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(54) **PREPARATION OF 7-DEHYDROCHOLESTEROL AND/OR THE BIOSYNTHETIC INTERMEDIATES AND/OR SECONDARY PRODUCTS THEREOF IN TRANSGENIC ORGANISMS**

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This patent is subject to a terminal disclaimer.

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(60) Continuation of application No. 14/583,418, filed on Dec. 26, 2014, which is a continuation of application No. 13/548,898, filed on Jul. 13, 2012, which is a continuation of application No. 12/607,017, filed on Oct. 27, 2009, which is a division of application No. 10/503,044, filed as application No. PCT/EP03/00592 on Jan. 22, 2003, now Pat. No. 7,608,421.

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Jan. 29, 2002 (DE) ..... 10 203 352

(51) **Int. Cl.**

**C12P 33/00** (2006.01)  
**C12P 5/00** (2006.01)  
**C12N 9/90** (2006.01)  
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**C12N 15/81** (2006.01)  
**C12N 9/04** (2006.01)  
**C12N 9/10** (2006.01)

(52) **U.S. Cl.**

CPC ..... **C12P 33/00** (2013.01); **C12N 9/001** (2013.01); **C12N 9/0004** (2013.01); **C12N 9/0006** (2013.01); **C12N 9/0071** (2013.01); **C12N 9/0073** (2013.01); **C12N 9/1007** (2013.01); **C12N 9/90** (2013.01); **C12N 15/52** (2013.01); **C12N 15/81** (2013.01); **C12Y 101/01088** (2013.01); **C12Y 103/01072** (2013.01); **C12Y 114/1307** (2013.01); **C12Y 114/13132** (2013.01); **C12Y 114/19** (2013.01); **C12Y 201/01041** (2013.01); **C12Y 503/03005** (2013.01)

(58) **Field of Classification Search**  
CPC ..... C12N 9/90; C12N 9/0004; C12P 33/00; C12P 5/007  
USPC ..... 435/252.3, 189, 134, 155, 233  
See application file for complete search history.

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(74) *Attorney, Agent, or Firm* — Eric J. Fechter

(57) **ABSTRACT**

The present invention relates to a method for preparing 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof by culturing organisms, in particular yeasts. Furthermore, the invention relates to the preparation of the nucleic acid constructs required for preparing the genetically modified organisms and to said genetically modified organisms, in particular yeasts, themselves.

**21 Claims, 10 Drawing Sheets**

**Specification includes a Sequence Listing.**

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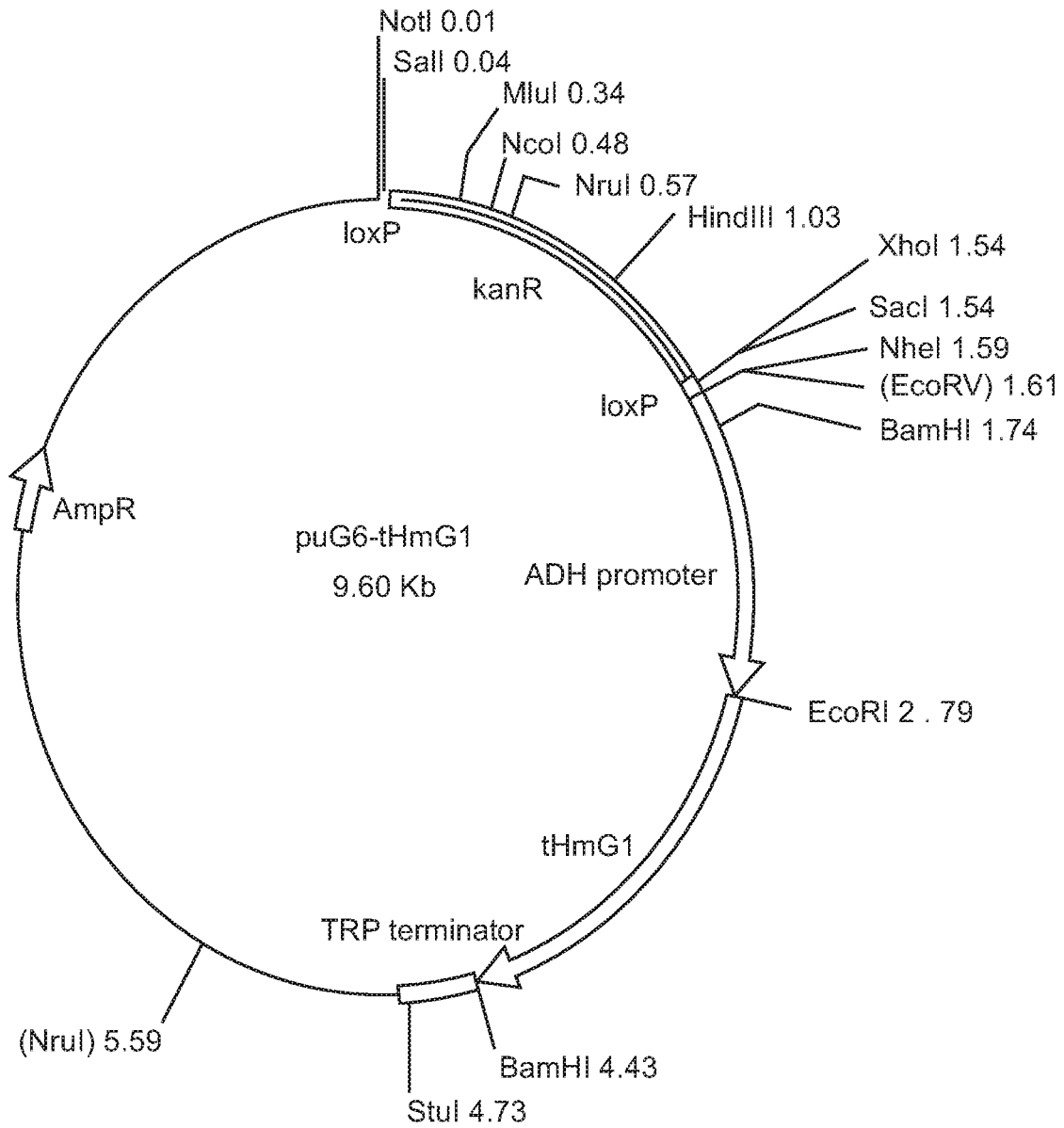


