of lipid bilayer, and is transported in plant body by transpiration stream (e.g., see nonpatent document 5). In the meantime, it was known that nutrient conditions of boron, which are suited for growth, differ significantly among species and cultivars. Although absorption, translocation and difference of use efficiency were exemplified as possible causes, molecules of the contributing factors were unknown. In recent years, transportation via channels has been proposed (e.g., see nonpatent document 6), but the evidence was only in vitro experiments using an expression system or a membrane vesicle in *Xenopus laevis* oocytes, and it was not shown whether these channel molecules were involved in the boron transportation in actual individual plants. Further, the presence of active transport by a transporter was suggested from absorption experiments in roots of sunflower roots (e.g., see nonpatent document 7), however, the responsible transporter was not identified.

The present inventors isolated an efflux boron tolerance protein BOR1 from a plant model, *Arabidopsis thaliana* for the first time in animate nature (e.g., see patent document 1). It is thought that BOR1 is responsible for an active boron transportation to vessels under nutrient conditions of lower boron (e.g., see nonpatent document 8). Further, YNL275w of yeast, aside from BOR1 is known as tolerance being responsible for boron transportation (e.g., see nonpatent document 9).

Further, as described above, Boron (B) is an essential trace nutrient for plants (e.g., see nonpatent document 10) and animals (e.g., see nonpatent document 11), but toxic at high concentrations (e.g., see nonpatent documents 12 and 13). Naturally occurring soils containing high concentration of B are distributed across the world and human activities such as fertilization with B, fossil combustion, and irrigation using B-containing water created an environment of high boron concentration (e.g., see nonpatent documents 12 and 13).

Symptoms of B toxicity in plants include chlorosis in leaf margin (e.g., see nonpatent document 13) and fruit disorder and/or bark necrosis (e.g., see nonpatent document 14). Excess B reduces the yield and quality of crops. B toxicity is a major obstruction of agricultural production worldwide. B is also toxic to animals and microorganisms at high concentration. The lethal dose of B is estimated to be about 140 mg/kg for adults and about 270 mg/kg for infants (e.g., see nonpatent documents 15 and 16). Long term-high B intake leads to poor appetite, nausea, weight loss, and decreased sexual activity for humans (e.g., see nonpatent document 17). At present, the acceptable safe intake of B for adults is suggested to be 13 mg per day (e.g., see nonpatent document 18). B has been contained in food preservatives for its sterilization effect on microorganisms (e.g., see nonpatent document 19). In addition, B has been used as insecticides for many years, especially against cockroaches (e.g., see nonpatent document 20).

In the last several decades since B toxicity has been recognized, a number of studies were conducted to investigate toxic effects of B. Those were mostly physiological studies. For example, in soybean leaves, the activity of allantoate amidohydrolase is decreased by boric acid (e.g., see nonpatent

document 21). The inhibitions of malate dehydrogenase and isocitrate dehydrogenase activities by B were observed in *Chara corallina* (e.g., see nonpatent document 22). A negative correlation between placental B levels and delta-aminolevulinic acid dehydratase activities involved in synthesis of porphobilinogen (an intermediate of porphyrin synthesis) in newborns has been also reported (e.g., see nonpatent document 23).

Solubilized borates are thought to play a major role in B toxicity. Boric acids in cells are partially converted into borates due to the higher internal pH. When boric acids with high concentration are supplied to cells, intracellular borate concentration rises to form borate complexes with a variety of cis-diol containing intracellular molecules. These cis-diols containing molecules include NAD⁺, ATP, S-Ado Met, RNA and several sugars (e.g., see nonpatent documents 24 and 25). Since these molecules are used as coenzymes and/or substrates for a number of enzymes, binding of borates is likely to induce loss of function or alteration of enzyme activities, inhibition of biochemical reactions, and finally metabolic disorders. Despite of the accumulation of biochemical and physiological analysis and speculation related to the toxic effect of B, molecular mechanism of B toxicity that leads to cell death has not been elucidated.

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Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

By introducing a gene that confers a boric acid tolerance to yeast into a plant, it has possibilities to generate plants having tolerance against excessive boron. It is thought that plant having boron tolerance can contribute to increase crop yields in places suffering from damages caused by excessive boron. Further, algae or bacteria wherein these genes have been introduced and boron tolerance has increased can be used to absorb boron contained in industrial water and to remove it, thus contributing to environmental cleanup. The present invention may provide a gene or protein conferring a boric acid tolerance to organisms, which has possibilities to generate plants having tolerance against excessive boron. Further, the present invention may provide a method for screening a gene conferring a boric acid tolerance effectively, by elucidating the toxicity mechanism of boric acid.

The present inventors devoted themselves to solve the above object and found 5 types of genes that can confer a boric acid tolerance to yeast, that is, AtPAB2, AtRBP47, AtRPS20B, AtMYB13 and AtMYB68, by expressing several genes of the higher plant *Arabidopsis thaliana* in yeast, which is an organism model of eukaryote. The present invention has been thus completed based on this knowledge. Further, the present inventors found that a key toxicity mechanism of boric acid exists in specific inhibition of splicing, and a gene related to enhancement of splicing efficiency also confers a boric acid tolerance, thus have completed the present invention.

That is, the present invention relates to (1) a DNA encoding a protein that may have an activity of conferring a boric acid tolerance and may consist of the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28

or 30; (2) a DNA encoding a protein that may consist of the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30 and has an activity of conferring a boric acid tolerance; (3) a gene DNA conferring a boric acid tolerance, which may consist of the base sequence shown by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29 or a complementary sequence thereof; (4) a DNA encoding a protein that consists of a base sequence wherein one or a few bases may be deleted, substituted or added in the base sequence shown by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29, and may have an activity of conferring a boric acid tolerance; (5) a DNA encoding a protein that may hybridize with the DNA according to "3" under stringent conditions and may have an activity of conferring a boric acid tolerance; (6) a protein that may have an activity of conferring a boric acid tolerance, which may consist of the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30; (7) a protein consisting of an amino sequence wherein one or a few amino acids may be deleted, substituted or added in the amino sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30; and may have an activity of conferring a boric acid tolerance; (8) a recombinant vector including the DNA according to any one of "1" to "5", which may express a protein conferring a boric acid tolerance; (9) a transformant wherein the recombinant vector according to "8" is introduced, which may express a protein conferring a boric acid tolerance; (10) the transformant according to "9" wherein the transformant may be yeast; (11) the transformant according to "9" wherein the transformant may be a plant; (12) a method for screening a gene conferring a boric acid tolerance, which may comprise the steps of transforming a YNL275w-disrupted yeast which is deficient in and not expressing YNL275w gene by using a gene library, culturing the obtained transformed YNL275w-disrupted yeast in medium containing boric acid, and measuring/evaluating an activity of conferring a boric acid tolerance of the transformed YNL275w-disrupted yeast; (13) a method for screening a gene conferring a boric acid tolerance wherein an enhancement level of splicing efficiency may be measured/evaluated by targeting a specific inhibition of splicing by boric acid; (14) the method for screening a gene conferring a boric acid tolerance according to "13", which may comprise the steps of expressing a test substance in yeast cells, culturing the expressed test substance in the presence of boric acid, and measuring/evaluating an improvement level of a specific inhibition of splicing by boric acid in an intron-containing gene in yeast, as an enhancement level of splicing efficiency; (15) the method for screening a gene conferring a boric acid tolerance according to "14" wherein the gene containing intron in yeast may be a gene RPL7B in *Saccharomyces cerevisiae* genome; (16) use of the DNA according to any one of "1" to "5" as a gene conferring a boric acid tolerance; (17) use of the DNA according to any one of "1" to "5" for producing a plant or yeast conferred a boric acid tolerance; (18) use of the protein according to "6" or "7" as a protein having an activity of conferring a boric acid tolerance; and (19) use of the protein according to "6" or "7" for producing a plant or yeast conferred a boric acid tolerance.

By introducing a gene that confers a boric acid tolerance of the present invention into a plant, it has possibilities to generate plants having tolerance against excessive boron. It is thought that plant having boron tolerance can contribute to increase crop yields in places suffering from damages caused by excessive boron. Algae or bacteria wherein these genes have been introduced and boron tolerance has increased can

be used to absorb boron contained in industrial water and to remove it, thus contributing to environmental cleanup.

Accordingly, it is an object of the invention to not encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. 112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product.

It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawing, in which:

FIG. 1 is a set of pictures showing the results of performance test of boric acid tolerance using yeast strain 1169. Yeast strain 1169 was transformed with pYES2 "2" and pYES2-BORI "7". Each yeast was streaked in SD solid medium containing 0 to 100 mM boric acid. The results after culturing at 26.5° C. for 16 days are shown.

FIG. 2 is a set of pictures showing the growth results of yeast strain 1169 in excessive boric acid medium. Yeast strain 1169 was transformed with 46, 72, 84, 86 and 87. Each yeast was spotted in SD medium containing 80 mM boric acid after the liquid culture. The spots were diluted by 1/5 at a time from left to right. The results after culturing at 26.5° C. for 9 days are shown.

FIG. 3 is a set of graphs showing the results of boric acid tolerance test of yeast strain 1169 in liquid medium. Yeast strain 1169 was transformed with 46, 72, 84, 86 and 87. Each yeast was subcultured to an OD₆₀₀ of 0.1 in SD medium containing 80 mM boric acid after the liquid culture. The values of OD₆₀₀ were measured after culturing at 30° C. for 4 days. The experiments of the test were performed in triplicate. The mean of the measurements and the standard deviation are shown using graph.

FIG. 4 is a set of pictures showing the growth results of yeast strain BY4741 in excessive boric acid medium. Yeast strain BY4741 was transformed with 46, 72, 84, 86 and 87. Each yeast was spotted on SD medium containing 100 mM boric acid after the liquid culture. The spots were diluted by 1/5 at a time from left to right. The results after culturing at 26.5° C. for 10 days are shown.

FIG. 5 is a set of graphs showing the results of boric acid tolerance test of yeast strain BY4741 in liquid medium. Yeast

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strain BY4741 was transformed with 46, 72, 84, 86 and 87. Each yeast was subcultured to an OD₆₀₀ of 0.1 in SD medium containing 80 mM boric acid after the liquid culture. The values of OD₆₀₀ were measured after culturing at 30° C. for 4 days. The experiments of the test were performed 3 times. The mean of the measurements and the standard deviation are shown using graphs.

FIG. 6 is a set of pictures and graphs showing the results of boric acid tolerance test for AtRBP47c'-related genes-transformed yeast cells. (A) Phylogenetic tree of AtRBP47c'-related family proteins. The dendrogram indicates relative evolutionary distance among the AtRBP47c'-related family proteins and was prepared by using NJ method. The bar indicates the genetic distance for 0.1 amino acid substitutions/site. (B) Boric acid tolerance in solid medium. Yeast cells were grown to an OD₆₀₀ of 1.0, serially diluted, and then 10 µl of the diluent was spotted in SD plate added with 0 or 80 mM boric acid. The growth was recorded after culturing for 10 days. Yeast cells transformed with an empty pFL61 vector were used as a control. (C) Boric acid tolerance in liquid medium. Yeast cells were grown to an OD₆₀₀ of 1.0, and then diluted to an OD₆₀₀ of 0.1 in SD medium added with 0 or 80 mM boric acid. The diluted yeast cells were cultured at 30° C. and the values of OD₆₀₀ in indicated time after the dilution were recorded. Vertical bars represent the standard deviation of the mean±the mean of three replicate measurements.

FIG. 7 is a figure showing the effect of boric acid on splicing. (A) Schematic representations of splicing of RPL7B. Three types of mRNA can be generated from pre-mRNA of RPL7B by splicing. Arrowheads indicate the locations of primers used for RT-PCR. (B) The effect of boric acid on splicing of RPL7B. Yeast cells were grown to an OD₆₀₀ of 1.0, and then boric acid was added to reach 80 mM at final concentrations. 24 hours later, the yeast cells were harvested and total RNA was isolated. cDNA was synthesized from the total RNA and was used as a template for splicing analysis by PCR. In this analysis, yeast strain BY4741 (Wild Type) transformed with empty pFL61 vector or AtRBP47c'-expression vector (AtRBP47c') was used. (C) The effect of boric acid on splicing of RPL7A. Splicing of RPL7A was analyzed by RT-PCR in BY4741 transformed with pFL61.

FIG. 8 is a set of pictures and graphs showing the results of boric acid tolerance test for RPL7A- or RPL7B-disrupted yeast cells. (A) Boric acid tolerance in solid medium. Yeast cells were grown to an OD₆₀₀ of 1.0, serially diluted, and then 10 µl of the diluent was spotted in SD plate added with 0 or 80 mM boric acid. The growth was recorded after culturing for 7 days. (B) Boric acid tolerance in liquid medium. Yeast cells were grown to an OD₆₀₀ of 1.0, and then diluted to an OD₆₀₀ of 0.1 in SD medium added with 0 or 80 mM boric acid. The diluted yeast cells were cultured at 30° C. for 21 hours (SD) and 60 hours (SD+80 mM boric acid) after the dilution, and then the values of OD₆₀₀ were recorded. Vertical bars represent the standard deviation of the mean±the mean of three replicate measurements. Δrpl7a and Δrpl7b represent RPL7A-disruption mutant (Y04443) and RPL7B-disruption mutant (Y01094), respectively. (C) The effect of over-expression of RPL7B on boric acid tolerance in RPL7A-disrupted yeast. Yeast cells were grown to an OD₆₀₀ of 1.0, serially diluted, and then 10 µl of the diluent was spotted in SD plate added with 0 or 80 mM boric acid. The growth was recorded after culturing for 5 days. Yeast cells transformed with an empty pDR195 vector were used as a control.

FIG. 9 is a set of pictures showing the effect of boric acid on splicing of genes containing noncanonical branchpoint sequences. Yeast were grown to an OD₆₀₀ of 1.0, and then boric acid was added to reach 80 mM at final concentrations.

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24 hours later, the yeast cells were harvested, and total RNA was isolated to use as a template for splicing analysis by PCR. In this analysis, yeast strain BY4741 (Wild Type) transformed with empty pFL61 vector or AtRBP47c'-expression vector (AtRBP47c') was used. White and black arrowheads indicate unspliced and spliced fragments, respectively.

FIG. 10 is a set of pictures showing the effects of salt on growth of AtRBP47c'-related genes-transformed yeast cells and on splicing of RPL7B. (A) Salt tolerance in solid medium. Yeast cells were grown to an OD₆₀₀ of 1.0, serially diluted, and then 10 µl of the diluent was spotted in SD plate containing 0, 1.75 or 2 M NaCl. The growth was recorded after culturing for 7 days. Yeast cells transformed with an empty pFL61 vector were used as a control. (B) The effect of salt on splicing of RPL7B. Yeast cells were grown to an OD₆₀₀ of 1.0, and then NaCl or boric acid was added to reach 2 M or 80 mM at final concentrations, respectively. 24 hours later, the yeast cells were harvested and total RNA was isolated. cDNA was synthesized from the total RNA and was used as a template for splicing analysis by PCR.

DETAILED DESCRIPTION

As for a gene DNA of the present invention, it is not especially limited as long as it is a gene conferring a boric acid tolerance consisting of the following: (A) a DNA encoding a protein that has an activity of conferring a boric acid tolerance and consists of the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30; (B) a DNA encoding a protein that consists of the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30 and has an activity of conferring a boric acid tolerance; (C) a gene DNA conferring a boric acid tolerance, which consists of the base sequence shown by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29 or a complementary sequence thereof; (D) a DNA encoding a protein that consists of a base sequence wherein one or a few bases are deleted, substituted or added in the base sequence shown by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29, and has an activity of conferring a boric acid tolerance; or (E) a DNA encoding a protein that hybridizes with a DNA conferring a boric acid tolerance which consists of the base sequence shown by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29 under stringent conditions and has an activity of conferring a boric acid tolerance.

Further, as for a protein of the present invention, it is not especially limited as long as the protein is the following: (A) a protein having an activity of conferring a boric acid tolerance, which consists of the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30; or (B) a protein consisting of an amino sequence wherein one or a few amino acids are deleted, substituted or added in the amino sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30; and having an activity of conferring a boric acid tolerance. Here, the term "a gene conferring a boric acid tolerance" relates to a gene that can confer a boric acid tolerance to a living organism, and the term "a protein conferring a boric acid tolerance" relates to a protein that can confer a boric acid tolerance to a living organism.

The above-mentioned phrase "a protein which has an activity of conferring a boric acid tolerance" relates to a protein having an activity that can confer tolerance against boric acid in a living organism such as yeast and plant, and the yeast and plant highly-expressing the protein can be grown even in the presence of boric acid in high concentration.

AtPAB2 gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 1, AtPAB2 as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 2, AtRBP47c' gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 3, AtRBP47c' as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 4, AtRPS20B gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 5, AtRPS20B as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 6, AtMYB13 gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 7, AtMYB13 as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 8, AtMYB68 gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 9, AtMYB68 as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 10, AtRBP45a gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 11, AtRBP45a as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 12, AtRBP45b gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 13, AtRBP45b as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 14, AtRBP45c gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 15, AtRBP45c as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 16, AtRBP45d gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 17, AtRBP45d as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 18, AtRBP47a gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 19, AtRBP47a as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 20, AtRBP47b gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 21, AtRBP47b as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 22, AtRBP47c gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 23, AtRBP47c as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 24, AtUBP1a gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 25, AtUBP1a as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 26, AtUBP1b gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 27, AtUBP1b as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 28, AtUBP1c gene as a gene conferring a boric acid tolerance consisting of the base sequence shown by SEQ ID NO: 29, AtUBP1c as a protein conferring a boric acid tolerance consisting of the amino acid sequence shown by SEQ ID NO: 30, can be exemplified respectively.

The above-mentioned phrase "an amino sequence wherein one or a few amino acids are deleted, substituted or added" relates to an amino sequence wherein, for example, any number of 1 to 20, preferably 1 to 15, more preferably 1 to 10, furthermore preferably 1-5 amino acids are deleted, substituted or added. Further, the above-mentioned phrase "a base

sequence wherein one or a few bases are deleted, substituted or added" relates to a base sequence wherein, for example, any number of 1 to 20, preferably 1 to 15, more preferably 1 to 10, furthermore preferably 1 to 5 bases are deleted, substituted or added.