FIG. 1

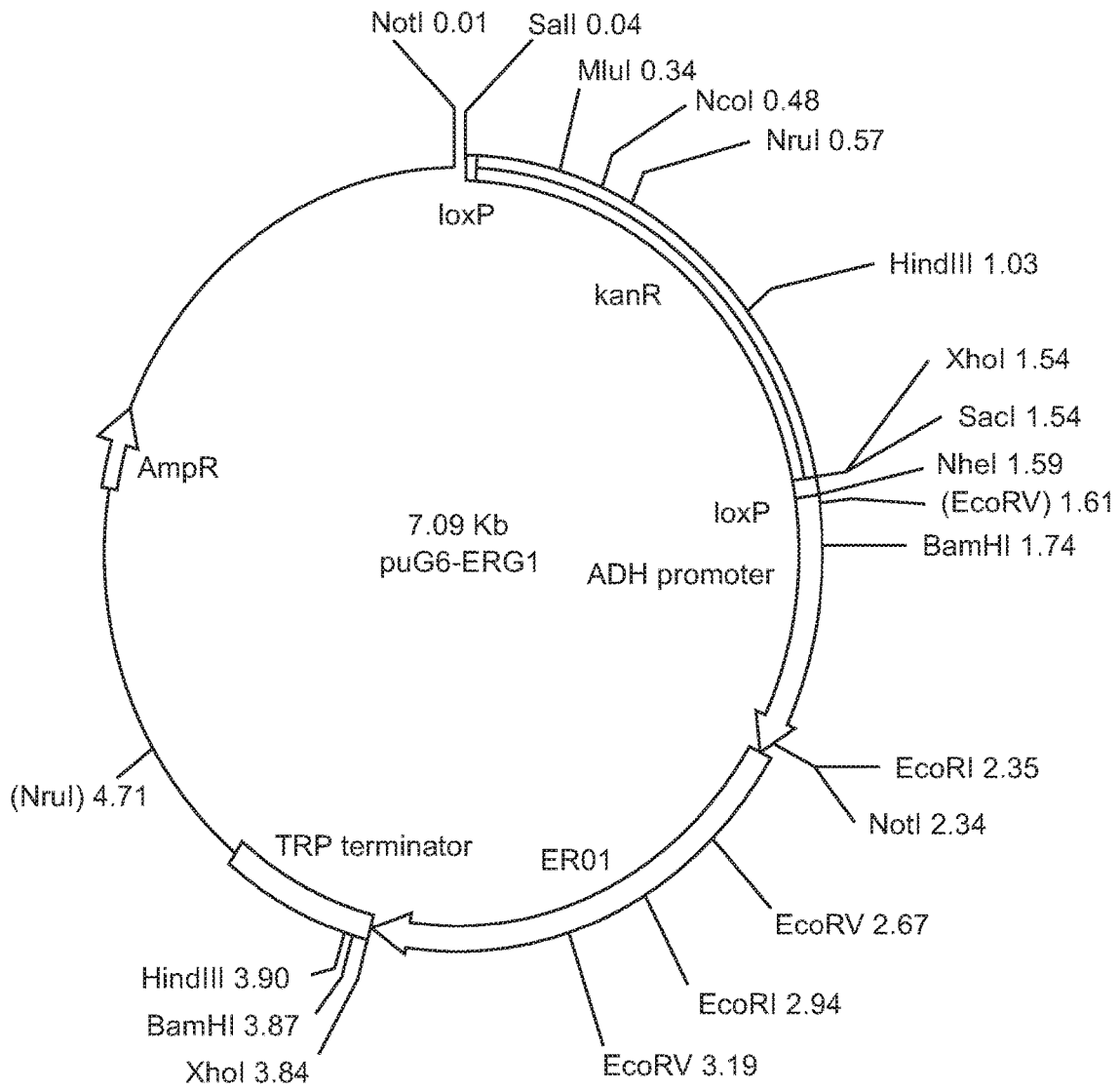
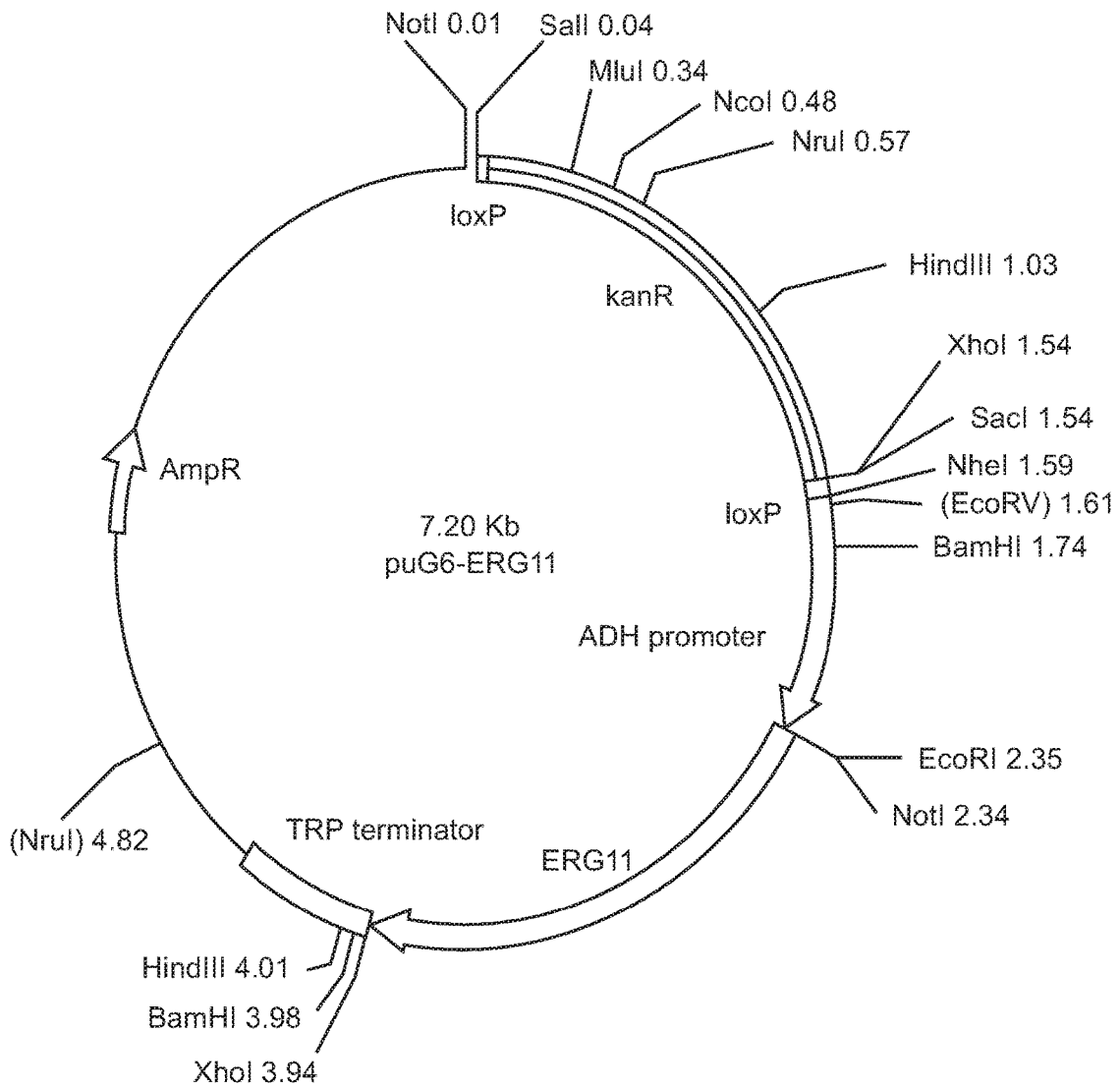


FIG. 2



**FIG. 3**

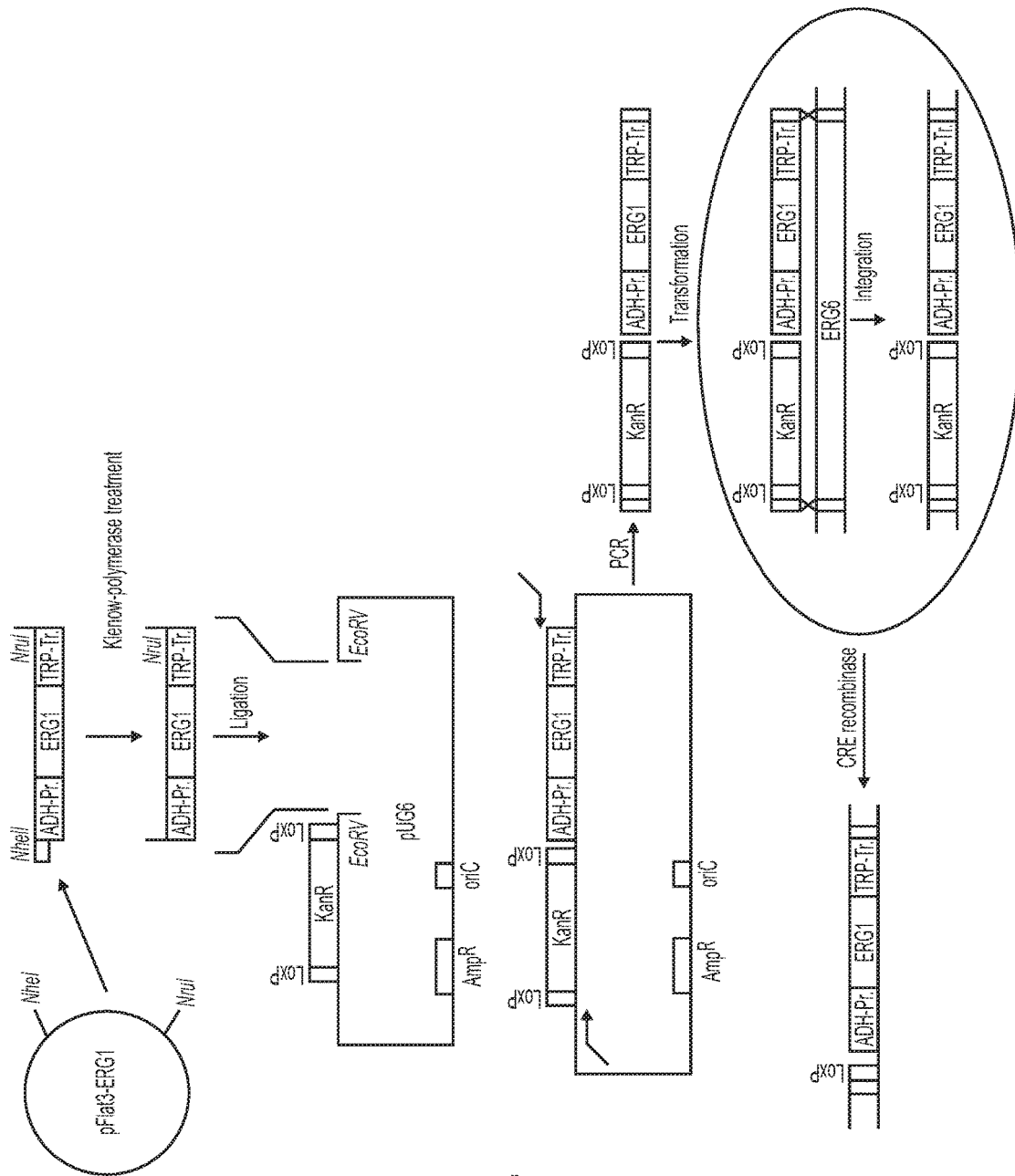


FIG. 4