For example, a DNA, which consists a base sequence wherein one or a few bases are deleted, substituted or added (mutated DNA), can be produced by any methods such as chemical synthesis, genetic engineering method and mutagenesis, which are known to those skilled in the art. Specifically, a mutated DNA can be obtained by introducing a mutation into a DNA that consists of the base sequence shown by SEQ ID NO: 1, 3, 5, 7 or 9, with the use of methods such as a method of allowing to contact and react an agent to be a mutagen, a method of irradiating ultraviolet and a genetic engineering method. Site-specific mutagenesis which one of the genetic engineering methods is a useful method that can introduce a specific mutant into a specific site, and can be performed according to methods described previously such as Molecular Cloning, A laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989 (hereinafter, abbreviated as "Molecular Cloning 2nd Ed."); Current Protocols in Molecular Biology, Supplement 1-38, John Wiley & Sons (1987-1997). By expressing this mutated DNA with the use of a suitable expression system, a protein encoded by an amino sequence wherein one or a few amino acids are deleted, substituted or added can be obtained.

The above-mentioned phrase "a base sequence which hybridizes under stringent conditions" relates to a base sequence obtained by using methods such as colony hybridization, plaque hybridization, and Southern blotting, with the use of nucleic acids such as DNA and RNA as a probe. Specifically, DNA that can be identified by hybridizing by using a filter-immobilized DNA derived from a colony or a plaque, or a fragment thereof, at 65° C. in the presence of 0.7-1.0 M NaCl; by washing the filter under the condition of 65° C. with the use of SSC solution of approximately 0.1-2.0-fold concentration (one-fold concentration of SSC solution is composed of 150 mM NaCl and 15 mM sodium citrate); can be exemplified. Hybridization can be performed according to the method described in Molecular Cloning 2nd Ed. and the like.

For example, as a DNA that can hybridize under stringent conditions, a DNA having above a certain level of homology with a base sequence of DNA used as a probe can be exemplified, and a DNA having, for example, 60% or more, preferably 70% or more, more preferably 80% or more, furthermore preferably 90% or more, especially preferably 95% or more, most preferably 98% or more of homology, can be exemplified.

Methods for obtaining and preparing genes of the present invention are not especially limited; and it can be prepared by isolating the desired genes through preparing a suitable probe or primer based on the base sequence information shown by SEQ ID NO: 1, 3, 5, 7 or 9, or the amino sequence information shown by SEQ ID NO: 2, 4, 6, 8 or 10 disclosed in the present specification, and screening a cDNA library wherein the presence of the genes are expected with the use of the above probe or primer; or by chemical synthesis according to ordinary methods.

Specifically, a gene of the present invention can be obtained by preparing a cDNA library from *Arabidopsis thaliana* from where the gene of the present invention was isolated, according to ordinary methods; and selecting the desired clone with the use of a specific and appropriate probe for the gene of the present invention. As the origin of the above cDNA, a variety of cells and tissues derived from the

above plant can be exemplified; and further, isolation of all RNA from these cells or tissues, purification and isolation of mRNA, obtaining cDNA and the cloning thereof, and the like, can all be performed according to ordinary methods. As for a method for screening genes of the present invention from a cDNA library, for example, methods which are generally used by those skilled in the art such as methods described in Molecular Cloning 2nd Ed., and the like, can be exemplified.

Furthermore, a mutated gene or homologous gene of the present invention which consists of the base sequence shown by any one of the above (B) to (F) can be isolated, with the use of a DNA fragment having, the base sequence shown by SEQ ID NO: 1, 3, 5, 7 or 9, or part thereof, by screening a homolog of the DNA under appropriate conditions from other organisms and the like. Furthermore, it can be prepared by the above-mentioned methods for preparing the mutated DNA.

Methods for obtaining and preparing proteins of the present invention are not especially limited, and any one of the following proteins can be used: a natural occurring protein, a chemical synthetic protein, or a recombinant protein prepared by transgenesis. When obtaining a natural occurring protein, a protein of the present invention can be obtained from the cells or tissues expressing the protein, by combining appropriately the methods of isolation/purification of protein. When preparing a protein by chemical synthesis, for example, a protein of the present invention can be synthesized according to chemical synthesis such as Fmoc method (fluorenylmethyloxycarbonyl method) and tBoc method (t-butyloxycarbonyl method). Further, a protein of the present invention can be also synthesized with the use of various types of peptide synthesizer being marketed. When preparing a protein by transgenesis, a protein of the present invention can be prepared by introducing a DNA that consists of a base sequence encoding the protein into a preferable expression system. Among the above methods, preparation by transgenesis which manipulation is relatively easy and by which a large amount of preparation can be available, is preferable.

For example, when preparing a protein of the present invention by transgenesis, known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin chromatography, and preferably high-performance liquid chromatography are used for collecting and purifying the protein from cell culture. Particularly, as for a column to use for affinity chromatography, for example, by using a column bound with antibodies such as monoclonal antibodies against a protein of the present invention; when a normal peptide tag is added to the above protein of the present invention, by using a column bound with certain materials that have an affinity for the peptide tag, purified products of these proteins can be obtained. Further, when a protein of the present invention is expressed on a cell membrane, purified preparations can be obtained by performing the above purification treatment after allowing to act a cell membrane catabolic enzyme.

In addition, a protein consisting of an amino acid sequence wherein one or a few amino acids are deleted, substituted or added in the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30, or a protein consists of the amino acid sequence having 60% or more of homology with the amino acid shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30 can be prepared or obtained conveniently by those skilled in the art according to the base sequence information shown by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29 which shows one of the examples of the base sequences encoding the amino

acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30 respectively. For example, a homolog of a DNA having the base sequence shown by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29, or part thereof can be isolated from organisms other than *Arabidopsis thaliana* by screening under appropriate conditions with the use of the DNA as a probe. A protein encoded by the homolog DNA can be prepared by integrating into an expression vector to express in an appropriate host after cloning a full length of the homolog DNA.

As for a recombinant vector of the present invention, it is not especially limited as long as it is a recombinant vector that contains the above gene of the present invention and can express a protein conferring a boric acid tolerance, and a recombinant vector of the present invention can be constructed by integrating the gene of the present invention appropriately into an expression vector. As for an expression vector, a vector that can self-replicate in host cells or can be integrated in chromosomes of host cells, is preferable; moreover, vectors which contain regulatory sequences such as promoter, enhancer and terminator at a position where a gene of the present invention can be expressed, can be used preferably. As for an expression vector, an expression vector for yeast, an expression vector for plant cells, an expression vector for bacteria, an expression vector for animal cells and the like can be used; however, a recombinant vector using an expression vector for yeast or expression vector for plant cells is preferable.

As for an expression vector for yeast, pYES2 (Invitrogen), YEpl3 (ATCC37115), YEpl24 (ATCC37051), Ycp50 (ATCC37419), pHS19 and pHS15 can be exemplified. As for a promoter for yeast, PHO5 promoter, PGK promoter, GAP promoter, ADH promoter, GAL1 promoter, GAL10 promoter, heat shock protein promoter, MF α 1 promoter and CUP1 promoter can be specifically exemplified.

As for an expression vector for plant cells, plasmids such as Ti plasmid (Tumor inducing plasmid), pSPORT1, pT7Blue-T vector, pIG121-Hm [Plant Cell Report, 15, 809-814(1995)], pBI121 [EMBO J. 6, 3901-3907(1987)], or plant viral vectors such as tobacco mosaic virus, cauliflower mosaic virus and geminivirus can be exemplified. As for a promoter for plant cells, cauliflower mosaic virus 35S promoter [Mol.Gen. Genet (1990) 220, 389-392] and ribulose biphosphate carboxylase small subunit promoter can be exemplified, and as for a terminator, nopaline synthase gene terminator can be exemplified.

Further, as for a transformant of the present invention, it is not especially limited as long as it is a transformant wherein the above recombinant vector of the present invention is introduced and which expresses a protein conferring a boric acid tolerance. Transgenic yeasts, transgenic plants (cells, tissues, individuals), transgenic bacteria, transgenic animals (cells, tissues, individuals), can be exemplified, while transgenic yeasts and transgenic plants (cells, tissues, individuals) are preferable.

As for a host yeast to use for producing a transgenic yeast, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Trichosporon pullulans* and *Schwanniomyces alluvius* can be exemplified. As for a method for introducing a recombinant vector to host yeast, for example, electroporation, spheroplast method and lithium acetate method can be exemplified.

As for a host plant (cell, tissue, individual) to use for producing a transgenic plant (cell, tissue, individual), species is not especially limited, and it can be appropriately selected from plants such as flowers and ornamental plants, fruit plants, vegetables, root crops, cereals, foliage plants and trees

including fruit trees, for example, plants belonging to Solanaceae, Poaceae, Brassicaceae, Asteraceae, Pedaliaceae, Oleaceae, Myrtaceae, Rosaceae, Leguminosae, Palmae or rubiaceae, and cultured cells and tissues thereof (seed, callus and the like) To produce a transgenic plant, a method for introducing a gene DNA of the present invention into genomic DNA within plant cells, by introducing the above recombinant vector into plant cells with the use of the recombinant vector of the present invention containing a gene of the present invention can be used. Transformation of a plant can be performed by appropriately using known methods such as leaf disk cocultivation method, electroporation, *Agrobacterium* method and particle gun method, according to species of the plant. Other methods for producing transgenic plant, including a method by directly incorporating a recombinant vector of the present invention into a receptor cell can be also used, by physically or chemically enhancing the permeability of plant cells.

As for a method for screening a gene conferring a boric acid tolerance of the present invention is not especially limited as long as it is a method for measuring/evaluating an activity of conferring a boric acid tolerance of the transformed YNL275w-disrupted yeast by transforming a YNL275w-disrupted yeast which is deficient in and not expressing YNL275w gene with the use of a gene library such as a variety of plants or yeasts, and by culturing the obtained transformed YNL275w-disrupted yeast in medium containing boric acid. As for a measurement/evaluation of an activity of conferring a boric acid tolerance, a measurement/evaluation of a level of growth/proliferation of transgenic yeast in culture medium containing boric acid can be exemplified. Further, as for a YNL275w-disrupted strain, *Saccharomyces cerevisiae* strain 1169 (Winzler, E. A.; Shoemaker, D. D.; Astromoff, A.; Liang, H.; Anderson, K.; Andre, B.; Bangham, R.; Benito, R.; Boeke, J. D.; Bussey, H.; Chu, A. M.; Connolly, C.; Davis, K.; Dietrich, F.; Dow, S. W.; El Bakkoury, M.; Foury, F.; Friend, S. H.; Gentalen, E.; Giaever, G.; Hege-
mann, J. H.; Jones, T.; Laub, M.; Liao, H.; Liebundguth, N.; Lockhart, D. J.; Lucau-Danila, A.; Lussier, M.; M'Rabet, N.; Menard, P.; Mittmann, M.; Pai, C.; Rebischung, C.; Revuelta, J. L.; Riles, L.; Roberts, C. J.; Ross-MacDonald, P.; Scherens, B.; Snyder, M.; Sookhai-Mahadeo, S.; Storms, R. K.; Veronneau, S.; Voet, M.; Volckaert, G.; Ward, T. R.; Wysocki, R.; Yen, G. S.; Yu, K. X.; Zimmermann, K.; Philippsen, P.; Johnston, M.; Davis, R. W. (1999) Functional characterization of the *Saccharomyces cerevisiae* genome by gene deletion and parallel analysis. *Science* 285: 901-906) can be preferably exemplified. As for yeast to use for screening, it is not limited to YNL275w-disrupted strains, and wild types can be used.

Further, as for a screening method of a gene conferring a boric acid tolerance of the present invention, a method for measuring/evaluating an enhancement level of splicing efficiency can be exemplified, for example, a method for measuring/evaluating an improvement level of a specific inhibition of splicing by boric acid in an intron-containing gene in yeast by expressing a test substance in yeast cells and culturing the expressed test substance in the presence of boric acid, as an enhancement level of splicing efficiency, can be exemplified. As for an intron-containing gene in yeast, specifically RPL7B gene (SEQ ID NO: 33) which is a gene encoding large subunit protein of essential ribosome in *Saccharomyces cerevisiae* genome, can be exemplified. The improvement level of specific inhibition of splicing by boric acid can be measured, for example, by RT-PCR, and at that time, AtRBP47c' gene, which is a gene conferring a boric acid tolerance is preferably used as a positive control.

In the present invention, use of (a method for) using the above DNA of the present invention as a gene conferring a boric acid tolerance, use of (a method for) using the above DNA of the present invention for producing plants or yeast conferred a boric acid tolerance, use of (a method for) using the above protein of the present invention as a protein having an activity of conferring a boric acid tolerance, and use of (a method for) using the above protein of the present invention for producing plants or yeast conferred a boric acid tolerance are included. Therefore, using the above gene conferring a boric acid tolerance and the above protein having an activity of conferring a boric acid tolerance (protein conferring a boric acid tolerance) for producing plants or yeast conferred a boric acid tolerance are included in the embodiments of the present invention.

The invention will now be further described by way of the following non-limiting examples which further illustrate the invention, and are not intended, nor should they be interpreted to, limit the scope of the invention.

EXAMPLES

Example 1

1.1. Test Yeasts and Plasmids

As for yeasts, *Saccharomyces cerevisiae* strain 1169 (purchased from Research Genetics) and *Saccharomyces cerevisiae* strain BY4741 (purchased from Research Genetics) are used. Genotypes for strain 1169 are MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, YNL275w, kanMX4; and MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0 for strain BY4741 respectively. As for plasmids, pYES2 (purchased from Invitrogen Genetics) and pFLM61 (provided from Dr. Nicolaus von Wiren in Hohenheim University, Germany; Minet M., Dufour M. -E., and Lacroute F. (1992) Complementation of *Saccharomyces cerevisiae* auxotrophic mutants by *Arabidopsis thaliana* cDNAs. *Plant J.* 2, 417-422) were used. pFL61 was used to produce an *Arabidopsis thaliana* expression library. Boric acid tolerance test on yeast-strain 1169

The performance of boric acid tolerance in the used yeast strain 1169 was evaluated. A single colony of yeast strain 1169 which was transformed with pYES2 and pYES2-BOR1 (to which inserted CDS of BOR1, a boron tolerance gene of *Arabidopsis thaliana* downstream of GAL1 promoter of pYES2 vector) was picked by a platinum loop, and shaking cultured to an OD₆₀₀ of around 1.0 in SD liquid medium. The culture solution was respectively streaked in SD solid medium containing 0, 20, 30, 40, 50, 60, 70, 80, 90 or 100 mM boric acid, and cultured at 26.5° C. for 16 days. It was then evaluated whether or not the yeast can form colony in each medium.

1.2. Screening of Genes Conferring Boric Acid Tolerance

Yeast strain 1169 was transformed with lithium acetate method with the use of *Arabidopsis thaliana* expression library (provided from Dr. Nicolaus von Wiren in Hohenheim University, Germany; Schaaf G., Catoni E., Fits M., Schwacke R., Schneider A., von Wiren N., and Frommer W.B. (2002) A putative role for the vacuolar calcium/manganese proton antiporter AtCAX2 in heavy metal detoxification. *Plant Biol.* 4; 612-618). The transgenic yeast was streaked in SD medium added 80 mM boric acid (6.7 g/l yeast nitrogen base without amino acids, 5 g/l ammonium sulfate, 20 g/l glucose, 2 g/l histidine, 2 g/l methionine, 3 g/l leucine, 20 g/l agar, pH 5.5) and cultured at 26.5° C. After 10 to 14 days, plasmids were collected from the yeast that formed a colony.

The collected plasmids were introduced into yeast again and the repeatability of the performance of boric acid tolerance was identified.

1.3. Boric Acid Tolerance Tests

Spot assays and tests in liquid culture were performed. Spot assays were performed by the following procedures. Each of the yeast was shaking cultured to an OD₆₀₀ of 0.5-1.0 at 30° C. in SD liquid medium. Each yeast culture was diluted until the values of OD₆₀₀ are equal in SD medium. 1/5, 1/25, 1/125 or 1/625 diluted diluent which values of OD₆₀₀ are equal was prepared for each yeast culture medium. Each diluent was spotted with 5 µl at a time by pipetman (Gilson) in SD solid medium with boric acid, and in SD solid medium without boric acid as a control. It was also spotted from left to right to lower the concentration for the same. The plate spotted yeast was cultured at 30° C. for around 10 days and growth states of the yeast were observed.

Test in liquid culture was performed as follows. Each yeast was shaking cultured to an OD₆₀₀ of around 1.0 at 30° C. in SD medium. Each culture medium was subcultured in SD solid medium with boric acid, and in SD solid medium without boric acid as a control, to an OD₆₀₀ of 0.1, and shaking cultured at 30° C., then the values of OD₆₀₀ were measured every 24 hours.

1.4. Sequences of Genes Conferring Boric Acid Tolerance

Analysis of the base sequences of 6 cDNA clones obtained by screening was performed as follows. The base sequences were analyzed by performing sequence reaction using fluorescent dye-terminator terminator, with the use of ABI 310 genetic analyzer. A gene encoding the base sequences was identified by BLAST search of TAIR (see the website for The Arabidopsis Information Resource) from the obtained base sequences.

1.5. Screening Results of Genes Conferring Boric Acid Tolerance

First, the performance of boric acid tolerance in yeast strain 1169 used in the present experiments was evaluated. The yeast 1169 was transformed with pYES2 and pYES2-BOR1. pYES2 and pYES2-BOR1 were used for the transformation, because these vectors retain URA3 that is the same one as vector pFL61, which is used in the *Arabidopsis thaliana* expression library that is used for the following screening, as a selection marker. Further, in SD medium, as the expression of BOR1 gene of pYES-BOR1 is not induced, the same level of boric acid tolerance as in transformant of pYES2 should be induced. The yeasts transformed with pYES2 and pYES2-BOR1 were named "2" and "7", respectively. "2" and "7" were shaking cultured in SD liquid medium, and streaked in SD solid medium containing 0, 20, 30, 40, 50, 60, 70, 80, 90 or 100 mM boric acid. As a result, it was revealed that the transformant by either vector could not also be grown in SD medium containing 80 mM or more of boric acid (FIG. 1).