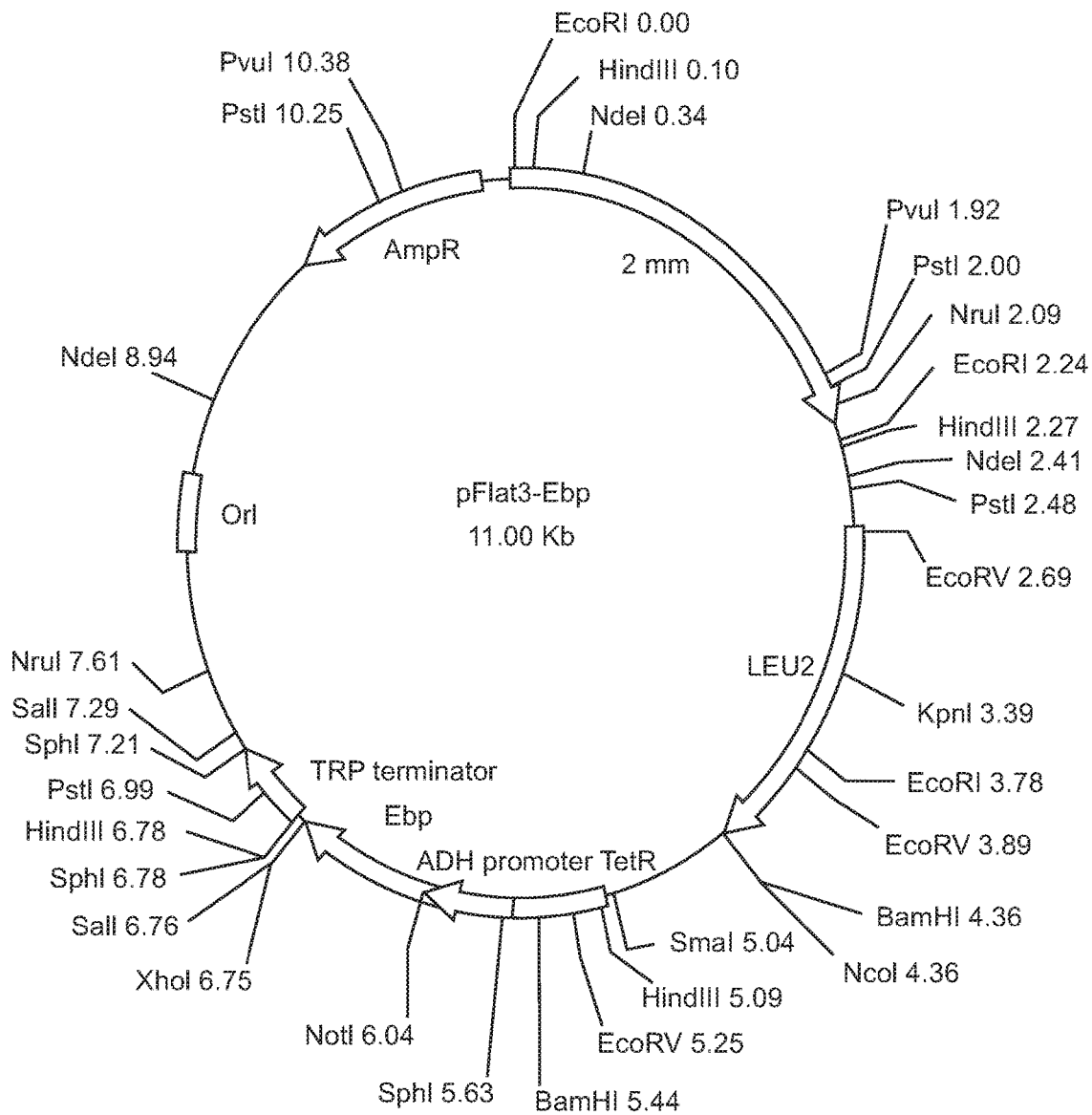


FIG. 5a