To isolate ones inferring a boric acid tolerance, *Arabidopsis thaliana* genes that can grow the yeast in SD medium containing 80 mM boric acid by expressing the genes in yeast strain 1169 were searched in the present experiment. Therefore, around 1.2 million yeasts transformed in *Arabidopsis thaliana* expression library were streaked in SD medium containing 80 mM boric acid. As a result, 6 transgenic yeasts: 46, 66, 72, 84, 86 and 87 that induce tolerance against 80 mM boric acid were obtained. The performances of boric acid tolerance in transgenic yeasts: 46, 72, 84, 86 and 87 by spot assays are shown in FIG. 2 (Since 66 encodes the same gene as 46 does, it is shown in the following, only the result of 46 is shown). Yeast strain 1169 can hardly form colony in SD

medium containing 80 mM boric acid, as it is shown in the upper half of FIG. 2. On the other hand, any of these transgenic yeasts could form more colonies compared to 1169 strain. Next, test in liquid culture was performed. In the liquid culture, 46, 72, 86 and 87 showed around 3-fold growth potential compared to strain 1169 in boric acid medium, as shown in FIG. 3. However, 84 had variable growth rates and no significant difference was observed compared to strain 1169 in boric acid tolerance. Further, these genes could confer a boric acid tolerance when they were introduced into yeast strain BY4741 as well as when they were introduced into 1169 strains. The results from spot assays are shown in FIG. 4, and the results from liquid culture are shown in FIG. 5. When they were introduced into strains BY4741, in all of the transformed yeasts, significant differences were also observed in boric acid tolerance in the liquid culture (FIG. 5).

1.6. Sequences of Genes Conferring Boric Acid Tolerance

6 base sequences of the cDNA clones obtained from screening were determined, and genes encoding them were identified by BLAST searches. As a result, it was revealed that 46 and 66, 72, 84, 86, and 87 matched AtPAB2, AtMYB68, AtMYB13, AtRPS20B, and AtRBP47, respectively. The respective sequences of the genes are shown in the following sequence listing. AtPAB2, AtMYB13 and AtMYB68, AtRPS20, and AtRBP47 are genes encoding polyA-binding protein, Myb-like transcription factor, ribosomal protein, and RNA-binding protein, respectively.

Example 2

2.1. Yeast Strains and Screening

Saccharomyces cerevisiae strain BY4741 (MATa his3D1 leu2D0 met15D0 ura3D0), Y01169 (MATa his3D1 leu2D0 met15D0 ura3D0 YNL275W::kanMX4), Y04443 (MATa his3D1 leu2D0 met15D0 ura3D0 YGL076C::kanMX4), and Y01094 (MATa his3D1 leu2D0 met15D0 ura3D0 YPL198W::kanMX4), were used in this study. Strains: Y01169, Y04443, and Y01094 were constructed from BY4741 by insertional mutagenesis (Winzeler et al., 1999) and obtained from EUROSCARF.

Yeast competent cells were transformed with an *Arabidopsis thaliana* cDNA library cloned in the expression plasmid pFL61 (Minet et al., 1992) by using the lithium acetate method (Gietz and Schiestl, 1995). The strain Y01169 was used as a host because it lacks YNL275W (hereinafter, referred to as BOR1), an efflux B transporter, and sensitive to boric acid compared with the corresponding wild type strain (data not shown). Transformants were screened on SD solid medium (Sherman, 1991) containing 80 mM boric acid at 26.5° C. SD medium contained 2% glucose, 0.67% yeast nitrogen base without amino acids, 0.05% ammonium sulfate, and the amino acids (20 mg/L His, 30 mg/L Leu, and 20 mg/L Met), which are required for the growth of the mutant, and the pH was adjusted to 5.5 with Tris. Agar (2% w/v) was added for making the solid medium. Colony formation of the nontransformed Y01169 (Δ bor1) cells was completely suppressed by addition of 80 mM boric acid. Among the transformed cells, those that formed colonies on media containing 80 mM boric acid after two-week incubation at 26.5° C. were selected and their tolerance were confirmed by testing their growth in the presence of 80 mM boric acid. To confirm that the phenotype was conferred by the plasmids, plasmids were isolated from the positive isolates and re-transformed into the yeast strain Y01169. Tolerant isolates were subjected to fluoro-orotic acid-induced plasmid loss (Boeke, J. D., LaC-

route, F., & Fink, G. R. (1984) Mol. Gen. Genet. 197, 345-346) to select only those clones showing plasmid-dependent boric acid tolerance.

2.2. Construction of Plasmids

ORF sequences of AtRBP47c"-related genes and RPL7B (see SEQ ID NO: 34) were amplified by PCR using the primer sets listed in Table 1. The amplified products were sub-cloned into pGEM-T easy vector (Promega). These plasmids were treated with NotI, and the resultant ORF fragments of AtRBP45a, AtRBP47b, AtRBP47c, AtRBP47c' and AtUBP1 were cloned into the NotI site of the pFL61 expression vector (Minet et al., 1992), and the ORF fragments of RPL7B were cloned into the NotI site of the pDR195 expression vector (Rentsch et al., 1995). pFL61 and pDR195 carry PGK and PMA1 promoters for expression, respectively.

TABLE 1

(SEQ ID NOS 35-48, respectively, in order of appearance)	
Gene	Primer sequences
AtRBP45a	5' -AAAAAGCAGGCTTAATGCAGCAACCACCGTCAAACGC C-3' 5' -AGAAAGCTGGGTTTCACTGACGTTGCTGCTGATAGT T-3'
AtRBP47a	5' -AAAAAGCAGGCTTAATGCAGACACCAACAACAACGG T-3' 5' -AGAAAGCTGGGTTTCAAGAAGCTCCCGGGACTGCAG C-3'
AtRBP47b	5' -AAAAAGCAGGCTTAATGCAGACAACCAACGGCTCAGA T-3' 5' -AGAAAGCTGGGTTTCAATTCTCCCATGATAGTTGT T-3'
AtRBP47c	5' -AAAAAGCAGGCTTAATGGCAGACGTCAGATTCAATC C-3' 5' -AGAAAGCTGGGTTTCACTAAGTGTGCTGATGAC C-3'
AtRBP47c'	5' -AAAAAGCAGGCTTAATGGCAGACGTCAGATTCAATC C-3' 5' -AGAAAGCTGGGTTTCACTAAGTGTGCTGATGAC C-3'
AtUBP1a	5' -AAAAAGCAGGCTTAATGCAGAATCAAAGGCTTATTAA G-3' 5' -AGAAAGCTGGGTTTACTGATAGTACATGAGCTGCT G-3'
RPL7B	5' -AAAAAGCAGGCTTAATGTCCACTGAAAAATCTT-3' 5' -AGAAAGCTGGGTTTGTTCATGCTTAACCA-3'

2.3. Boric Acid Tolerance Assays

For boric acid tolerance assay of AtRBP47c"-related family genes, the expression plasmids were introduced into the *Saccharomyces cerevisiae* strain BY4741. As controls, empty vectors without insert were also introduced into BY4741. The transformants were grown to stationary phase in the SD liquid medium, and then cell densities of the cultures were adjusted to OD₆₀₀=1.0. These cell density-adjusted cultures were diluted to 1/5, 1/25, 1/125, and 1/625 with the SD liquid medium and 10 µL of diluted cultures were dropped on the SD solid medium with or without 80 mM boric acid and incubated at 30° C. for 7 days.

For analysis in liquid culture, the transformants were grown to stationary phase in the SD liquid medium, and then diluted in the SD liquid medium with or without 80 mM boric acid to adjust the value of OD₆₀₀ to 0.1 for the performance test of high concentration boric acid tolerance.

For analysis of boric acid tolerance of Δrpl7a (Y04443) and Δrpl7b (Y01094) mutants, SD medium containing 2% glucose, 0.67% yeast nitrogen base without amino acids, and 0.05% ammonium sulfate was used, adjusting to pH 5.5 with Tris, and the required amino acids (20 mg/L His, 30 mg/L Leu, 20 mg/L Met, and 20 mg/L Ura) were added. The mutants were obtained from EUROSCARF. To further examine the role of RPL7B in boric acid tolerance, RPL7B was over-expressed in the yeast strain Y04443. Boric acid tolerance assays were carried out as described above.

2.4 Detection of Unspliced Transcripts by RT-PCR

Yeast cells were grown to exponential phase (OD₆₀₀=0.5-1.0) in SD liquid medium, and then boric acid was added to be 80 mM at final concentration. After 24h incubation at 30° C., one-ml of samples were taken, and the cells were collected by centrifugation, frozen in liquid nitrogen, and stored at -80° C. until use.

Total RNA was extracted from the yeast cells by using an RNeasy Mini Kit (Qiagen), and 1 µg of total RNA was reverse-transcribed by using MuLV reverse-transcriptase (Applied Biosystems) and oligo (dT)₁₆ primer. One-fifteenth of the RT products was subjected to PCR with the following cycle: 40-50 times at 94° C. for 30 sec, 45° C. for 30 sec, and 72° C. for 1 min. PCR was carried out with a Smart Cycler (Cepheid) using a DNA polymerase, Ex taq (Takara). Primer sets used in this analysis are listed in Table 2, which is published as expanded information on the PNAS web site. Amplified transcripts were separated on 2% agarose gel and detected after staining with Etd bromide.

TABLE 2

(SEQ ID NOS 49-134, respectively, in order of appearance)		
Gene	Primer sequences	
SNR17A	5' -AATCTGTGTCGACGTAATTC-3' 5' -AGAAGTACATAGGATGGGTC-3'	(Forward) (Reverse)
SNR17B	5' -AAAAATTGTCGACGTAATTC-3' 5' -AAAGGAAGTTATCACAATTG-3'	(Forward) (Reverse)
YBR230C	5' -CCAGCATCTATGTCTGCAAC-3' 5' -CGTATCTGGAGTAGTATTTC-3'	(Forward) (Reverse)
VMA10	5' -GCAAGGTATACAAAGCAGAA-3' 5' -TCATCCTTTTCTCTCTGC-3'	(Forward) (Reverse)
SEC27	5' -GACACGATGAAGTTGGATAT-3' 5' -TGACTGTCAAATCCTACTG-3'	(Forward) (Reverse)
YNL050C	5' -CAGTATAAAAAATGTCTGAAT-3' 5' -TGGTTGATTATTTCTTCTTC-3'	(Forward) (Reverse)
RPL7B	5' -ATCAACGTCATAATGTCCAC-3' 5' -TACCAGAGTTGATTCTTGTCT-3'	(Forward) (Reverse)
MUD1	5' -ACCTAAAGAAACCATGTGAC-3' 5' -TATCAAGGTTGTACGTTTCG-3'	(Forward) (Reverse)
SNC1	5' -ATGTACAGTCTAAGTCAAGG-3' 5' -GACTAAAGTGAACAGCAATG-3'	(Forward) (Reverse)
POP8	5' -GAGAATGGCAATATTTCAAG-3' 5' -TGTTCTTCTTCTTCCATTAC-3'	(Forward) (Reverse)
ARP2	5' -TGGACCCACATAATCCAATT-3' 5' -TTTCGAACATTACCTCACAC-3'	(Forward) (Reverse)
CNB1	5' -GTGGATGGTCTTTTAGAAGA-3' 5' -AACTCCTCGAACTTAAACG-3'	(Forward) (Reverse)

TABLE 2-continued

(SEQ ID NOS 49-134, respectively, in order of appearance)	
Gene	Primer sequences
RPS22B	5'-TATTGAGACCTTCTTCCAAG-3' (Forward) 5'-AAGATTTTACCGAAACGTG-3' (Reverse)
YML025C	5'-GACGATAAAAAGAAATTTGGTG-3' (Forward) 5'-CTCAAGCGTTGTTGAAAG-3' (Reverse)
TUB3	5'-GAGAGAGGTCATTAGTATTA-3' (Forward) 5'-TTTTCTAATAACAGGAACC-3' (Reverse)
STO1	5'-GTTTAATAGAAAAGAAGAGGAG-3' (Forward) 5'-TAGTTCATCAACTAAAACATGG-3' (Reverse)
RPS16A	5'-AGCTGTCCCAAGTGTTCAA-3' (Forward) 5'-ACCCTTACCACCGAATTTC-3' (Reverse)
SAR1	5'-GTTGGGATATTTTTGGTTGG-3' (Forward) 5'-AAAGGAACGTCTTCAATTC-3' (Reverse)
PM140	5'-AACAGCTGTTTCAGGTTAGA-3' (Forward) 5'-GGTTTGTGATTATCATCAGG-3' (Reverse)
RPL7A	5'-AATTAAGATCACAATGGCCG-3' (Forward) 5'-CTTGTAACCTTGGACGAATG-3' (Reverse)
YBL091C-A	5'-CAGAAAAGCTGGTGTCAAG-3' (Forward) 5'-TGATTCTGCATCGTGGTTTC-3' (Reverse)
RPL19A	5'-TTGATTAAGAACTCCAAGC-3' (Forward) 5'-TCTTCTCAAGACACGTAATC-3' (Reverse)
PCH2	5'-AGATGAGGTTGAAGCAATAG-3' (Forward) 5'-CAAGGGCAATTTCTTATTG-3' (Reverse)
RPS9B	5'-TAAGACTAAGCAACATGCC-3' (Forward) 5'-AAACCAACTTGTAGACTTG-3' (Reverse)
YBR230C	5'-GCATCTCATAATATGTCTGC-3' (Forward) 5'-TTGTTGCTAAGACTGTAGAG-3' (Reverse)
YDR381C-A	5'-CAAATCCATTTCAAATATAGG-3' (Forward) 5'-CTCCTCTATCTAAAAACC-3' (Reverse)
YRA1	5'-AAGAAGAGTTGGTAAGCAAG-3' (Forward) 5'-CACCCTTTTGAATGTGATG-3' (Reverse)
UBC8	5'-AGCGTAATACGAAAGATGAG-3' (Forward) 5'-AGCTTCGTTATTCAAGGAT-3' (Reverse)
MND1	5'-GTATCATAAACATTCAACAATG-3' (Forward) 5'-CGGACTCTGTTTATTCTC-3' (Reverse)
MER3	5'-AAACAAGTTTGTATCGCCTG-3' (Forward) 5'-TCGTGCTCAAACATTTCTTC-3' (Reverse)
ERV1	5'-AAAATGACGGATAATCCACC-3' (Forward) 5'-TTCAAAGTCTTTAGCACACC-3' (Reverse)
SRB2	5'-CAATCCATCATGGGAAAATC-3' (Forward) 5'-CTTGGACGACAAAATAGTGT-3' (Reverse)
MOB1	5'-AGGACTTCAATTTCCATGTC-3' (Forward) 5'-AGTGTCACTCCACAATTTG-3' (Reverse)
RPS21A	5'-GAAAACGATAAGGGCCAATT-3' (Forward) 5'-CGTCTTTAACAACCATCG-3' (Reverse)
NYV1	5'-TACCAATGAAACGCTTTAATG-3' (Forward) 5'-TCTTCATGGAAGAGTCTAG-3' (Reverse)
YLR211C	5'-ATGGAATGAGTACTTTAGCG-3' (Forward) 5'-CTTCATTTCCGAGTTTTTGG-3' (Reverse)
TAD3	5'-AATAGAAAATCGGCTTCTGC-3' (Forward) 5'-TATTTGATCATTTGGGTTGC-3' (Reverse)

TABLE 2-continued

(SEQ ID NOS 49-134, respectively, in order of appearance)	
Gene	Primer sequences
ERV41	5'-GATTGAAGACATTTGATGCG-3' (Forward) 5'-TCGCCACTAATCTATTAC-3' (Reverse)
SPO1	5'-ACCATTTTCAGGTACAATGTC-3' (Forward) 5'-CTTCGAAATATCGAATTCC-3' (Reverse)
YOL048C	5'-CTGAAACGATACCAACAATG-3' (Forward) 5'-TTTGTGGTTTAGGCAATACC-3' (Reverse)
RPS9A	5'-ATACAAAAGTATACAACATGCC-3' (Forward) 5'-TTTCCAAGAAATCTTCGACC-3' (Reverse)
CIN2	5'-CTTTACTGCGAAGATAAAGG-3' (Forward) 5'-GCCACTATAATCTGTTGTTG-3' (Reverse)
YRP098C	5'-TCAAACACTACGGCTCATTGG-3' (Forward) 5'-TGAAACAAAAGACTCAATCCG-3' (Reverse)

2.4. Salt Tolerance Assays

25 Salt tolerance assay were carried out as in the above-described. boric acid tolerance assays, except that SD media containing 1.75 M or 2 M NaCl were used.

2.5. Accession Numbers

30 The GenBank accession numbers for the sequences described in Example 2 are as follows: *Arabidopsis thaliana* sequences AtRBP45a, MN124872; AtRBP45b, MN101037; AtRBP45c, MN118834; AtRBP45d, MN121940; AtRBP47a, MN103848; AtRBP47b, MN112800; AtRBP47c, MN103642; AtRBP47c', MN103643; AtUBP1a, MN104285; AtUBP1b, MN101598; AtUBP1c, MN112266; and *Saccharomyces cerevisiae* sequences RPL7A, X62627; RPL7B, Z73554.

2.6. Result of Isolation of *Arabidopsis thaliana* cDNA Clones that Confer High Boric Acid Tolerance to Yeast

40 *Saccharomyces cerevisiae* strain Y01169 was transformed with an *Arabidopsis thaliana* cDNA expression library (Minet, M., Dufour, M. -E., & Lacroute, F. (1992) Plant J. 2, 417-422) and the transformants were selected on dishes containing 80 mM of boric acid. Boric acid at this concentration completely suppressed the formation of colonies of Y01169 cells even after two-week incubation at 26.5° C. In this screening, several colonies of yeast which showed enhanced boric acid tolerances were isolated. It was shown that one of the cDNA clones encodes an RNA binding protein, AtRBP47c'.

2.7. Expression of AtRBP47c'-Related Genes from *Arabidopsis thaliana* Confers Boric Acid Tolerance to Yeast

55 AtRBP47c' has three RNA recognition motifs (RRM). In *Arabidopsis thaliana* genome, there are eleven genes encoding a protein which has three RRM and 100 or more of sequence identity scores to AtRBP47c' in BLASTP program. The phylogenetic tree of these AtRBP47c'-related family proteins is shown in FIG. 6A.

60 To investigate whether or not the expression of these *Arabidopsis thaliana* genes confers a boric acid tolerance to yeast, ORF sequences corresponding to 6 genes AtRBP45a, AtRBP47a, AtRBP47b, AtRBP47c, AtRBP47c', and AtUBP1a) were cloned into pFL61 expression vector. The plasmids were introduced into the yeast strain BY4741 and boric acid tolerances of these transformants were investi-

gated. As shown in FIG. 6B, all of the 6 constructs conferred the ability to the yeast strains to grow on 80 mM boric acid-containing SD solid medium to varying extents. To compare the level of boric acid tolerances among those transformants, their growth rates in the presence of boric acid were analyzed in liquid culture. All transformants showed faster growth rate than the control. In the graph, the AtRBP47c'-expressing line showed the fastest growth rate (FIG. 6C).

2.8. Boric Acid Treatment Inhibits Splicing of RPL7B, but not RPL7A, in Yeast

The present inventors found that the over-expression of AtRBP47c'-related genes conferred a boric acid tolerance. Although roles of these genes in *A. thaliana* are still unknown, similar genes in other plant species were characterized. *Nicotiana plumbaginifolia* RBP45 (Simpson, C. G., Jennings, S. N., Clark, G. P., Thow, G., & Brown, J. W. S. (2004) Plant J. 37, 82-91) and UB1 (Lambermon, M. H., Simpson, G. G., Wiczorek Kirk, D. A., Hemmings-Mieszczak, M., Klahre, U., & Filipowicz, W. (2000) EMBO J. 19, 1638-1649) were shown to enhance splicing efficiency. This led the present inventors to investigate the effect of boric acid on splicing of randomly selected 20 intron-containing genes in *Saccharomyces cerevisiae* by RT-PCR. Among the 6317 nuclear genes in the *Saccharomyces cerevisiae* genome, only 231 genes contain introns (see the website for the Munich Information Center for Protein Sequences. Among the 20 genes investigated, the increase by boric acid treatment in the accumulation of unspliced fragments compared to that of spliced fragments was observed in RPL7B, a gene encoding an essential ribosomal large subunit protein. This suggests that the splicing of RPL7B was inhibited in boric acid-treated yeast (FIG. 7B).

The RPL7B contains two introns. The size of unspliced fragments indicated that these fragments were derived from splicing of either one of the first and second introns (see FIG. 7A). To determine which intron is more susceptible to boric acid, the unspliced fragments were cloned and DNA sequences of the eight clones were determined. Six and two clones contained the first intron and the second intron, respectively. This suggests that inhibition occurs both at the first and the second introns and the first intron is more susceptible to high boric acid than the second one. The results also indicate that one of the two introns were correctly spliced, i.e., those unspliced fragments did not derive from genome DNA contamination but from the reverse transcription reaction of RNA.

Moreover, the inhibition of splicing of RPL7B by boric acid was not observed in yeast expressing AtRBP47c' (FIG. 7B). This result suggest that AtRBP47c' elevate splicing efficiency of RPL7B in the presence of high boric acid. It is possible that enhancement of splicing efficiency may be the cause of boric acid tolerance in yeast.

RPL7B has a paralog, RPL7A (SEQ ID NO: 32), in the yeast genome. RPL7A gene (SEQ ID NO: 31) also has two introns as in RPL7B gene. The effect of boric acid on the

splicing of RPL7A was examined. The splicing inhibition by boric acid was not observed unlike in the case of RPL7B (FIG. 7C).

2.9. Disruption of RPL7A in Yeast Reduces Boric Acid Tolerance

RPL7A and RPL7B Double Disruption Mutant is Lethal (see the website for the *Saccharomyces* Genome Database), indicating that RPL7 proteins are essential for yeast growth. Considering the differential sensitivity of boric acid to splicing between the two genes, it is possible that the boric acid tolerances of RPL7A- and RPL7B-disruption mutants differ. The Δ rpl7b (Y01094) showed a similar level of boric acid tolerance to the wild type *Saccharomyces cerevisiae*, whereas a boric acid tolerance of the Δ rpl7a (Y04443) was lower than the wild type (FIG. 8A). The difference in a boric acid tolerance was also evident in liquid culture (FIG. 8B). These results suggest that the inhibition of RPL7B splicing by boric acid is caused by reduction in a boric acid tolerance of Δ rpl7a.

2.10. Expression of Intronless RPL7B in RPL7A-Disrupted Yeast Increases Boric Acid Tolerance

If the reduction in a boric acid tolerance of Δ rpl7a is due to the reduction in the level of RPL7 protein by inhibition of RPL7B splicing, expression of intronless RPL7B cDNA should increase the tolerance of Δ rpl7a.

It was examined whether the expression of intronless RPL7B in Δ rpl7a increases boric acid tolerance. ORF sequence of RPL7B was cloned into pDR195 expression vector. The plasmid was then introduced into the Δ rpl7a and a boric acid tolerance in the transformant was investigated. As shown in FIG. 8C, the expression of intronless RPL7B increased boric acid tolerance in Δ rpl7a. This result indicates that the inhibition of RPL7B splicing is the cause of growth cessation by highly concentrated boric acid in Δ rpl7a.

2.11. Analysis of Splicing Inhibition in Genes Containing Noncanonical Branchpoint Sequences by Boric Acid Treatment

RPL7B has a noncanonical branchpoint sequence in its first intron (see Table 3). 28 genes containing such noncanonical branchpoint sequences among 231 nuclear intron-containing genes were found. Among the 28 genes, increase in the level of unspliced fragments by boric acid treatment compared to that of spliced fragments was observed in nine genes (FIG. 9). These genes are ERV1, ERV41, NYV1, RPS9A, RPS9B, SRB2, YOL048C, YPR098C, and YRA1.

TABLE 3

	5' splice site	Branchpoint	3' splice site
Consensus sequence	GUAUGU-----	UACUAAC-----	YAG
First intron	GUAUGU-----	U [⊗] CUAAC-----	UAG
Second intron	GUAUGU-----	UACUAAC-----	UAG

Table 3 shows three consensus sequences, 5' splice site, branchpoint, and 3' splice site, that were recognized in yeast. A transition point from A to G in branchpoint of the first intron is represented by white letter in black background. Y represents pyrimidine ribonucleotides (C or U).

The effects of over-expression of AtRBP47c' on the splicing inhibition of those genes by boric acid was analyzed. As shown in FIG. 9, the level of splicing inhibition of NYV1 and SRB2 was impaired in yeast expressing AtRBP47c'. NYV1

and SRB2 encode v-SNARE protein and RNA polymerase II holoenzyme protein, respectively. These results strongly suggest that the mechanism of conferring a boric acid tolerance to yeast by over-expression of AtRBP47c' is the enhancement of splicing efficiency.

2.12. Effects of Salt Treatment are Different from those of Boric Acid Treatment

Over-expression of splicing factor genes confers salt tolerance to yeast and/or plants (Forment, J., Naranjo, M. A., Roldan, M., Serrano, R., & Vicente, O. (2002) *Plant J.* 30, 511-519, 2002; Serrano, R., Gaxiola, R., Rios, G., Forment, J., Vicente, O., & Ros, R. (2003) *Monatsh. Chem.* 134, 1445-1464). It was examined whether AtRBP47c'-related genes also confer salt tolerance to yeast. All six AtRBP47c'-related genes tested in this study did not increase the salt tolerance in yeast (FIG. 10A). Furthermore, inhibition of splicing of RPL7B was not observed in cells exposed to high salt (FIG. 10B). These results suggest that AtRBP47c'-related genes do not function in salt tolerance and that inhibition of RPL7B splicing is likely to be unique to boric acid treatment.

2.13. Discussion

AtRBP47c' was isolated from *Arabidopsis thaliana* as a gene that confers a boric acid tolerance to yeast cells by yeast complementation. In yeast genome, there are seven genes encoding a protein-which has three RRM and 100 or more of sequence identity scores to AtRBP47c' in BLASTP program. Among these genes, the most similar gene to AtRBP47c' is NAM8. Although NAM8 was originally isolated as a suppressor of mitochondrial splicing deficiencies (Ekwall, K., Kermorgant, M., Dujardin, G., Groudinsky, O., & Slonimski, P. P. (1992) *Mol. Gene. Genet.* 233, 136-144), subsequent analysis showed that NAM8 interacts with U1snRNA and that NAM8 is indispensable for efficient 5' splice site recognition when this process is impaired as a result of the presence of noncanonical 5' splice sites (Gottschalk, A., Tang, J., Puig, O., Salgado, J., Neubauer, G., Colot, H. V., Mann, M., Seraphin, B., Rosbash, M., Luhrmann, R., & Fabrizio, P. (1998) *RNA* 4, 374-393.; Puig, O., Gottschalk, A., Fabrizio, P., & Seraphin, B. (1999) *Gene. Dev.* 13, 569-580). From these observations, it was hypothesized that AtRBP47c' might play a similar role with NAM8 in a boric acid tolerance. However, over-expression of NAM8 did not confer a boric acid tolerance to yeast and NAM8-disrupted mutants were tolerant to boric acid as well as wild type, indicating that AAAtRBP47c' has possibilities to be involved in an another step of splicing processes and/or other reaction(s) in boric acid tolerance.

In this study, it was found that boric acid could inhibit splicing of RPL7B among randomly selected 20 genes in yeast (FIG. 7B). By analysis of the DNA sequence in the first intron of this gene, it became clear that the first intron has a transition in the consensus sequence of the branchpoint. As shown in Table 1, the second A in the branchpoint consensus sequence is converted to G in the first intron of RPL7B. The binding of branchpoint bridging protein (BBP) to the branchpoint is a critical step in splicing progression (Abovich and Rosbash, 1997). Affinity between BBP and branchpoint sequence is known to be an important factor for splicing efficiency (Champion-Arnaud, et al., 1995). It has been reported that especially, this type of transition from A to G in second nucleotide of branchpoint sequence showed an approximately 10% decrease in the affinity with BBP (Berglund, J. A., Chua, K., Abovich, N., Reed, R., & Rosbash, M. (1997) *Cell* 89, 781-787). Therefore, it is likely that RPL7B is one of the genes with low splicing efficiency.

It is reported that the second step of splicing is inhibited by boric acid treatment in HeLa cell in vitro splicing system

(Shomron, N., & Ast, G. (2003) *FEBS Lett.* 552, 219-224). The second step of splicing is a process in which the treated 3' end of an exon is ligated to 5' end of the next exon. Considering that boric acid binds to cis-diol in ribose (Ralston, N. V. C., & Hunt, C. D. (2000) *FASEB J.* 14, A538; Nicholas et al., 2001; Ricardo, A., Carrigan, M. A., Olcott, A. N., & Benner, S. A. (2004) *Science* 303, 196), it is likely that the ligation reaction in second step of splicing is inhibited by the binding of boric acid to the 3' end of an exon. The above Shomron and Ast (2003) has been reported that inhibition of splicing by boric acid at the second step is a general phenomenon, as five different mRNA precursors exhibited a similar pattern of inhibition. In that case, inhibition of the splicing in yeast should occur similarly with all introns. However, among the 20 genes tested in the initial step of this study, the only gene in which inhibition was observed was RPL7B (FIG. 7B).

A possible explanation of this specific inhibition is as follows. The inhibition of splicing in the second step by boric acid takes place with all intron-containing genes in yeast. At this step, intron-including splicing intermediates, which should be rapidly degraded when the splicing progresses normally, accumulate. The accumulation of intermediates inhibits normal turnover. In such a situation, genes having introns with low splicing efficiency are likely to be more susceptible to the inhibition of splicing by boric acid. As one of such genes, a gene containing a noncanonical branchpoint sequence such as RPL7B can be exemplified. This speculation was verified by analysis of the inhibition of splicing by high boric acid on other genes having the same feature (FIG. 9). In the analysis, it was found that high boric acid treatment inhibits splicing of nine genes containing noncanonical branchpoint sequences except for RPL7B. This result clearly indicates that one of the toxic mechanisms of boric acid is inhibition of splicing of genes having introns with low splicing efficiency. Moreover, it was found that the splicing inhibitions of two genes among those nine genes were impaired by over-expression of AtRBP47c' (FIG. 9). This result suggests that a boric acid tolerance by over-expression of AtRBP47c' may be achieved by the enhancement of splicing efficiency of part of genes among many genes of which splicing is inhibited during high boric acid treatment. Hence, splicing inhibition of a limited number of genes might be a cause of growth inhibition.

The AtRBP47c'-related proteins have three RRM. RNA binding activity of RBP45, RBP47, and UBP1 of *N. plumbaginifolia* has been confirmed. All of these proteins tend to bind with U-rich sequence (Lambermon, M. H., Simpson, G. G., Wieczorek Kirk, D. A., Hemmings-Mieszczak, M., Klahre, U., & Filipowicz, W. (2000) *EMBO J.* 19, 1638-1649; Lorkovic, Z. J., Wieczorek Kirk, D. A., Klahre, U., Hemmings-Mieszczak, M., & Filipowicz, W. (2000) *RNA* 6, 1610-1624). Moreover, deletion analysis of RBP45 in *N. plumbaginifolia* indicated that at least two RRM are required for interaction with RNA (Lorkovic et al., 2000). Although an RRM was thought to be involved in RNA binding, it was shown that an RRM of a certain protein participates in interaction with other proteins (Kielkopf, C. L., Lucke, S., & Green, M. R. (2004) *Gene Dev.* 18, 1513-1526). Especially, yeast U2AF⁶⁵, a splicing factor containing three RRM, is reported that the third RRM is bound to BBP (Rain, J. C., Rafi, Z., Rhani, Z., Legrain, P., & Kramer, A. (1998) *RNA* 4, 551-565). These results suggest that AtRBP47c' may also interact with BBP. Furthermore, analysis of RBP7B first intron and SRB2 intron sequences revealed that there are U-rich sequences at the 3' side of the branchpoint. Taking the results together, it was hypothesized that AtRBP47c' stabilizes the interaction of BBP with branchpoint and the U-rich

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sequence of the branchpoint by binding with BBP, and as a result, the efficiency of splicing is increased.

It is reported that splicing is also inhibited by salt stress. Furthermore, over-production of several splicing factors such as SR protein have been also reported to increase salt tolerance in yeast and plants (Forment, J., Naranjo, M. A., Roldan, M., Serrano, R., & Vicente, O. (2002) *Plant J.* 30,511-519, 2002; Serrano, R., Gaxiola, R., Rios, G., Forment, J., Vicente, O., & Ros, R. (2003) *Monatsh. Chem.* 134, 1445-1464). In the present study, however, over-expression of AtRBP47c'-related genes did not confer salt tolerance to yeast (FIG. 10A), and inhibition of splicing of RPL7B was not detected after salt treatment (FIG. 10B). These results suggest that the mechanism of splicing inhibition is different between salt treatment and boric acid treatment.

Example 2 is the first report showing that the key of the toxic mechanisms of boric acid is the specific inhibition of splicing and that genes involved in enhancement of splicing efficiency lead to the boric acid tolerance. However, the toxic mechanisms other than the inhibition of splicing should exist, since-toxic effect of boric acid is observed in the prokaryotes in which splicing are not performed.

The invention is further described by the following numbered paragraphs:

1. A DNA encoding a protein that has an activity of conferring a boric acid tolerance and consists of the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30.

2. A DNA encoding a protein that consists of the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30 and has an activity of conferring a boric acid tolerance.

3. A gene DNA conferring a boric acid tolerance, which consists of the base sequence shown by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29 or a complementary sequence thereof.

4. A DNA encoding a protein that consists of a base sequence wherein one or a few bases are deleted, substituted or added in the base sequence shown by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29, and has an activity of conferring a boric acid tolerance.

5. A DNA encoding a protein that hybridizes with the DNA according to paragraph 3 under stringent conditions and has an activity of conferring a boric acid tolerance.

6. A protein having an activity of conferring a boric acid tolerance, which consists of the amino acid sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 or 30.

7. A protein consisting of an amino sequence wherein one or a few amino acids are deleted, substituted or added in the amino sequence shown by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14,

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16, 18, 20, 22, 24, 26, 28 or 30; and having an activity of conferring a boric acid tolerance.

8. A recombinant vector including the DNA according to any one of paragraphs 1 to 5, which can express a protein conferring a boric acid tolerance.

9. A transformant wherein the recombinant vector according to paragraph 8 is introduced, which can express a protein conferring a boric acid tolerance.

10. The transformant according to paragraph 9 wherein the transformant is yeast.

11. The transformant according to paragraph 9 wherein the transformant is a plant.

12. A method for screening a gene conferring a boric acid tolerance, comprising the steps of transforming a YNL275w-disrupted yeast which is deficient in and not expressing YNL275w gene by using a gene library, culturing the obtained transformed YNL275w-disrupted yeast in a medium containing boric acid, and measuring/evaluating an activity of conferring a boric acid tolerance of the transformed YNL275w-disrupted yeast.

13. A method for screening a gene conferring a boric acid tolerance wherein an enhancement level of splicing efficiency is measured/evaluated by targeting a specific inhibition of splicing by boric acid.

14. The method for screening a gene conferring a boric acid tolerance according to paragraph 13, comprising the steps of expressing a test substance in yeast cells, culturing the expressed test substance in the presence of boric acid, and measuring/evaluating an improvement level of a specific inhibition of splicing by boric acid in an intron-containing gene in yeast, as an enhancement level of splicing efficiency.

15. The method for screening a gene conferring a boric acid tolerance according to paragraph 14 wherein the gene containing intron in yeast is a gene RPL7B in *Saccharomyces cerevisiae* genome.

16. Use of the DNA according to any one of paragraphs 1 to 5 as a gene conferring a boric acid tolerance.

17. Use of the DNA according to any one of paragraphs 1 to 5 for producing a plant or yeast conferred a boric acid tolerance.

18. Use of the protein according to paragraph 6 or 7 as a protein having an activity of conferring a boric acid tolerance.