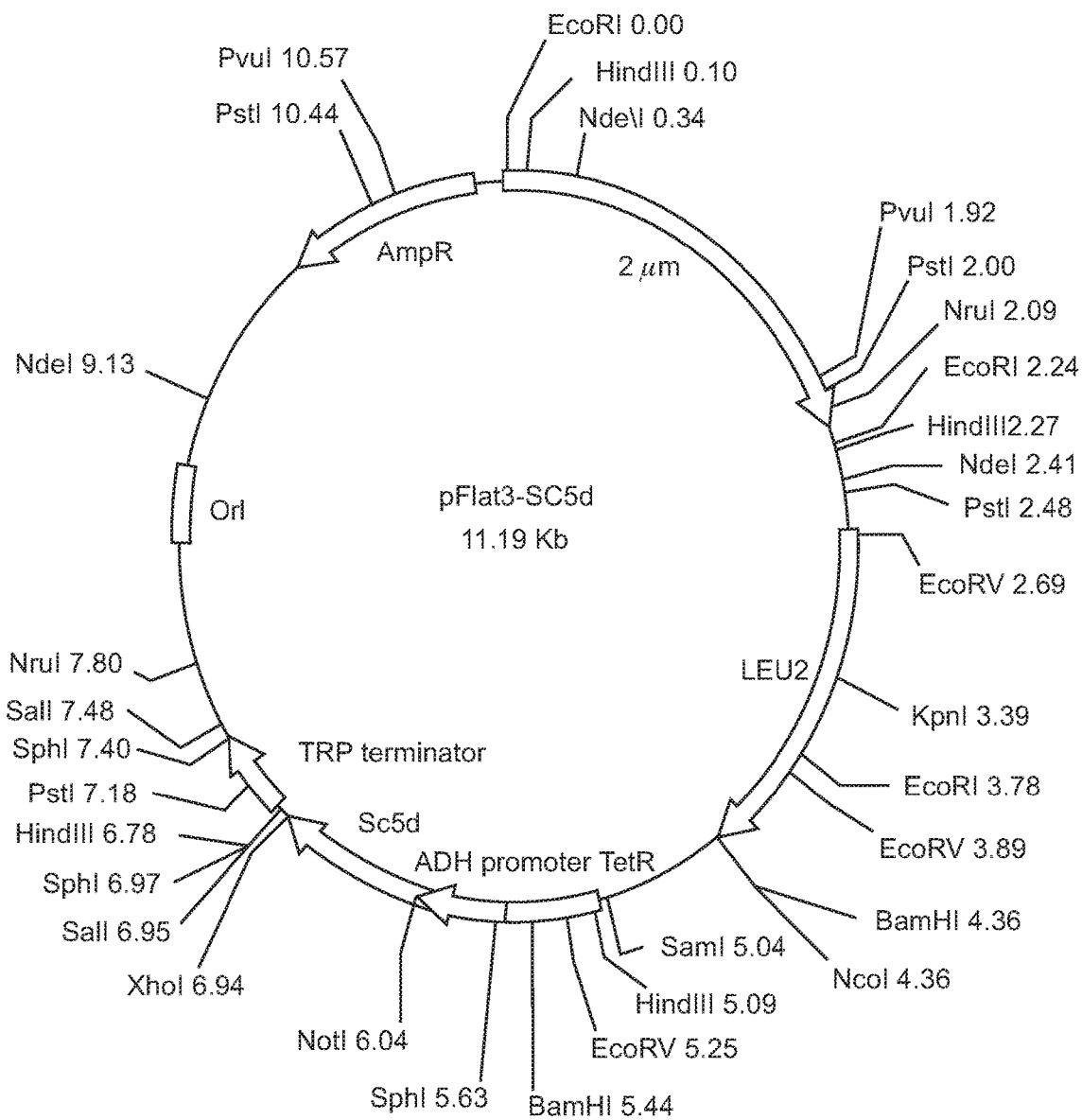
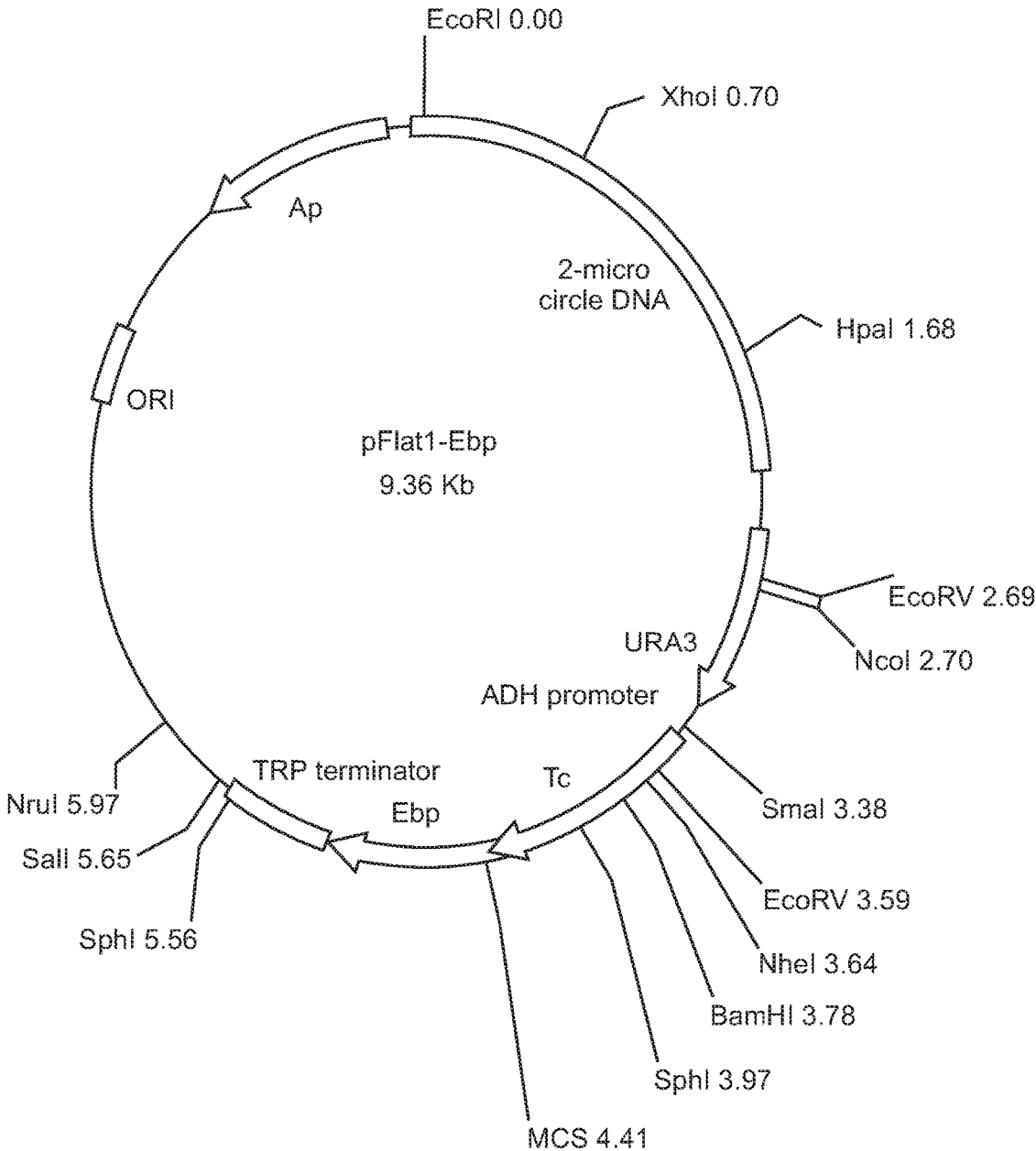