19. Use of the protein according to paragraph 6 or 7 for producing a plant or yeast conferred a boric acid tolerance.

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

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Gln	Thr	Glu	Val	Leu	His	Leu	Leu	Glu	Ser	Pro	Glu	Ala	Leu	Lys	Ala	405	410	415	
Lys	Val	Ala	Glu	Ala	Met	Asp	Val	Leu	Arg	Ser	Val	Ala	Ala	Gly	Gly	420	425	430	
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Pro Pro His Trp Met Arg Tyr Pro Pro Val Leu Met Pro Gln Met Met
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Tyr Ala Pro Pro Pro Pro Met Pro Phe Ser Pro Tyr His Gln Tyr Pro
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Ser Val Lys Ala Ala Lys Val Val Leu Asp Ala Asn Thr Gly Arg Ser
 225 230 235 240

Lys Gly Tyr Gly Phe Val Arg Phe Gly Asp Glu Asn Glu Arg Thr Lys
 245 250 255

Ala Met Thr Glu Met Asn Gly Val Lys Cys Ser Ser Arg Ala Met Arg
 260 265 270

Ile Gly Pro Ala Thr Pro Arg Lys Thr Asn Gly Tyr Gln Gln Gln Gly
 275 280 285

Gly Tyr Met Pro Ser Gly Ala Phe Thr Arg Ser Glu Gly Asp Thr Ile
 290 295 300

Asn Thr Thr Ile Phe Val Gly Gly Leu Asp Ser Ser Val Thr Asp Glu
 305 310 315 320

Asp Leu Lys Gln Pro Phe Ser Glu Phe Gly Glu Ile Val Ser Val Lys
 325 330 335

Ile Pro Val Gly Lys Gly Cys Gly Phe Val Gln Phe Val Asn Arg Pro
 340 345 350

Asn Ala Glu Glu Ala Leu Glu Lys Leu Asn Gly Thr Val Ile Gly Lys
 355 360 365

Gln Thr Val Arg Leu Ser Trp Gly Arg Asn Pro Ala Asn Lys Gln Pro
 370 375 380

Arg Asp Lys Tyr Gly Asn Gln Trp Val Asp Pro Tyr Tyr Gly Gly Gln
 385 390 395 400

Phe Tyr Asn Gly Tyr Gly Tyr Met Val Pro Gln Pro Asp Pro Arg Met
 405 410 415

Tyr Pro Ala Ala Pro Tyr Tyr Pro Met Tyr Gly Gly His Gln Gln Gln
 420 425 430

Val Ser

<210> SEQ ID NO 5
 <211> LENGTH: 369
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 5

atggcgtatg aaccgatgaa gccaccgaaa gctggtttgg aggctcctct ggagcagatt 60

cataagatca ggatcactct ctcttcaaaa aatgtgaaga acttgaaaa agtgtgcact 120

gatttggtcc gtggagctaa ggataagaga cttagagtta agggaccagt gagaatgcc 180

actaaggttc ttaagatcac taccagaaag gcaccttgtg gtgaaggtac caatacttgg 240

gacaggtttg agctcagggt tcacaagcgt gtcatogatc tcttcagctc cctgacggt 300

gttaagcaaa tcacgtctat caccattgag cccggtgttg aggtogaggt cactattgct 360

gactcttag 369

<210> SEQ ID NO 6
 <211> LENGTH: 122
 <212> TYPE: PRT
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 6

Met Ala Tyr Glu Pro Met Lys Pro Thr Lys Ala Gly Leu Glu Ala Pro
 1 5 10 15

Leu Glu Gln Ile His Lys Ile Arg Ile Thr Leu Ser Ser Lys Asn Val
 20 25 30

Lys Asn Leu Glu Lys Val Cys Thr Asp Leu Val Arg Gly Ala Lys Asp

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	35				40						45								
Lys	Arg	Leu	Arg	Val	Lys	Gly	Pro	Val	Arg	Met	Pro	Thr	Lys	Val	Leu				
	50					55					60								
Lys	Ile	Thr	Thr	Arg	Lys	Ala	Pro	Cys	Gly	Glu	Gly	Thr	Asn	Thr	Trp				
65					70					75					80				
Asp	Arg	Phe	Glu	Leu	Arg	Val	His	Lys	Arg	Val	Ile	Asp	Leu	Phe	Ser				
				85					90					95					
Ser	Pro	Asp	Val	Val	Lys	Gln	Ile	Thr	Ser	Ile	Thr	Ile	Glu	Pro	Gly				
			100					105					110						
Val	Glu	Val	Glu	Val	Thr	Ile	Ala	Asp	Ser										
		115					120												

<210> SEQ ID NO 7
 <211> LENGTH: 741
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 7

atggggagaa gaccatgctg tgagaagata ggattgaaga aagggccatg gagtgctgaa	60
gaagatcgaa tcttgatcaa ttatattagt ctccatggcc atcccaattg gagagctctc	120
cctaaactag cggggtact tcgggtcgga aaaagttgca ggcttcgctg gattaattat	180
ttgagaccag acatcaaacg tggcaatttc actcctcatg aagaagatac tatcatcagc	240
ttacatcaac tcttaggcaa cagatggtct gcgatagctg caaaattgcc tggacgaaca	300
gacaacgaaa ttaaaaatgt ttggcacact catttaaaga aaagactcca ccacagtcaa	360
gatcaaaaca acaaggaaga tttcgtctct actacagctg cggagatgcc aacctctccg	420
caacaacaat ctagtagtag tgccgacatt tcagcaatta caacattggg aaacaacaat	480
gacatctcca atagcaacaa agactccgcg acgtcatccg aagatgttct tgcaattata	540
gatgagagct tttggtcaga agtgggtattg atggactgtg acatttcagg aaatgagaag	600
aatgagaaaa agatagagaa ttgggagggc tcaactagata gaaacgataa gggatataac	660
catgacatgg agttttggtt tgaccatctc actagtagta gttgtataat tggagaaatg	720
tccgacattt ctgagttttg a	741

<210> SEQ ID NO 8
 <211> LENGTH: 246
 <212> TYPE: PRT
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 8

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

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Lys Lys Arg Leu His His Ser Gln Asp Gln Asn Asn Lys Glu Asp Phe
 115 120 125
 Val Ser Thr Thr Ala Ala Glu Met Pro Thr Ser Pro Gln Gln Gln Ser
 130 135 140
 Ser Ser Ser Ala Asp Ile Ser Ala Ile Thr Thr Leu Gly Asn Asn Asn
 145 150 155 160
 Asp Ile Ser Asn Ser Asn Lys Asp Ser Ala Thr Ser Ser Glu Asp Val
 165 170 175
 Leu Ala Ile Ile Asp Glu Ser Phe Trp Ser Glu Val Val Leu Met Asp
 180 185 190
 Cys Asp Ile Ser Gly Asn Glu Lys Asn Glu Lys Lys Ile Glu Asn Trp
 195 200 205
 Glu Gly Ser Leu Asp Arg Asn Asp Lys Gly Tyr Asn His Asp Met Glu
 210 215 220
 Phe Trp Phe Asp His Leu Thr Ser Ser Ser Cys Ile Ile Gly Glu Met
 225 230 235 240
 Ser Asp Ile Ser Glu Phe
 245

<210> SEQ ID NO 9

<211> LENGTH: 1125

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 9

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atgggaagag caccgtgttg tgataaggcc aacgtgaaga aagggccttg gtctcctgag    60
gaagacgcca aactcaaaga ttacatcgag aatagtggca caggaggcaa ctggattgct    120
ttgcctcaga aaattggttt aaggagatgt ggggaagagtt gcaggctaag gtggctcaac    180
tatttgagac caaacatcaa acatggtggc ttctccgagg aagaagacaa catcatttgt    240
aacctctatg ttactattgg tagcaggtgg tctataattg ctgcacaatt gccgggaaga    300
accgacaacg atatcaaaaa ctattggaac acgaggctga agaagaagct tctgaacaaa    360
caaaggaaa agttccaaga agcgcgaatg aagcaagaga tggatgatgat gaaaaggcaa    420
caacaaggac aaggacaagg tcaaagtaat ggtagtacgg atctttatct taacaacatg    480
tttgatcat caccatggcc attactacca caacttcctc ctccacatca tcaaatacct    540
cttggatga tggaaccaac aagctgtaac tactacaaa cgacaccgtc ttgtaaccta    600
gaacaaaagc cattgatcac actcaagaac atggtcaaga ttgaagaaga acaggaaagg    660
acaaaacctg atcatcatca tcaagattct gtcacaaaacc cttttgattt ctctttctct    720
cagcttttgt tagatcccaa ttactatctg ggatcaggag ggggaggaga aggagathtt    780
gctatcatga gcagcagcac aaactcacca ttaccaaaaca caagtagtga tcaacatcca    840
agtcaacagc aagagattct tcaatgggtt gggagcagta actttcagac agaagcaatc    900
aacgatatgt tcataaacaa caacaacaac atagtgaatc ttgagaccat cgagaacaca    960
aaagtctatg gagaagcctc agtagccgga gccgctgtcc gagcagcttt gggcggaggg   1020
acaacgagta catcgcgcca tcaaaagtaca ataagttggg aggatataac ttctctagtt   1080
aattccgaag atgcaagtta cttcaatgcy ccaaatcatg tgtaa                               1125

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<210> SEQ ID NO 10

<211> LENGTH: 374

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

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<400> SEQUENCE: 10

Met Gly Arg Ala Pro Cys Cys Asp Lys Ala Asn Val Lys Lys Gly Pro
 1 5 10 15
 Trp Ser Pro Glu Glu Asp Ala Lys Leu Lys Asp Tyr Ile Glu Asn Ser
 20 25 30
 Gly Thr Gly Gly Asn Trp Ile Ala Leu Pro Gln Lys Ile Gly Leu Arg
 35 40 45
 Arg Cys Gly Lys Ser Cys Arg Leu Arg Trp Leu Asn Tyr Leu Arg Pro
 50 55 60
 Asn Ile Lys His Gly Gly Phe Ser Glu Glu Glu Asp Asn Ile Ile Cys
 65 70 75 80
 Asn Leu Tyr Val Thr Ile Gly Ser Arg Trp Ser Ile Ile Ala Ala Gln
 85 90 95
 Leu Pro Gly Arg Thr Asp Asn Asp Ile Lys Asn Tyr Trp Asn Thr Arg
 100 105 110
 Leu Lys Lys Lys Leu Leu Asn Lys Gln Arg Lys Glu Phe Gln Glu Ala
 115 120 125
 Arg Met Lys Gln Glu Met Val Met Met Lys Arg Gln Gln Gln Gly Gln
 130 135 140
 Gly Gln Gly Gln Ser Asn Gly Ser Thr Asp Leu Tyr Leu Asn Asn Met
 145 150 155 160
 Phe Gly Ser Ser Pro Trp Pro Leu Leu Pro Gln Leu Pro Pro Pro His
 165 170 175
 His Gln Ile Pro Leu Gly Met Met Glu Pro Thr Ser Cys Asn Tyr Tyr
 180 185 190
 Gln Thr Thr Pro Ser Cys Asn Leu Glu Gln Lys Pro Leu Ile Thr Leu
 195 200 205
 Lys Asn Met Val Lys Ile Glu Glu Glu Gln Glu Arg Thr Asn Pro Asp
 210 215 220
 His His His Gln Asp Ser Val Thr Asn Pro Phe Asp Phe Ser Phe Ser
 225 230 235 240
 Gln Leu Leu Leu Asp Pro Asn Tyr Tyr Leu Gly Ser Gly Gly Gly Gly
 245 250 255
 Glu Gly Asp Phe Ala Ile Met Ser Ser Ser Thr Asn Ser Pro Leu Pro
 260 265 270
 Asn Thr Ser Ser Asp Gln His Pro Ser Gln Gln Gln Glu Ile Leu Gln
 275 280 285
 Trp Phe Gly Ser Ser Asn Phe Gln Thr Glu Ala Ile Asn Asp Met Phe
 290 295 300
 Ile Asn Asn Asn Asn Asn Ile Val Asn Leu Glu Thr Ile Glu Asn Thr
 305 310 315 320
 Lys Val Tyr Gly Asp Ala Ser Val Ala Gly Ala Ala Val Arg Ala Ala
 325 330 335
 Leu Gly Gly Gly Thr Thr Ser Thr Ser Ala Asp Gln Ser Thr Ile Ser
 340 345 350
 Trp Glu Asp Ile Thr Ser Leu Val Asn Ser Glu Asp Ala Ser Tyr Phe
 355 360 365
 Asn Ala Pro Asn His Val
 370

<210> SEQ ID NO 11

<211> LENGTH: 1164

<212> TYPE: DNA

-continued-

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 11

atcagcaaac caccgtcaaa cgcggccgga gctggacaga taccatcagg acaacagcat

120 tctgtgtagta tcatgcaaca gacagcagcag cagcagcaga tgcagtgtc tgcggcgcca

180 ctaggctaac atcagtagcgg tcttgatct cagaatccag gatccgctag cgaagttaa

240 tctgtgtgga tctggagactt gacagcaatgg atggacgaga actaacatc gatcgtctt

300 gctcagctc gcnaggtcac atcagctaaa gtcattgta ataacgtac gggacaatc

360 gaaggtatg gatcattga gttcgtcagc cactctgtag cagagcgggt ttgacagact

420 taacatggtg ctccatgccc gagcactgaa cagacgttta ggtccaactg gctccaagct

480 ggggtctgag aagaacgat ccaagctgaa gggcctgacc ataacattt cgtaggtgac

540 ttggcaactg aggtgactga ctatatgctc tgcgacacat tcaagaatgt gtagggctc

600 gtcaaaagggg ctaagttgt gcttgcacag accacttgaa gtrccaagg gtagggtrt

660 gttaggttgc cgtatgaaaa tgcagatg cgtgccaatg atgtaataaa tggtaatac

720 tgcctcgaaca ggcctatgcy tcttggtccg gctgccaata agaattgctc tccgtagcaa

780 ccagctatgt atcaaacac tcaagagca aatgcttgag ataatgatcc taataacaca

840 acaattttgc ttggaggtct gtagtcta at gttacagcgt atgaaataaa gtcatttt

900 ggtcaattgc tctgaactct tcatgtgaaa atacctccag gaaaacgttg tggattcgtt

960 caatagcca acaagggtct tgcagagcat gcacttccg tgcgtatg aacaacatba

1020 ggtggaacaa gctaccgtct tctgtgggga cgtagttccaa acaagcagtc tgatcaagcg

1080 caatggaaag gctggtagta ctatgtagc cctccacagc cacagggcgg ctatggttat

1140 gacgtcaac caccactca agaccctaat gctactatg gctggtacac tggctatggc

1164 aactatcagc agcaacgtca gtga

<210> SEQ ID NO 12

<211> LENGTH: 387

<212> TYPE: PR1

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 12

Met Gln Gln Pro Ser Asn Ala Ala Gly Ala Gly Gln Ile Pro Ser

1 5 10 15 20 25 30 35 40 45

Gly Gln Gln His Leu Trp Met Met Gln Gln Gln Gln Gln

50 55 60 65 70 75 80

Gly Ser Gln Asn Pro Gly Ser Ala Ser Asp Val Lys Ser Leu Trp Ile

85 90 95

Ala Gln Ser Gly Gln Ala Thr Ser Ala Lys Val Ile Arg Asn Lys Leu

100 105 110

Thr Gly Gln Ser Gln Gly Tyr Gly Phe Ile Gln Phe Val Ser His Ser

115 120 125

Val Ala Gln Arg Val Leu Gln Thr Tyr Asn Gly Ala Pro Met Pro Ser

130 135 140

Thr Gln Gln Thr Phe Arg Leu Asn Trp Ala Gln Ala Gly Gln

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

<210> SEQ ID NO 13

<211> LENGTH: 1218

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 13

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atgatgcagc agccaccacc cggaggatc cttccacatc acgctcctcc tccttctgcg      60
caacaacagt acggttacca acaaccttac gggattgctg gagctgctcc accaccacca      120
cagatgtgga atcctcaagc ggcggcgccg ccatcagttc agcctacgac cgctgaogag      180
atccggactc tttggatcgg ggacttacag tattggatgg atgagaatth cctctacggt      240
tgctttgctc ataccggaga gatggtttct gctaaagtga ttcgtaacaa gcaaaccggt      300
caagttgaag gatacggttt cattgaattc gcatctcatg ctgctgctga aagagttcta      360
caaacattca acaacgctcc tatcccgagc tttcctgata agctcttag actgaactgg      420
gcatcattga gttcaggaga taaacgagac gattcaccgg actacacgat atttgcggt      480
gatctggctg ctgatgttac ggattatata ttacttgaga cgttcagagc ctcttatccg      540
tcagtgaagg gtgcaaagg tgttattgac agagtcactg gacgtacaaa aggatatggg      600
tttgtaggt tttctgatga aagtgaacag atccgtgcta tgacggagat gaatggcggt      660
cctgttctca ctagacctat gagaattggt cccgctgcta gcaagaaagg tgtaactggt      720

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caaagagatt cataccagag ctctgctgca ggggtaacaa ctgataatga tccaaataac 780
acaactgttt ttgttggtgg attagatgca tctgtcacgg atgatcatct gaagaatgtc 840
tttagccaat atggtgagat tgtgcatgtg aaaataccgg ctggaaagcg ctgtggattc 900
gttcagtttt ccgagaagag ctgtgcagag gaagctctta gaatgctgaa tggagtgcaa 960
ttaggcggaa caaccgtcag gctctcatgg ggccgaagtc cttcgaacaa acagtcgggg 1020
gatccgagcc agttttacta cggtggtgat ggacaaggac aggagcagta tgggtacacg 1080
atgcctcaag accctaagc atattacgga ggctactctg gtggaggata cagcggtggt 1140
taccagcaga caccacaggc aggacagcaa ccaccacaac agccaccaca gcagcaacaa 1200
gtcgggttta gctactaa 1218

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<210> SEQ ID NO 14

<211> LENGTH: 405

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 14

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

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275
 His Val Lys Ile Pro Ala Gly Lys Arg Cys Gly Phe Val Gln Phe Ser
 290
 305 Gln Lys Ser Cys Ala Gln Ala Leu Arg Met Leu Asn Gly Val Gln
 310
 315
 320 Leu Gly Gly Thr Val Arg Leu Ser Trp Gly Arg Ser Pro Ser Asn
 325
 330
 335 Lys Gln Ser Gly Asp Pro Ser Gln Phe Tyr Gly Tyr Gly Gln
 340
 345
 350 Gly Gln Gln Tyr Gly Tyr Thr Met Pro Gln Asp Pro Asn Ala Tyr
 355
 360
 365 Tyr Gly Gly Tyr Ser Gly Tyr Ser Gly Tyr Gln Thr
 370
 375
 380 Pro Gln Ala Gly Gln Pro Gln Pro Gln Gln Gln Gln
 385
 390
 395 Val Gly Phe Ser Tyr
 405