FIG. 5b



**FIG. 5c**

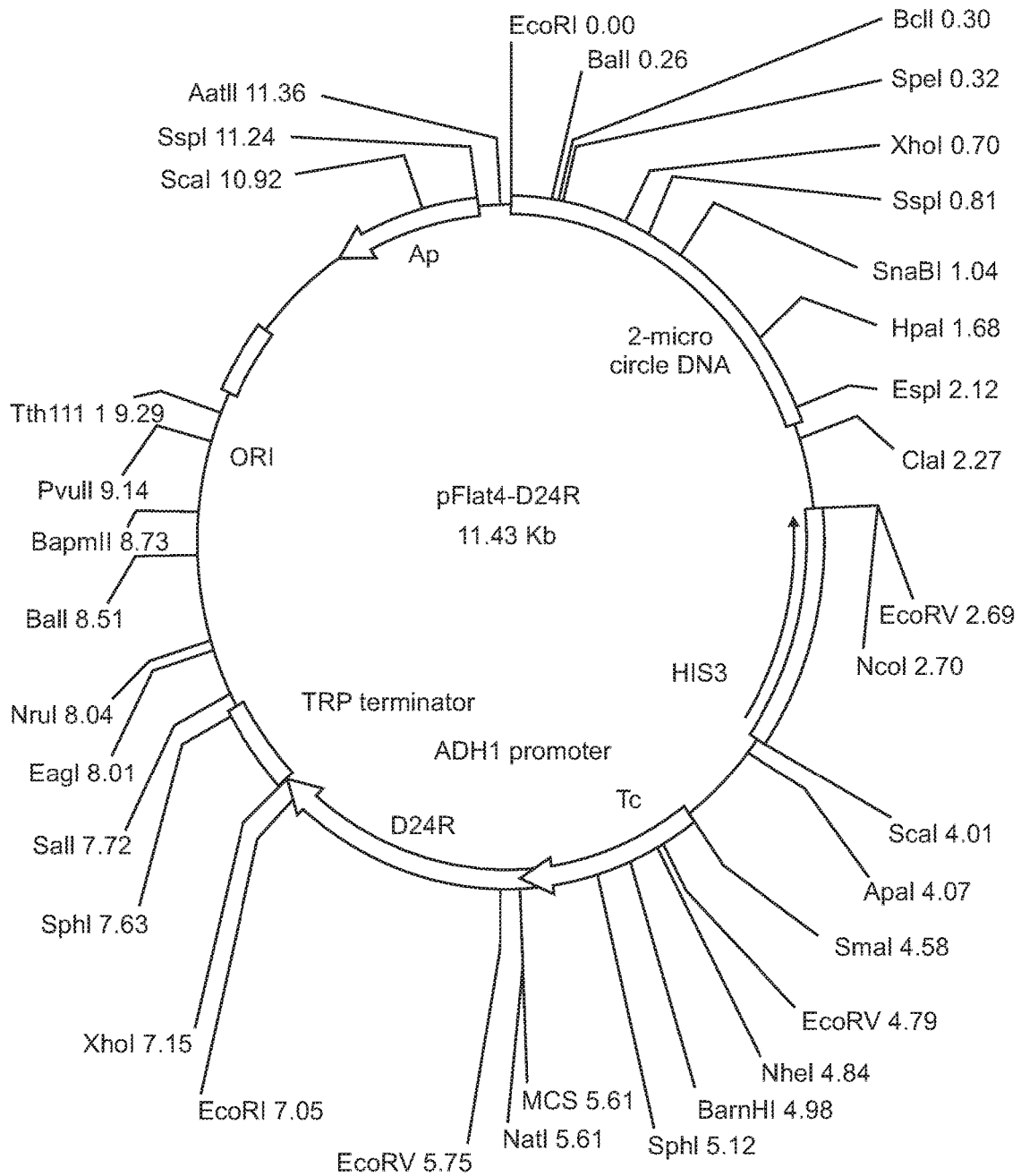


FIG. 5d

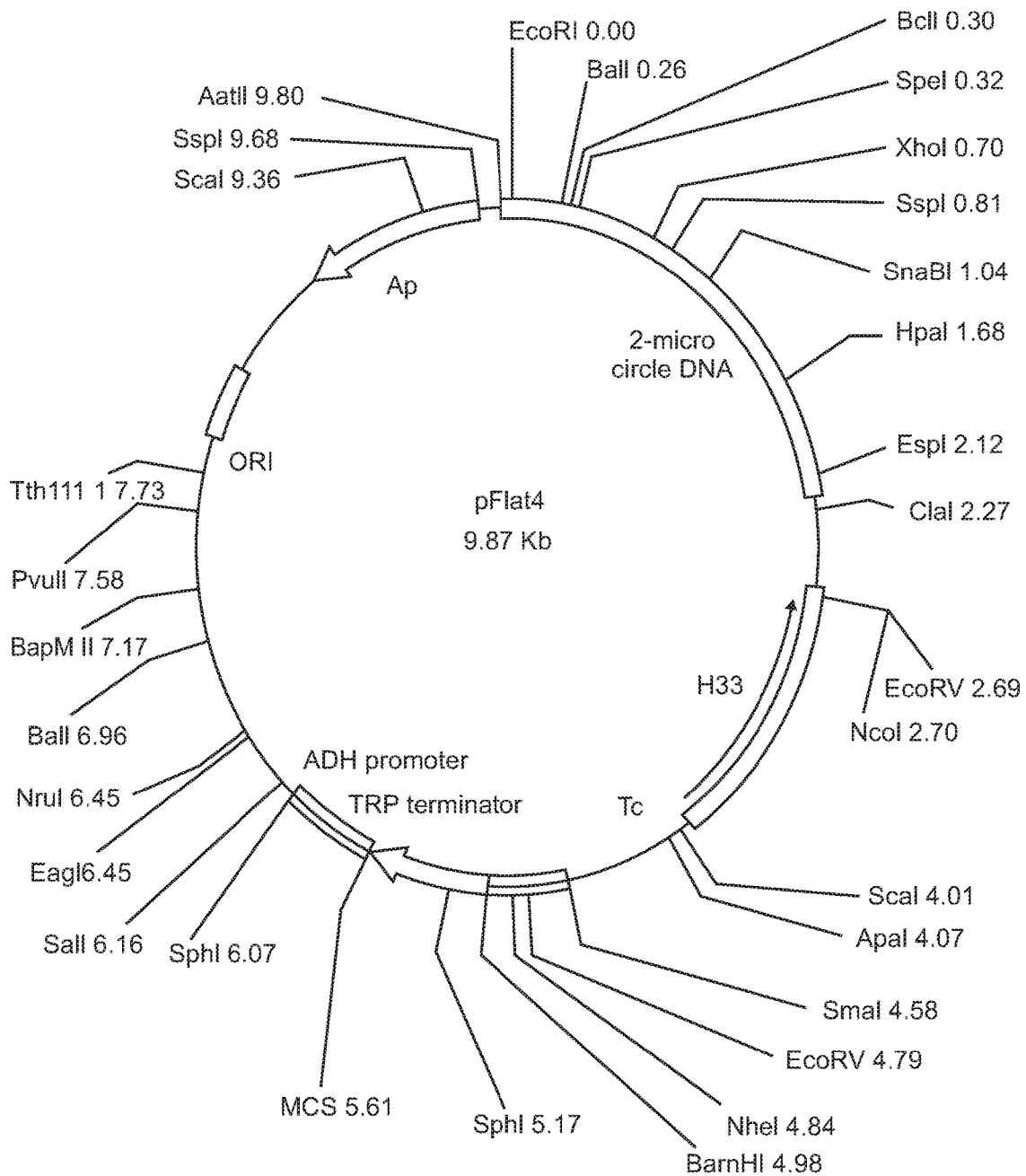


FIG. 6

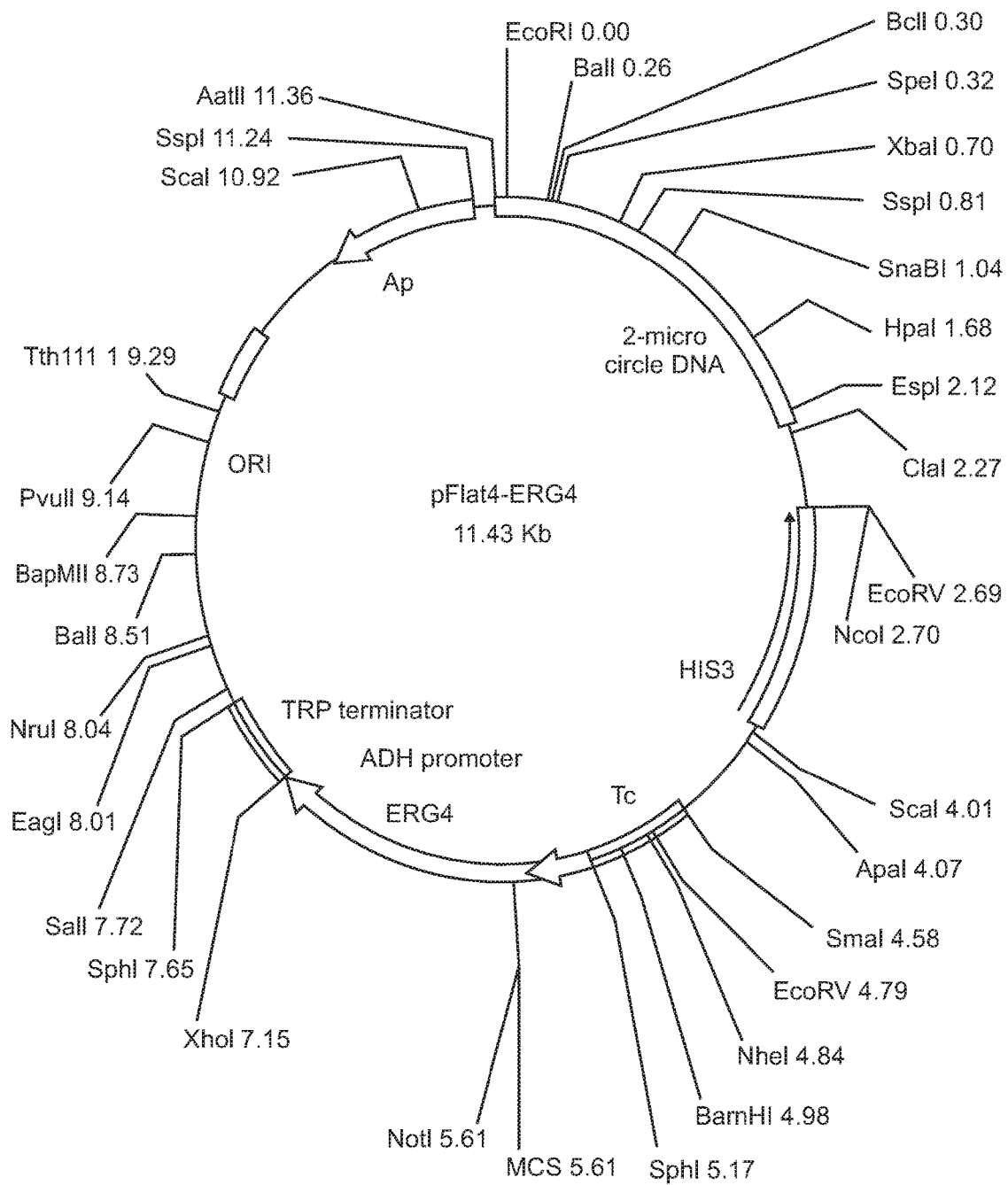


FIG. 7

**PREPARATION OF  
7-DEHYDROCHOLESTEROL AND/OR THE  
BIOSYNTHETIC INTERMEDIATES AND/OR  
SECONDARY PRODUCTS THEREOF IN  
TRANSGENIC ORGANISMS**

CROSS REFERENCE TO RELATED  
APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 14/583,418 filed Dec. 26, 2014, now abandoned, which is a continuation of U.S. patent application Ser. No. 13/548,898 filed Jul. 13, 2012, now abandoned, which is a continuation of U.S. patent application Ser. No. 12/607,017 filed Oct. 27, 2009, now abandoned, which is a divisional of U.S. patent application Ser. No. 10/503,044, now U.S. Pat. No. 7,608,421, which is a 35 U.S.C. 371 national application of PCT/EP2003/000592 filed Jan. 22, 2003, which claims priority from German application 10203352.8 filed Jan. 29, 2002.

The present invention relates to a method for preparing 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof by culturing organisms, in particular yeasts. Furthermore, the invention relates to the preparation of the nucleic acid constructs required for preparing the genetically modified organisms and to said genetically modified organisms, in particular yeasts, themselves.

7-Dehydrocholesterol, also referred to as cholesta-5,7-dienol or provitamin D<sub>3</sub>, its biosynthetic intermediates of the sterol metabolism, such as, for example, zymosterol, farnesol, geraniol, squalene, lanosterol, cholesta-5,7,24-trienol and cholesta-5,7,22,24-tetraenol and its biosynthetic secondary products of the sterol metabolism, such as vitamin D<sub>3</sub> and cholesterol, are compounds of high economic value.

7-Dehydrocholesterol is economically important especially for obtaining vitamin D<sub>3</sub> from 7-dehydrocholesterol via UV irradiation.

Squalene is used as building block for the synthesis of terpenes. It is used in hydrogenated form as squalane in dermatology and cosmetics and also in various derivatives as an ingredient of skin and haircare products.

Furthermore, sterols such as zymosterol and lanosterol can be utilized economically, lanosterol being pivotal as crude and synthesis material for the chemical synthesis of saponins and steroid hormones. Due to its good skin penetration and spreading properties, lanosterol serves as emulsifier and active substance in skin creams.

An economic method for preparing 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof is therefore of great importance.

Particularly economic methods are biotechnological methods utilizing organisms which have been optimized by genetic modification and which produce 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof.

While the sterol metabolism in bacteria, fungi, yeasts and some insects essentially goes from zymosterol via fecosterol, episterol, ergosta-5,7-dienol and ergosta-5,7,22,24-tetraen-3 $\beta$ -ol to ergosterol (provitamin D<sub>2</sub>), the sterol metabolism in mammals essentially goes from zymosterol via cholesta-7,24-dienol, lanosterol to 7-dehydrocholesterol (provitamin D<sub>3</sub>).

7-Dehydrocholesterol (provitamin D<sub>3</sub>) is converted to cholesterol by 7-dehydrocholesterol reductase and chole-

terol is converted to steroid hormones, corticoids and bile acids, such as progesterone, testosterone, estradiol, aldosterone, cortisone and cholate.