<210> SEQ ID NO 15
 <211> LENGTH: 1248
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana
 <400> SEQUENCE: 15

60 atgctcagc agccaccctc agctcccaac ggtcgtcagca caggcccaag gcaagatcct
 120 tccgaaccaac aagctaccct ccagcagcag cagtcgtgga tgatcagcaca ccagcagca
 180 caacaaggctc agcccgctgc aggatgggat ccagcagctgc caccgctctc tggctcaaca
 240 ccagcagcagc agtatggtggt tggctggtat ccagatccag gatcagctgg tgaagatccg
 300 tccctcgtggc tccggtgcatc gcagccatgg atgatgagga actatctcat gaaagctctt
 360 ggtctaccatc gtcgaggtacc agcagctaaa gttatctcga ataacacagaa cggatattca
 420 gaaggttatc gcttatcga gttcgtcagc catgctlacg ctgagagggaa ttaccagact
 480 tacaatggtg ctcctgcatc agcagatgag ccagccttca ggtcagatc ggtctcagctt
 540 gtagctcggag agagagccca gctcgaaggg cctcgaagcaca cagttcttgg tggagacctg
 600 gcaacctgatc tcaaccgaca catgcttact gaaacctta agctcgtgta tccctcctgc
 660 aagggtgagc aagtctgtaa tgatagagct actggacggt ccaagggtta tggatcttgc
 720 aggtctggcg atgaaagtgca gcagatctgt gccatctgac aaatgatgg tcaatcactgc
 780 tcatcaaggc ctatgctgac tggctcctgc gccacaagaa agcctctac aatgcaaca
 840 gctctcatcc agaacactca agyaaatcga gtagaagtg atccaactaa cacaacaact
 900 tcttgctggag cctgctgatac aagtatgata gaaatatacc gtagaataac gctcgtcaata
 960 tcttgctgatac tagttcagat gaaatatacc gtagaataac gctcgtcaata
 1020 gccaataggg catgctgctga gcaagcactt cctgctgatac acctggagca actctggggg
 1080 caaagcattc gtcctctcag ggtcctcaga ccttcccaac aacaagctca accctgatca
 1140 gccccagtat gttggtggtg aggtatcctc ggttatcctc ctcaaggata tgaagcattca
 1200 ggtatctgac cctcctctca ggtaccctca ggtaccctca ggtaccctca ggtaccctca
 1248 atgctcagc agccaccctc agctcccaac ggtcgtcagca caggcccaag gcaagatcct

<210> SEQ ID NO 16
 <211> LENGTH: 415

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<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 16

Met Met Gln Gln Pro Pro Pro Ala Ser Asn Gly Ala Ala Thr Gly Pro
1      5      10      15

Gly Gln Ile Pro Ser Asp Gln Gln Ala Tyr Leu Gln Gln Gln Gln Ser
20      25      30

Trp Met Met Gln His Gln Gln Gln Gln Gly Gln Pro Pro Ala Gly
35      40      45

Trp Asn Gln Gln Ser Ala Pro Ser Ser Gly Gln Pro Gln Gln Gln Gln
50      55      60

Tyr Gly Gly Gly Gly Ser Gln Asn Pro Gly Ser Ala Gly Glu Ile Arg
65      70      75      80

Ser Leu Trp Ile Gly Asp Leu Gln Pro Trp Met Asp Glu Asn Tyr Leu
85      90      95

Met Asn Val Phe Gly Leu Thr Gly Glu Ala Thr Ala Ala Lys Val Ile
100     105     110

Arg Asn Lys Gln Asn Gly Tyr Ser Glu Gly Tyr Gly Phe Ile Glu Phe
115     120     125

Val Asn His Ala Thr Ala Glu Arg Asn Leu Gln Thr Tyr Asn Gly Ala
130     135     140

Pro Met Pro Ser Ser Glu Gln Ala Phe Arg Leu Asn Trp Ala Gln Leu
145     150     155     160

Gly Ala Gly Glu Arg Arg Gln Ala Glu Gly Pro Glu His Thr Val Phe
165     170     175

Val Gly Asp Leu Ala Pro Asp Val Thr Asp His Met Leu Thr Glu Thr
180     185     190

Phe Lys Ala Val Tyr Ser Ser Val Lys Gly Ala Lys Val Val Asn Asp
195     200     205

Arg Thr Thr Gly Arg Ser Lys Gly Tyr Gly Phe Val Arg Phe Ala Asp
210     215     220

Glu Ser Glu Gln Ile Arg Ala Met Thr Glu Met Asn Gly Gln Tyr Cys
225     230     235     240

Ser Ser Arg Pro Met Arg Thr Gly Pro Ala Ala Asn Lys Lys Pro Leu
245     250     255

Thr Met Gln Pro Ala Ser Tyr Gln Asn Thr Gln Gly Asn Ser Gly Glu
260     265     270

Ser Asp Pro Thr Asn Thr Thr Ile Phe Val Gly Ala Val Asp Gln Ser
275     280     285

Val Thr Glu Asp Asp Leu Lys Ser Val Phe Gly Gln Phe Gly Glu Leu
290     295     300

Val His Val Lys Ile Pro Ala Gly Lys Arg Cys Gly Phe Val Gln Tyr
305     310     315     320

Ala Asn Arg Ala Cys Ala Glu Gln Ala Leu Ser Val Leu Asn Gly Thr
325     330     335

Gln Leu Gly Gly Gln Ser Ile Arg Leu Ser Trp Gly Arg Ser Pro Ser
340     345     350

Asn Lys Gln Thr Gln Pro Asp Gln Ala Gln Tyr Gly Gly Gly Gly Gly
355     360     365

Tyr Tyr Gly Tyr Pro Pro Gln Gly Tyr Glu Ala Tyr Gly Tyr Ala Pro
370     375     380

Pro Pro Gln Asp Pro Asn Ala Tyr Tyr Gly Gly Tyr Ala Gly Gly Gly
385     390     395     400

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Thr	Leu	Gln	Thr	Tyr	Asn	Gly	Thr	Gln	Met	Pro	Gly	Thr	Glu	Leu	Thr
				85					90					95	
Phe	Arg	Leu	Asn	Trp	Ala	Ser	Phe	Gly	Ser	Gly	Gln	Lys	Val	Asp	Ala
			100					105					110		
Gly	Pro	Asp	His	Ser	Ile	Phe	Val	Gly	Asp	Leu	Ala	Pro	Asp	Val	Thr
		115					120					125			
Asp	Tyr	Leu	Leu	Gln	Glu	Thr	Phe	Arg	Val	His	Tyr	Ser	Ser	Val	Arg
	130					135					140				
Gly	Ala	Lys	Val	Val	Thr	Asp	Pro	Ser	Thr	Gly	Arg	Ser	Lys	Gly	Tyr
145					150					155					160
Gly	Phe	Val	Lys	Phe	Ala	Glu	Glu	Ser	Glu	Arg	Asn	Arg	Ala	Met	Ala
				165					170					175	
Glu	Met	Asn	Gly	Leu	Tyr	Cys	Ser	Thr	Arg	Pro	Met	Arg	Ile	Ser	Ala
		180						185					190		
Ala	Thr	Pro	Lys	Lys	Asn	Val	Gly	Val	Gln	Gln	Gln	Tyr	Val	Thr	Lys
		195					200						205		
Ala	Val	Tyr	Pro	Val	Thr	Val	Pro	Ser	Ala	Val	Ala	Ala	Pro	Val	Gln
	210					215					220				
Ala	Tyr	Val	Ala	Pro	Pro	Glu	Ser	Asp	Val	Thr	Cys	Thr	Thr	Ile	Ser
225					230					235					240
Val	Ala	Asn	Leu	Asp	Gln	Asn	Val	Thr	Glu	Glu	Glu	Leu	Lys	Lys	Ala
				245					250					255	
Phe	Ser	Gln	Leu	Gly	Glu	Val	Ile	Tyr	Val	Lys	Ile	Pro	Ala	Thr	Lys
		260						265					270		
Gly	Tyr	Gly	Tyr	Val	Gln	Phe	Lys	Thr	Arg	Pro	Ser	Ala	Glu	Glu	Ala
		275				280						285			
Val	Gln	Arg	Met	Gln	Gly	Gln	Val	Ile	Gly	Gln	Gln	Ala	Val	Arg	Ile
	290					295						300			
Ser	Trp	Ser	Lys	Asn	Pro	Gly	Gln	Asp	Gly	Trp	Val	Thr	Gln	Ala	Asp
305				310						315					320
Pro	Asn	Gln	Trp	Asn	Gly	Tyr	Tyr	Gly	Tyr	Gly	Gln	Gly	Tyr	Asp	Ala
				325					330					335	
Tyr	Ala	Tyr	Gly	Ala	Thr	Gln	Asp	Pro	Ser	Val	Tyr	Ala	Tyr	Gly	Gly
			340					345					350		
Tyr	Gly	Tyr	Pro	Gln	Tyr	Pro	Gln	Gln	Gly	Glu	Gly	Thr	Gln	Asp	Ile
		355					360					365			
Ser	Asn	Ser	Ala	Ala	Gly	Gly	Val	Ala	Gly	Ala	Glu	Gln	Glu	Leu	Tyr
	370					375					380				
Asp	Pro	Leu	Ala	Thr	Pro	Asp	Val	Asp	Lys	Leu	Asn	Ala	Ala	Tyr	Leu
385					390					395					400
Ser	Val	His	Ala	Ser	Ala	Ile	Leu	Gly	Arg	Pro	Met	Trp	Gln	Arg	Thr
			405						410					415	
Ser	Ser	Leu	Thr	Ser	Gln	Leu	Gly	Lys							
			420					425							

<210> SEQ ID NO 19

<211> LENGTH: 1338

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 19

atgcagacac caaacaacaa cggttcaaca gattcagtg taccaccaac atcagccgga 60

acaacaccac caccaccggt gcagcaatca acaccaccac cgcagcagca acaacaacaa 120

cagtggaac aacaacaaca atggatggct gcgatgcagc aataccctgc agctgctatg 180

gctatgatgc aacaacaaca gatgatgatg tatcctcacc ctcatacgc tccttaacaat
 240 caagtgctct atcaacagca tctcagtt caatacagctg cttatcaaca gcagcagcag
 300 caacatcaccc agagtcacagca gcagccacgc ggtgggatctg gttggtgatgta tgcacaagact
 360 ctttgggtct gttgatctct tcaatggatg gatggagactt atcctccatac cgttctctct
 420 cacacaacatg agtttctct tglgaaagt atacgcaaca agcaaatg tcaatctgaa
 480 gcatatgggt tgtttgatgt tcttcaagt tcagcagctg aggaagctct tcagagcttt
 540 agcgtgtgta caatgcccga cgcggaaacag ccttccgtt taaactcggc atcttccagt
 600 actggtgaga aaagagctc agaatgctt cctgaacctat ccaatctgt tggagatctg
 660 gctccagatg tgaagtgatgc tgccttgcct gagacctttg ctggtagata tccatctgct
 720 aaagtgctca aagttgtgat tgaatccaac actcggcgctt ccaaggtta cgggttctgt
 780 aggtttgctg atgaaatgga gcgatcaaga gctatgacag aaatgaaatg tgccttctgt
 840 tcaagcagcc aatgctggt tglatcgcga accccgaaaa ggcctgctgc tcaaggccaa
 900 caaatggtt cacaagctct tacactgct gttgacatg gagggaaatg tccaatgctc
 960 gatgtagaat caataatc aacaatatt gttggcgctc ttgatgctga tglatcagaa
 1020 gaagacctca tgaacctt tccgattt ggggaggttg ttcaagtgaa gatcccagta
 1080 ggaagatg tttgcttct caatctgct aacagggcaaa gttcctgagga agccatcggg
 1140 ggaagatg tttgcttct caatctgct aacagggcaaa gttcctgagga agccatcggg
 1140 aacttgacg ggcagatcat tggaaagaa actgtccgct ttcactgggg aagaagcccc
 1200 aacaacaact ggaagaatgca cctcggcaac caatggaatg gaggatattc aagaggtcaaa
 1260 ggtacaaca atgatatgc caatcagac tcaaacatgt acgtactgc agcgctgca
 1320 gtcaccgggag ctctctga 1338

<210> SEQ ID NO 20
 <211> LENGTH: 445
 <212> TYPE: PRT
 <213> ORGANISM: Arabidopsis thaliana
 <400> SEQUENCE: 20

Met Ala Ala Met Gln Gln Tyr Pro Ala Ala Met Ala Met Met Gln
 50 Met Ala Ala Met Gln Gln Tyr Pro Ala Ala Met Ala Met Met Gln
 55 Gln Gln Met Met Tyr Pro His Pro Gln Tyr Ala Pro Tyr Asn
 60 Gln Ala Ala Tyr Gln His Pro Gln Phe Gln Tyr Ala Ala Tyr Gln
 65 Gln Ala Ala Tyr Gln His Pro Gln Phe Gln Tyr Ala Ala Tyr Gln
 70 Gln Gln Gln Gln Gln His His Gln Ser Gln Gln Pro Arg Gly Gly
 75 Ser Gly Gly Asp Asp Val Lys Thr Leu Trp Val Gly Asp Leu Leu His
 80 Trp Met Asp Gln Thr Tyr Leu His Thr Cys Phe Ser His Thr Asn Gln
 85 Val Ser Ser Val Lys Val Ile Arg Asn Lys Gln Thr Cys Gln Ser Gln
 90 Val Ser Ser Val Lys Val Ile Arg Asn Lys Gln Thr Cys Gln Ser Gln
 95

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Gly	Tyr	Gly	Phe	Val	Glu	Phe	Leu	Ser	Arg	Ser	Ala	Ala	Glu	Glu	Ala
				165					170					175	
Leu	Gln	Ser	Phe	Ser	Gly	Val	Thr	Met	Pro	Asn	Ala	Glu	Gln	Pro	Phe
			180					185					190		
Arg	Leu	Asn	Trp	Ala	Ser	Phe	Ser	Thr	Gly	Glu	Lys	Arg	Ala	Ser	Glu
		195					200				205				
Asn	Gly	Pro	Asp	Leu	Ser	Ile	Phe	Val	Gly	Asp	Leu	Ala	Pro	Asp	Val
	210					215					220				
Ser	Asp	Ala	Val	Leu	Leu	Glu	Thr	Phe	Ala	Gly	Arg	Tyr	Pro	Ser	Val
225				230						235					240
Lys	Gly	Ala	Lys	Val	Val	Ile	Asp	Ser	Asn	Thr	Gly	Arg	Ser	Lys	Gly
			245					250						255	
Tyr	Gly	Phe	Val	Arg	Phe	Gly	Asp	Glu	Asn	Glu	Arg	Ser	Arg	Ala	Met
		260					265						270		
Thr	Glu	Met	Asn	Gly	Ala	Phe	Cys	Ser	Ser	Arg	Gln	Met	Arg	Val	Gly
		275					280					285			
Ile	Ala	Thr	Pro	Lys	Arg	Ala	Ala	Ala	Tyr	Gly	Gln	Gln	Asn	Gly	Ser
	290					295					300				
Gln	Ala	Leu	Thr	Leu	Ala	Gly	Gly	His	Gly	Gly	Asn	Gly	Ser	Met	Ser
305					310					315					320
Asp	Gly	Glu	Ser	Asn	Asn	Ser	Thr	Ile	Phe	Val	Gly	Gly	Leu	Asp	Ala
				325					330					335	
Asp	Val	Thr	Glu	Glu	Asp	Leu	Met	Gln	Pro	Phe	Ser	Asp	Phe	Gly	Glu
			340					345					350		
Val	Val	Ser	Val	Lys	Ile	Pro	Val	Gly	Lys	Gly	Cys	Gly	Phe	Val	Gln
		355					360					365			
Phe	Ala	Asn	Arg	Gln	Ser	Ala	Glu	Glu	Ala	Ile	Gly	Asn	Leu	Asn	Gly
	370					375					380				
Thr	Val	Ile	Gly	Lys	Asn	Thr	Val	Arg	Leu	Ser	Trp	Gly	Arg	Ser	Pro
385					390					395					400
Asn	Lys	Gln	Trp	Arg	Ser	Asp	Ser	Gly	Asn	Gln	Trp	Asn	Gly	Gly	Tyr
			405					410						415	
Ser	Arg	Gly	Gln	Gly	Tyr	Asn	Asn	Gly	Tyr	Ala	Asn	Gln	Asp	Ser	Asn
			420					425					430		
Met	Tyr	Ala	Thr	Ala	Ala	Ala	Ala	Val	Pro	Gly	Ala	Ser			
		435					440					445			

<210> SEQ ID NO 21

<211> LENGTH: 1308

<212> TYPE: DNA

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 21

atgcagacaa ccaacggctc agattcgacg ttggcaactt cgggagccac accgccgaat	60
caacaaacc ctcctccacc tcagcagtgg cagcagcagc aacagcaaca gcaacagtgg	120
atggctgcc tgaatatcc accagcggcg gcgatgatga tgatgcagca gcaacagatg	180
ctgatgtatc ctcatcaata tgttccgtat aatcaaggtc cttatcagca gcatcatcct	240
cagcttcacc aatacgggtc ttatcaacag caccagcacc agcaacacaa ggctattgac	300
cgtggatctg gagatgatgt caagactctt tgggttggtg atcttcttca ttggatggat	360
gagacttata tccattcttg cttttctcac accggcgagg tttcttctgt gaaagtata	420
cgtaacaagc tcacttctca atcagaaggg tatgggtttg ttgagtttct ttcacgtgct	480
gcagctgaag aagttcttca gaactatagt ggttcagtga tgccaaactc ggaccaacc	540

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ttccgtataa actgggcac ttttagtact ggtgaaaaa gagcagtgga aaatggcca 600
gacctatctg tttttgtggg agacttgtct ccagatgtca ctgacgtttt attgcatgag 660
accttttctg atagatatcc ttctgtcaaa agcgccaaaag ttgtgattga ttccaacacc 720
ggccgggtcca aaggttatgg ttttgtgagg ttcggtgatg aaaatgagag gtcaagggct 780
ttgacagaaa tgaatggagc ttactgttcg aacaggcaaa tgcgtgtagg tattgcaact 840
cccaaaagag cgattgctaa tcagcaacaa cattcttcac aagctgtgat tctggctggt 900
ggacatggat caaatggttc catgggttat ggctcgcagt ctgatggcga atcaactaac 960
gcaacaatat ttgttggcgg cattgaccct gatgttattg atgaagacct cagacaacct 1020
ttttccagc tgggagaggt tgtttcagtg aagatcccag tagggaaagg atgtggattt 1080
gtccaatttg ctgacaggaa gagtgtgaa gatgctatcg agagtttgaa cgggacagtc 1140
atcggcaaga aactgtcag actctcctgg ggacgaagcc caaacaagca gtggagagga 1200
gactcagggc agcagtggaa tggaggatac tcacgaggac atggttacia caatggagga 1260
ggatatgcta accaccacga ctccaacaac tatcatgggg agaattga 1308

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<210> SEQ ID NO 22

<211> LENGTH: 435

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 22

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Met Gln Thr Thr Asn Gly Ser Asp Ser Thr Leu Ala Thr Ser Gly Ala
1          5          10          15
Thr Pro Pro Asn Gln Gln Thr Pro Pro Pro Pro Gln Gln Trp Gln Gln
20          25          30
Gln Gln Gln Gln Gln Gln Trp Met Ala Ala Met Gln Tyr Pro Pro
35          40          45
Ala Ala Ala Met Met Met Met Gln Gln Gln Gln Met Leu Met Tyr Pro
50          55          60
His Gln Tyr Val Pro Tyr Asn Gln Gly Pro Tyr Gln Gln His His Pro
65          70          75          80
Gln Leu His Gln Tyr Gly Ser Tyr Gln Gln His Gln His Gln Gln His
85          90          95
Lys Ala Ile Asp Arg Gly Ser Gly Asp Asp Val Lys Thr Leu Trp Val
100         105         110
Gly Asp Leu Leu His Trp Met Asp Glu Thr Tyr Leu His Ser Cys Phe
115         120         125
Ser His Thr Gly Glu Val Ser Ser Val Lys Val Ile Arg Asn Lys Leu
130         135         140
Thr Ser Gln Ser Glu Gly Tyr Gly Phe Val Glu Phe Leu Ser Arg Ala
145         150         155         160
Ala Ala Glu Glu Val Leu Gln Asn Tyr Ser Gly Ser Val Met Pro Asn
165         170         175
Ser Asp Gln Pro Phe Arg Ile Asn Trp Ala Ser Phe Ser Thr Gly Glu
180         185         190
Lys Arg Ala Val Glu Asn Gly Pro Asp Leu Ser Val Phe Val Gly Asp
195         200         205
Leu Ser Pro Asp Val Thr Asp Val Leu Leu His Glu Thr Phe Ser Asp
210         215         220
Arg Tyr Pro Ser Val Lys Ser Ala Lys Val Val Ile Asp Ser Asn Thr
225         230         235         240

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aagcaacctt tcaatgaatt cggggaata gtctctgtca agattcctgt tggtaaagga 1020
tgccgatttg ttcagtttgt taacagacca aatgcagagg aggctttgga gaaactaaat 1080
gggactgtaa ttggaaaaca aacagttcgg ctttcttggg gacgtaatcc cgccaataag 1140
cagcctagag ataagtatgg aaaccaatgg gttgatccgt actatggagg acagttttac 1200
aatgggtatg gatacatggt acctcaacct gaccggagaa tgtatcccgc tgcaccttac 1260
tatccaatgt acggtggtca tcagcaacaa gttagctga 1299

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<210> SEQ ID NO 24

<211> LENGTH: 432

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 24

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

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305 310 315 320
 Lys Gln Pro Phe Asn Gln Phe Gly Gln Ile Val Ser Val Lys Ile Pro 325
 Val Gly Lys Gly Cys Gly Phe Val Gln Phe Val Asn Arg Pro Asn Ala 340
 Gln Gln Ala Leu Gln Lys Leu Asn Gly Thr Val Ile Gly Lys Gln Thr 355
 Val Arg Leu Ser Trp Gly Arg Asn Pro Ala Asn Lys Gln Pro Arg Asp 370
 Lys Tyr Gly Asn Gln Trp Val Asp Pro Tyr Gly Gly Gln Phe Tyr 385
 Asn Gly Tyr Gly Tyr Met Val Pro Gln Pro Asp Pro Arg Met Tyr Pro 405
 Ala Ala Pro Tyr Tyr Pro Met Tyr Gly Gly His Gln Gln Val Ser 420
 425 430 435

<210> SEQ ID NO 25
 <211> LENGTH: 1281
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana
 <400> SEQUENCE: 25

60 atcgagatc aaggtctat taagcagcaa caacaacaac acatcaaca
 120 gctatgctc aacaagctat gatgcacaac catcctctc ttatctatc tggltgltatg
 180 gctcctctc agatgagagc ttacaacagt ggaacaacct cctctggltt tgaatccaact
 240 actgcccgt a gtlgtatgct tggaaacat catagcaggg tcaagagat tcttctcaa
 300 gagatatttct ccaagtatctgg tccatttgaac agctgttaaac tcatcagaa gbatatgctc
 360 tcatatggat tgttctaca ccttgatcga agatgtgctc a gtaagctat aatgactctt
 420 aacggaaggc atatatgtg aacgacctatg aagttatatt gggcgtatg c aactggtcaa
 480 agggaaagata catcaagctc attcaacat tttgttggag atctatgacc agagttact
 540 gatgagcagc at tgtttgatag ctttctgct tttacaagct gctcggagcc aagagttatg
 600 tggagccaaag aactgtgagc ctcaagagcc ttttggltt tttccctccg taatcagcag
 660 gatgctcctc aaaa ctgcccataaa tgaatgatggg taagtagcag acaatcagaa
 720 tgcacaactggg cgaacaagg tgcatacttt ggccgagagcaa aacatagctc tgaatggaaaa
 780 agtltgtatg aactatcaa cggatctca gaggatgtgta gaggactgctc aatgaaagt
 840 gccccctgaaa acaatcctca atttacaact gttctatgtag gaaatctctc tccagaaagt
 900 aactcagctg atctacaacgg tctatctat acccttgggt cttgagtgat cgaagagctc
 960 cgtgtcccagc gaggacaagg gtttgglttt gtgagatata acactcatala cgaagctgct
 1020 ctgtctatcc agatggtggcaa cgtctcagct tctcctctta gtagacaagat aaggtgtctc
 1080 ttgggaaaca aaccaactcc atcaggtcaca gctctcaaac ccactctccc accagccccg
 1140 gcatcagctc cttctctctc tgcactggac ctcttagccr acgagagggc aactgctctca
 1200 gccaagatgc atcctcagcc tcaaacatct ctgagggcaag caggtcttgg agtccaatglt
 1260 gctggagagaa ctgcaagctat gttatgtagtt ggtctatcagag atgtatcagctg cggccccatcag
 1281 cagctcagct a actatcagta a

<210> SEQ ID NO 26
 <211> LENGTH: 426
 <212> TYPE: PRT

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<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 26

Met Gln Asn Gln Arg Leu Ile Lys Gln Gln Gln Gln Gln Gln Gln
 1 5 10 15
 Gln His Gln Gln Ala Met Ile Gln Gln Ala Met Met Gln Gln His Pro
 20 25 30
 Ser Leu Tyr His Pro Gly Val Met Ala Pro Pro Gln Met Glu Pro Leu
 35 40 45
 Pro Ser Gly Asn Leu Pro Pro Gly Phe Asp Pro Thr Thr Cys Arg Ser
 50 55 60
 Val Tyr Ala Gly Asn Ile His Thr Gln Val Thr Glu Ile Leu Leu Gln
 65 70 75 80
 Glu Ile Phe Ala Ser Thr Gly Pro Ile Glu Ser Cys Lys Leu Ile Arg
 85 90 95
 Lys Asp Lys Ser Ser Tyr Gly Phe Val His Tyr Phe Asp Arg Arg Cys
 100 105 110
 Ala Ser Met Ala Ile Met Thr Leu Asn Gly Arg His Ile Phe Gly Gln
 115 120 125
 Pro Met Lys Val Asn Trp Ala Tyr Ala Thr Gly Gln Arg Glu Asp Thr
 130 135 140
 Ser Ser His Phe Asn Ile Phe Val Gly Asp Leu Ser Pro Glu Val Thr
 145 150 155 160
 Asp Ala Ala Leu Phe Asp Ser Phe Ser Ala Phe Asn Ser Cys Ser Asp
 165 170 175
 Ala Arg Val Met Trp Asp Gln Lys Thr Gly Arg Ser Arg Gly Phe Gly
 180 185 190
 Phe Val Ser Phe Arg Asn Gln Gln Asp Ala Gln Thr Ala Ile Asn Glu
 195 200 205
 Met Asn Gly Lys Trp Val Ser Ser Arg Gln Ile Arg Cys Asn Trp Ala
 210 215 220
 Thr Lys Gly Ala Thr Phe Gly Glu Asp Lys His Ser Ser Asp Gly Lys
 225 230 235 240
 Ser Val Val Glu Leu Thr Asn Gly Ser Ser Glu Asp Gly Arg Glu Leu
 245 250 255
 Ser Asn Glu Asp Ala Pro Glu Asn Asn Pro Gln Phe Thr Thr Val Tyr
 260 265 270
 Val Gly Asn Leu Ser Pro Glu Val Thr Gln Leu Asp Leu His Arg Leu
 275 280 285
 Phe Tyr Thr Leu Gly Ala Gly Val Ile Glu Glu Val Arg Val Gln Arg
 290 295 300
 Asp Lys Gly Phe Gly Phe Val Arg Tyr Asn Thr His Asp Glu Ala Ala
 305 310 315 320
 Leu Ala Ile Gln Met Gly Asn Ala Gln Pro Phe Leu Phe Ser Arg Gln
 325 330 335
 Ile Arg Cys Ser Trp Gly Asn Lys Pro Thr Pro Ser Gly Thr Ala Ser
 340 345 350
 Asn Pro Leu Pro Pro Pro Ala Pro Ala Ser Val Pro Ser Leu Ser Ala
 355 360 365
 Met Asp Leu Leu Ala Tyr Glu Arg Gln Leu Ala Leu Ala Lys Met His
 370 375 380
 Pro Gln Ala Gln His Ser Leu Arg Gln Ala Gly Leu Gly Val Asn Val
 385 390 395 400

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Ala Gly Gly Thr Ala Ala Met Tyr Asp Gly Tyr Gln Asn Val Ala
 405 410 415
 Ala Ala His Gln Leu Met Tyr Tyr Gln
 420 425

<210> SEQ ID NO 27
 <211> LENGTH: 1260
 <212> TYPE: DNA
 <213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 27

atgcagaggt tgaagcagca gcaagcagca caaagatta tgaatcctatg 60

cagcaacagat cctcctaacca tccctggtctc cctgccccgc cacagataga accaatccca 120

agtggaatc tccccccctgg ttctgatcca agtacttgcc gcatgtgta cgttggaaac 180

atccatattc agtgcacgga accctctgctt caagaggttt ttgctgggac tggctccctgta 240

gaaagctgta aactaatag gaaagaaag tctccttatg gtrttgtgca ctaacttgat 300

cgaagatccg ctggtctctgc aatcccttct ccaatggaa ggcatttgtt tgggcaacct 360

atacaagtta actgggctta tgcgagtggtc cagagtggtg atatacctcaag tcaatcctcaat 420

atatgtgtg gggatrttag tccggaggtt actgatggca tgcgtttac tgcrtctctc 480

gtcctaccga ctgctcggga tgcagagtt atgtgggtatc agaaactgg gcgtccaaga 540

ggatrtggat ttgttctct cgttaacca cagatggccc agatctgcaat agatrtgatata 600

actgggaaat ggccttggttc cagcagatata cgttgcaact gggcgacaaga gggagccccact 660

tctggtgtagg acaaacagag ctctgatccc aaagcgtctg tggaaactac cagtgggtgctc 720

tggaggtatg gtaaaagtatc tactaatgtt gaagctcctg agaaactatgc tcaagtacaaca 780

actgtttacg tccgktaatct tgcctccagag gtgtcccaagg tgatcttca cggccacttc 840

catctcccctgg gtctgtgggtt catagagaa gtccgtgttc aaagagcaaa aggttctcgg 900

tttgtgatat actctactca tgtagagca gccctcgtca tccagatggg aacaacacat 960

tccactca gttggcagga aatgaaagt tcttggggaa gcaagccca tccagcagga 1020

acagctcaaa accgccttcc tccaaccagt ccttgcaacca tccgggatc ctcaagcagt 1080

gatcctcttg ctcaagtagg gcaactagc atgagcaaga tggcaggaat gaatccgatg 1140

atgcatcaccc cgcagggaca acatgtcttt aaacaagctg caatgggagc cacttggtca 1200

aaacaggaata tataratgagg tggttaccag aacggcagc agctcatgta ctaccagtaa 1260

<210> SEQ ID NO 28
 <211> LENGTH: 419
 <212> TYPE: PRT
 <213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 28

Met Gln Arg Leu Lys Gln Gln Gln Gln Gln Gln Val Met Gln 1

Gln Ala Leu Met Gln Gln Gln Ser Leu Tyr His Pro Gly Leu Ala 20

Pro Pro Gln Ile Gln Pro Ile Pro Ser Gly Asn Leu Pro Pro Gly Phe 35

Asp Pro Ser Thr Cys Arg Ser Val Tyr Val Gly Asn Ile His Ile Gln 50

Val Thr Gln Pro Leu Leu Gln Val Phe Ala Gly Thr Gly Pro Val 65

70 75 80

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gtgtatgttg gaaacatcca tacacaggtc acagagcctt tgcttcaaga gatttttaca 240
agcactggcc ctggtgaaag cagtaaactc atcagaaagg ataagtcatc atatggattt 300
gttcactact ttgatcgaag atccgctgct ctggctatac tgtctctgaa cggaaggcat 360
ctggtttggac agcctatcaa agtcaattgg gcgcatgcca ctggtcagag ggaagataca 420
tcaagtcatt tcaacatttt tgttgagat ctcagtccag aggtcactga tgcaacatta 480
tatcaaagct tttctgtctt ttccagttgt tcggatgcga gagttatgtg ggacaaaaaa 540
actggggcgt cgagaggcct tgggtttggt tccttccgca atcaacagga tgctcaaact 600
gccattaatg agatgaatgg taagtgggta agtagcagac aaatcagatg caactgggcc 660
acgaaggggc ctacttctgg tgatgataag ctcagttctg atggaaaaag tgttgggaa 720
cttacaactg gctcatcaga ggatggtaaa gagacattaa atgaggaaac acctgaaaat 780
aattctcagt ttaccactgt ttatgtggga aaccttgcct cagaggtaac tcagcttgat 840
ctacaccgtt acttccatgc tcttggcgtt ggagttattg aggaggtccg tgtccaacga 900
gacaaaaggct ttggtttctg gagatataac actcatcccg aagctgctct tgctattcag 960
atgggtaaca ctcagcctta cctctttaac agacagataa agtgctcatg gggaaacaag 1020
ccaactccac caggtacagc ctcaaaccca etccccccac ctgccccagc tccagttcct 1080
ggcttatctg cagctgatct cctaaactat gagaggcaat tggcacttag caagatggca 1140
agtgtgaatg cgtaaatgca tcaacagggt caacaccctc taaggcaggc tcatggaata 1200
aatgccgctg gagcaactgc agccatgtat gatggtggct ttcagaatgt agccgcgcga 1260
cagcaactca tgtactatca gtaa 1284

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<210> SEQ ID NO 30

<211> LENGTH: 427

<212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 30

```

Met Gln Asn Pro Arg Leu Lys Gln His Gln Gln Gln Gln Gln Gln
1 5 10 15
Ala Met Met Gln Gln Ala Leu Met Gln Gln His Ser Leu Tyr His
20 25 30
Pro Gly Val Leu Ala Pro Pro Gln Leu Glu Pro Val Pro Ser Gly Asn
35 40 45
Leu Pro Pro Gly Phe Asp Pro Ser Thr Cys Arg Ser Val Tyr Val Gly
50 55 60
Asn Ile His Thr Gln Val Thr Glu Pro Leu Leu Gln Glu Ile Phe Thr
65 70 75 80
Ser Thr Gly Pro Val Glu Ser Ser Lys Leu Ile Arg Lys Asp Lys Ser
85 90 95
Ser Tyr Gly Phe Val His Tyr Phe Asp Arg Arg Ser Ala Ala Leu Ala
100 105 110
Ile Leu Ser Leu Asn Gly Arg His Leu Phe Gly Gln Pro Ile Lys Val
115 120 125
Asn Trp Ala Tyr Ala Thr Gly Gln Arg Glu Asp Thr Ser Ser His Phe
130 135 140
Asn Ile Phe Val Gly Asp Leu Ser Pro Glu Val Thr Asp Ala Thr Leu
145 150 155 160
Tyr Gln Ser Phe Ser Val Phe Ser Ser Cys Ser Asp Ala Arg Val Met
165 170 175

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Trp Asp Gln Lys Thr Gly Arg Ser Arg Gly Phe Gly Phe Val Ser Phe
 180 185 190

Arg Asn Gln Gln Asp Ala Gln Thr Ala Ile Asn Glu Met Asn Gly Lys
 195 200 205

Trp Leu Ser Ser Arg Gln Ile Arg Cys Asn Trp Ala Thr Lys Gly Ala
 210 215 220

Thr Ser Gly Asp Asp Lys Leu Ser Ser Asp Gly Lys Ser Val Val Glu
 225 230 235 240

Leu Thr Thr Gly Ser Ser Glu Asp Gly Lys Glu Thr Leu Asn Glu Glu
 245 250 255

Thr Pro Glu Asn Asn Ser Gln Phe Thr Thr Val Tyr Val Gly Asn Leu
 260 265 270

Ala Pro Glu Val Thr Gln Leu Asp Leu His Arg Tyr Phe His Ala Leu
 275 280 285

Gly Ala Gly Val Ile Glu Glu Val Arg Val Gln Arg Asp Lys Gly Phe
 290 295 300

Gly Phe Val Arg Tyr Asn Thr His Pro Glu Ala Ala Leu Ala Ile Gln
 305 310 315 320

Met Gly Asn Thr Gln Pro Tyr Leu Phe Asn Arg Gln Ile Lys Cys Ser
 325 330 335

Trp Gly Asn Lys Pro Thr Pro Pro Gly Thr Ala Ser Asn Pro Leu Pro
 340 345 350

Pro Pro Ala Pro Ala Pro Val Pro Gly Leu Ser Ala Ala Asp Leu Leu
 355 360 365

Asn Tyr Glu Arg Gln Leu Ala Leu Ser Lys Met Ala Ser Val Asn Ala
 370 375 380

Leu Met His Gln Gln Gly Gln His Pro Leu Arg Gln Ala His Gly Ile
 385 390 395 400

Asn Ala Ala Gly Ala Thr Ala Ala Met Tyr Asp Gly Gly Phe Gln Asn
 405 410 415

Val Ala Ala Ala Gln Gln Leu Met Tyr Tyr Gln
 420 425

<210> SEQ ID NO 31

<211> LENGTH: 735

<212> TYPE: DNA

<213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 31

```

atggcgcgctg aaaaaatcctt gaccccagaa tctcagttga agaagtctaa ggctcaacaa    60
aagactgctg aacaagtcgc tgctgaaaga gctgctcgta aggctgctaa caaggaaaag    120
agagccatta ttttgaaag aaacgccgct taccaaaagg aatacgaac tgctgaaaga    180
aacatcattc aagctaagcg tgatgccaag gctgctggtt cctactacgt cgaagctcaa    240
cacaagttgg tcttctgtgt cagaatcaag ggtattaaca agatcccacc taagccaaga    300
aaggttctac aattgctaag attgacaaga atcaactctg gtacattcgt caaagttacc    360
aaggctactt tggaactatt gaagttgatt gaaccatacg ttgcttacgg ttacctatcg    420
tactctacta ttagacaatt ggtctacaag agaggtttcg gtaagatcaa caagcaaaga    480
gttcattgt cgcacaatgc tatcatcgaa gccaaactgg gtaagtatgg tatctgtgcc    540
attgacgatt tgattcacga aatcatcact gttggtccac acttcaagca agctaacaac    600
ttttgtggc cattcaagtt gtccaacca tctggtggtt ggggtgtccc aagaaagttc    660
aagcacttta tccaaggtgg ttctttcggg aacctgaag aattcatcaa caaattggtt    720

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aagtccatga actaa

735

<210> SEQ ID NO 32

<211> LENGTH: 244

<212> TYPE: PRT

<213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 32

Met Ala Ala Glu Lys Ile Leu Thr Pro Glu Ser Gln Leu Lys Lys Ser
1 5 10 15Lys Ala Gln Gln Lys Thr Ala Glu Gln Val Ala Ala Glu Arg Ala Ala
20 25 30Arg Lys Ala Ala Asn Lys Glu Lys Arg Ala Ile Ile Leu Glu Arg Asn
35 40 45Ala Ala Tyr Gln Lys Glu Tyr Glu Thr Ala Glu Arg Asn Ile Ile Gln
50 55 60Ala Lys Arg Asp Ala Lys Ala Ala Gly Ser Tyr Tyr Val Glu Ala Gln
65 70 75 80His Lys Leu Val Phe Val Val Arg Ile Lys Gly Ile Asn Lys Ile Pro
85 90 95Pro Lys Pro Arg Lys Val Leu Gln Leu Leu Arg Leu Thr Arg Ile Asn
100 105 110Ser Gly Thr Phe Val Lys Val Thr Lys Ala Thr Leu Glu Leu Leu Lys
115 120 125Leu Ile Glu Pro Tyr Val Ala Tyr Gly Tyr Pro Ser Tyr Ser Thr Ile
130 135 140Arg Gln Leu Val Tyr Lys Arg Gly Phe Gly Lys Ile Asn Lys Gln Arg
145 150 155 160Val Pro Leu Ser Asp Asn Ala Ile Ile Glu Ala Asn Leu Gly Lys Tyr
165 170 175Gly Ile Leu Ser Ile Asp Asp Leu Ile His Glu Ile Ile Thr Val Gly
180 185 190Pro His Phe Lys Gln Ala Asn Asn Phe Leu Trp Pro Phe Lys Leu Ser
195 200 205Asn Pro Ser Gly Gly Trp Gly Val Pro Arg Lys Phe Lys His Phe Ile
210 215 220Gln Gly Gly Ser Phe Gly Asn Arg Glu Glu Phe Ile Asn Lys Leu Val
225 230 235 240

Lys Ser Met Asn

<210> SEQ ID NO 33

<211> LENGTH: 735

<212> TYPE: DNA

<213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 33

atgtccactg aaaaaatctt gactcctgaa tctcaattga agaagactaa agctcaacaa 60

aagactgcag aacaaattgc tgcagagaga gctgcccgta aagccgctaa caaggaaaaa 120

agagctatta ttttggaaag aaacgocgct taccaaaagg aatacgaaac tgctgaaaga 180

aacatcattc aagctaagcg tgatgccaaag gctgctggtt cctactacgt cgaagctcaa 240

cacaagttgg tcttcgttgt cagaatcaag ggtattaaca agattccacc taagccaaga 300

aaggttctac aattgctaag attgacaaga atcaactctg gtacattcgt caaagttacc 360

aaggctactt tggaactatt gaagttgatt gaaccatacg ttgcttacgg ttaccatcc 420

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tactctacta ttagacaatt ggtctacaag agaggtttcg gtaagatcaa caagcaaaga 480
gttccattgt ccgacaatgc tatcatcgaa gcccaacttg gtaagtatgg tatcttgtcc 540
attgacgatt tgattcacga aatcatcact gttggtccac acttcaagca agctaacaac 600
ttttgtggc cattcaagtt gtccaacca tctggtggtt ggggtgtccc aagaaagttc 660
aagcatttca tccaaggtgg ttctttcggg aaccgtgaag aattcatcaa taaattggtt 720
aaggctatga actaa 735
```

```
<210> SEQ ID NO 34
<211> LENGTH: 244
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae
```

```
<400> SEQUENCE: 34
```

```
Met Ser Thr Glu Lys Ile Leu Thr Pro Glu Ser Gln Leu Lys Lys Thr
 1           5           10           15
Lys Ala Gln Gln Lys Thr Ala Glu Gln Ile Ala Ala Glu Arg Ala Ala
 20           25           30
Arg Lys Ala Ala Asn Lys Glu Lys Arg Ala Ile Ile Leu Glu Arg Asn
 35           40           45
Ala Ala Tyr Gln Lys Glu Tyr Glu Thr Ala Glu Arg Asn Ile Ile Gln
 50           55           60
Ala Lys Arg Asp Ala Lys Ala Ala Gly Ser Tyr Tyr Val Glu Ala Gln
 65           70           75           80
His Lys Leu Val Phe Val Val Arg Ile Lys Gly Ile Asn Lys Ile Pro
 85           90           95
Pro Lys Pro Arg Lys Val Leu Gln Leu Leu Arg Leu Thr Arg Ile Asn
 100          105          110
Ser Gly Thr Phe Val Lys Val Thr Lys Ala Thr Leu Glu Leu Leu Lys
 115          120          125
Leu Ile Glu Pro Tyr Val Ala Tyr Gly Tyr Pro Ser Tyr Ser Thr Ile
 130          135          140
Arg Gln Leu Val Tyr Lys Arg Gly Phe Gly Lys Ile Asn Lys Gln Arg
 145          150          155          160
Val Pro Leu Ser Asp Asn Ala Ile Ile Glu Ala Asn Leu Gly Lys Tyr
 165          170          175
Gly Ile Leu Ser Ile Asp Asp Leu Ile His Glu Ile Ile Thr Val Gly
 180          185          190
Pro His Phe Lys Gln Ala Asn Asn Phe Leu Trp Pro Phe Lys Leu Ser
 195          200          205
Asn Pro Ser Gly Gly Trp Gly Val Pro Arg Lys Phe Lys His Phe Ile
 210          215          220
Gln Gly Gly Ser Phe Gly Asn Arg Glu Glu Phe Ile Asn Lys Leu Val
 225          230          235          240
Lys Ala Met Asn
```

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<210> SEQ ID NO 35
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer
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<400> SEQUENCE: 35
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aaaaagcagg cttaatgcag caaccaccgt caaacgcc 38

<210> SEQ ID NO 36
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 36

agaaagctgg gtttcaactga cgttgctgct gatagtt 37

<210> SEQ ID NO 37
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 37

aaaaagcagg cttaatgcag acaccaaca acaacggt 38

<210> SEQ ID NO 38
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 38

agaaagctgg gtttcaagaa gctcccggga ctgcagc 37

<210> SEQ ID NO 39
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 39

aaaaagcagg cttaatgcag acaaccaacg gctcagat 38

<210> SEQ ID NO 40
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 40

agaaagctgg gtttcaattc tcccacatgat agttggt 37

<210> SEQ ID NO 41
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 41

aaaaagcagg cttaattggca gacgtcaaga ttcaatcc 38

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<210> SEQ ID NO 42
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 42

agaaagctgg gtttcagcta acttggtgct gatgacc 37

<210> SEQ ID NO 43
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 43

aaaaagcagg cttaatggca gacgtcaagg ttcaatcc 38

<210> SEQ ID NO 44
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 44

agaaagctgg gtttcagcta acttggtgct gatgacc 37

<210> SEQ ID NO 45
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 45

aaaaagcagg cttaatgcag aatcaaaggc ttattaag 38

<210> SEQ ID NO 46
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 46

agaaagctgg gttttactga tagtacatga gctgctg 37

<210> SEQ ID NO 47
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 47

aaaaagcagg cttaatgtcc actgaaaaaa tctt 34

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<210> SEQ ID NO 48
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 48

agaaagctgg gttttagttc atagccttaa cca 33

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 49

aatctgtgtc gacgtacttc 20

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 50

agaagtacat aggatgggtc 20

<210> SEQ ID NO 51
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 51

aaaaattgtc gacgtacttc 20

<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 52

aaaggaagtt atcacaattg 20

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 53

ccagcatcta tgtctgcaac 20

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<210> SEQ ID NO 54
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 54

cgtatctgga gtagtatttc 20

<210> SEQ ID NO 55
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 55

gcaaggtata caaagcagaa 20

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 56

tcacccctttt tcttctctgc 20

<210> SEQ ID NO 57
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 57

gacacgatga agttggatat 20

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 58

tgactgtcaa atcatcactg 20

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 59

cagtataaaa atgtctgaat 20

<210> SEQ ID NO 60

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 60

tggttgatta tttcttcttc 20

<210> SEQ ID NO 61
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 61

atcaacgtca taatgtccac 20

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 62

taccagagtt gattcttctg 20

<210> SEQ ID NO 63
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 63

acctaagaa accatgtcag 20

<210> SEQ ID NO 64
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 64

tatcaaggtt gtacgtttcg 20

<210> SEQ ID NO 65
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 65

atgtacagtc taagtcaagg 20

<210> SEQ ID NO 66
<211> LENGTH: 20

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 66
gactaaagtg aacagcaatg 20

<210> SEQ ID NO 67
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 67
gagaatggca atatttcaag 20

<210> SEQ ID NO 68
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 68
tggttttctt cttccattac 20

<210> SEQ ID NO 69
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 69
tggaccacaca taatccaatt 20

<210> SEQ ID NO 70
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 70
tttcgaacat tacctcacac 20

<210> SEQ ID NO 71
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 71
gtggatggtc ttttagaaga 20

<210> SEQ ID NO 72
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 primer

 <400> SEQUENCE: 72

 aactcctcga aacttaaacy 20

 <210> SEQ ID NO 73
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 primer

 <400> SEQUENCE: 73

 tattgagacc ttcttccaag 20

 <210> SEQ ID NO 74
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 primer

 <400> SEQUENCE: 74

 aagattttac cggaaacgtg 20

 <210> SEQ ID NO 75
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 primer

 <400> SEQUENCE: 75

 gacgataaaa agaaatttgg tg 22

 <210> SEQ ID NO 76
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 primer

 <400> SEQUENCE: 76

 ctcaaagcgt tgmtgaaag 19

 <210> SEQ ID NO 77
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
 primer

 <400> SEQUENCE: 77

 gagagaggtc attagtatta 20

 <210> SEQ ID NO 78
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence

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<220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

 <400> SEQUENCE: 78
 ttttctaata acaggaacc 20

<210> SEQ ID NO 79
 <211> LENGTH: 23
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

 <400> SEQUENCE: 79
 gtttaataga aaaagaagag gag 23

<210> SEQ ID NO 80
 <211> LENGTH: 23
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

 <400> SEQUENCE: 80
 tagttcatca actaaaaaca tgg 23

<210> SEQ ID NO 81
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

 <400> SEQUENCE: 81
 agctgtcca agtgttcaa 19

<210> SEQ ID NO 82
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

 <400> SEQUENCE: 82
 acccttacca cgaatttc 19

<210> SEQ ID NO 83
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

 <400> SEQUENCE: 83
 gttggatat ttttggttg 20

<210> SEQ ID NO 84
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 84

aaaggaacgt ccttcaattc 20

<210> SEQ ID NO 85
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 85

aacaagctgt tcaggtaga 20

<210> SEQ ID NO 86
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 86

ggtttgatgat tatcatcagg 20

<210> SEQ ID NO 87
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 87

aattaaagat cacaatggcc g 21

<210> SEQ ID NO 88
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 88

cttgtaact ttgacgaatg 20

<210> SEQ ID NO 89
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 89

cagaaaagct ggtgttcaag 20

<210> SEQ ID NO 90
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

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primer

<400> SEQUENCE: 90

tgattctgca tegtggtttc 20

<210> SEQ ID NO 91
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 91

ttgattaaga actccaaagc 20

<210> SEQ ID NO 92
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 92

tcttctcaag acacgtaatc 20

<210> SEQ ID NO 93
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 93

agatgaggtt gaagcaatag 20

<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 94

caagggcaat ttccttattg 20

<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 95

taagactaag caacaatgcc 20

<210> SEQ ID NO 96
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

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<400> SEQUENCE: 96

aaacccaact tgtagacttg 20

<210> SEQ ID NO 97

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 97

gcatttcata atatgtctgc 20

<210> SEQ ID NO 98

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

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ttggtgctaa gactgtagag 20

<210> SEQ ID NO 99

<211> LENGTH: 22

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 99

caaatccatt tcaaaatata gg 22

<210> SEQ ID NO 100

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 100

ctcctcctat ctaaaaaacc 20

<210> SEQ ID NO 101

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 101

aagaagagtt ggtaagcaag 20

<210> SEQ ID NO 102

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

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<400> SEQUENCE: 102
caccgttttt gaatgtgatg 20

<210> SEQ ID NO 103
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 103
agcgtaatac gaaagatgag 20

<210> SEQ ID NO 104
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 104
agcttcgtta ttcaagggat 20

<210> SEQ ID NO 105
<211> LENGTH: 22
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 105
gtatcataaa cattcaacaa tg 22

<210> SEQ ID NO 106
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 106
cggatctggt gtttattctc 20

<210> SEQ ID NO 107
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 107
aaacaaagtt tgatgcctc 20

<210> SEQ ID NO 108
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 108

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tcgtgctcaa acattttctc 20

<210> SEQ ID NO 109
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 109

aaaatgacgg ataatccacc 20

<210> SEQ ID NO 110
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 110

ttcaaagtct ttagcacacc 20

<210> SEQ ID NO 111
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 111

caatccatca tgggaaaatc 20

<210> SEQ ID NO 112
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 112

cttgacgac aaaatagtgt 20

<210> SEQ ID NO 113
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 113

aggacttcaa tttccatgtc 20

<210> SEQ ID NO 114
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 114

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agtgatcatct ccacaatttg 20

<210> SEQ ID NO 115
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 115

gaaaacgata agggccaatt 20

<210> SEQ ID NO 116
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 116

cgttctttaa caaacctcg 20

<210> SEQ ID NO 117
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 117

taccaaatga aacgctttaa tg 22

<210> SEQ ID NO 118
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 118

tcttcatgga aagagtctag 20

<210> SEQ ID NO 119
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 119

atggaatgag tacttttagcg 20

<210> SEQ ID NO 120
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 120

cttcatttcc gagtttttgg 20

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<210> SEQ ID NO 121
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 121

aatagaaaat cggcttctgc 20

<210> SEQ ID NO 122
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 122

tatttgatca ttggggttgc 20

<210> SEQ ID NO 123
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 123

gattgaagac atttgatgcg 20

<210> SEQ ID NO 124
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 124

tcgccactaa ctctatttac 20

<210> SEQ ID NO 125
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 125

accatttcag gtacaatgtc 20

<210> SEQ ID NO 126
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 126

cttcggaaat atcgaattcc 20

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<210> SEQ ID NO 127
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 127

ctgaaacgat accaacaatg 20

<210> SEQ ID NO 128
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 128

tttgtggttt aggcaatacc 20

<210> SEQ ID NO 129
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 129

atacaaaagt atacaacatg cc 22

<210> SEQ ID NO 130
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 130

tttccaagaa atcttcgacc 20

<210> SEQ ID NO 131
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 131

ctttactgcg aagataaagg 20

<210> SEQ ID NO 132
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
primer

<400> SEQUENCE: 132

gccactataa tctggtgttg 20

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<210> SEQ ID NO 133
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        primer

<400> SEQUENCE: 133

tcaaaactac ggctcatttg                                20
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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        primer

<400> SEQUENCE: 134

tgaacaaaag actcaatccg                                20
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What is claimed is:

1. A method for conferring boric acid tolerance to a yeast comprising:

i) introducing the DNA of SEQ ID NO: 3 operably linked to a promoter into the yeast;

25 ii) culturing the yeast in the presence of boric acid and expressing the DNA of SEQ ID NO: 3 to confer boric acid tolerance to the yeast; and
 iii) selecting the yeast in which tolerance to 80 mM boric acid is conferred.

* * * * *