Some genes of the 7-dehydrocholesterol metabolism in mammals are known and have been cloned, such as, for example,

nucleic acids encoding a human  $\Delta 8$ - $\Delta 7$ -isomerase (also referred to as emopamil-binding protein (EBP)), ACCES-  
SION NM\_006579, and a murine  $\Delta 8$ - $\Delta 7$ -isomerase (Braver-  
man, N. et al., (1999): Mutations in the gene encoding  
3 $\beta$ -hydroxysteroid- $\Delta 8$ , $\Delta 7$ -isomerase cause X-linked domi-  
nant Conradi-Hunermann syndrome. Nat. Genet. 22(3),291-  
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nucleic acids encoding a human  $\Delta 5$ -desaturase (also  
referred to as sterol C5-desaturase), ACCESSION  
AB016247 and a murine  $\Delta 5$ -desaturase (Nishi, S. et al.,  
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Acta 1490(1-2),106-108),

nucleic acids encoding a human  $\Delta 24$ -reductase (also  
referred to as 24-dehydrocholesterol reductase (DHCR24)),  
ACCESSION NM\_014762 and a murine  $\Delta 24$ -reductase  
(Waterham, H. R. et al. (2001): Mutations in the 3 $\beta$ -hydrox-  
ysterol  $\Delta 24$ -reductase gene cause desmosterolosis, an auto-  
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nucleic acids encoding a human sterol acyltransferase  
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murine sterol acyltransferase (Uelmen, P. J.: Tissue-specific  
expression and cholesterol regulation of acylcoenzyme  
A:cholesterol acyltransferase (ACAT) in mice. Molecular  
cloning of mouse ACAT cDNA, chromosomal localization,  
and regulation of ACAT in vivo and in vitro, J. Biol. Chem.  
1995 Nov. 3; 270(44):26192-201).

The genes of the ergosterol metabolism in yeast are  
essentially known and have been cloned, such as, for  
example,

nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase (ERG2) (Ash-  
man, W. H. et al. (1991): Cloning and disruption of the yeast  
C-8 sterol isomerase gene. Lipids. August; 26(8):628-32.),

Nucleic acids encoding a  $\Delta 5$ -desaturase (ERG3)  
(Arthington, B. A. et al. (1991): Cloning, disruption and  
sequence of the gene encoding yeast C-5 sterol desaturase.  
Gene. June 15; 102(1):39-44.),

nucleic acids encoding a  $\Delta 24$ -reductase (ERG 4) (Lai, M.  
H. et al., (1994): The identification of a gene family in the  
*Saccharomyces cerevisiae* ergosterol biosynthesis pathway.  
Gene. March 11; 140(1):41-9.),

nucleic acids encoding an HMG-CoA reductase (HMG)  
(Bason M. E. et al, (1988) Structural and functional conser-  
vation between yeast and human 3-hydroxy-3-methylglu-  
taryl coenzyme A reductases, the rate-limiting enzyme of  
sterol biosynthesis. Mol Cell Biol 8:3797-3808,

nucleic acids encoding a truncated HMG-CoA reductase  
(t-HMG) (Polakowski T, Stahl U, Lang C. (1998)  
Overexpression of a cytosolic hydroxymethylglutaryl-  
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Appl Microbiol Biotechnol. January; 49(1):66-71,

nucleic acids encoding a lanosterol C14-demethylase  
(ERG11) (Kalb V F, Loper J C, Woods C W, Sutter  
T R (1986) Isolation of a cytochrome P-450 structural gene  
from *Saccharomyces cerevisiae*. Gene 45(3):237-45,

nucleic acids encoding a squalene synthetase (ERG9)  
(Jennings, S. M., (1991): Molecular cloning and character-

ization of the yeast gene for squalene synthetase. Proc Natl Acad Sci USA. July 15; 88(14):6038-42),

nucleic acids encoding a sterol acyltransferase (SAT1) and (SAT2) (Yang, H.: Sterol esterification in yeast: a two-gene process. Science. 1996 May 31; 272(5266):1353-6.) and a further sterol acyltransferase (J. Biol. Chem. 1996, Sep. 27; 271(39):24157-63), nucleic acids encoding a squalene epoxidase (ERG1) (Jandrositz, A., et al (1991) The gene encoding squalene epoxidase from *Saccharomyces cerevisiae*: cloning and characterization. Gene 107:155-160),

nucleic acids encoding a C24-methyltransferase (ERG6) (Hardwick, K. G. et al.: SED6 is identical to ERG6, and encodes a putative methyltransferase required for ergosterol synthesis. Yeast. February; 10(2):265-9) and

nucleic acids encoding a  $\Delta 22$ -desaturase (ERG5) (Skaggs, B. A. et al.: Cloning and characterization of the *Saccharomyces cerevisiae* C-22 sterol desaturase gene, encoding a second cytochrome P-450 involved in ergosterol biosynthesis. Gene. 1996 Feb. 22; 169(1):105-9.).

Furthermore, methods are known whose aim is an increase in the content of specific intermediates and final products of the sterol metabolism in yeasts and fungi.

EP 486 290 discloses that the content of squalene and other specific sterols such as, for example, zymosterol, in yeasts can be increased by increasing the rate of expression of HMG-CoA reductase and simultaneously interrupting the metabolic pathway of zymosterol C24-methyltransferase (ERG6) and ergosta-5,7,24(28)-trienol 22-dehydrogenase (ERG5).

From T. Polakowski, Molekularbiologische Beeinflussung des Ergosterolstoffwechsels der Hefe *Saccharomyces cerevisiae* [Influencing the ergosterol metabolism of the yeast *Saccharomyces cerevisiae* by molecular biological means], Shaker Verlag Aachen, 1999, pages 59 to 66, it is known that increasing the rate of expression of HMG-CoA reductase alone, without interrupting the downstream metabolic flow as in EP 486 290, merely leads to a slight increase in the content of early sterols and of squalene, while the content of later sterols such as ergosterol does not change substantially and, in the case of ergosterol, even tententially decreases.

WO 99/16886 describes a method for preparing ergosterol in yeasts which overexpress a combination of genes tHMG, ERG9, SAT1 and ERG1.

Tainaka et al., J. Ferment. Bioeng. 1995, 79, 64-66, further describe that overexpression of ERG11 (lanosterol C14-demethylase) leads to accumulation of 4,4-dimethylzymosterol but not of ergosterol. Compared to the wild type, the transformant showed an increase in the zymosterol content by a factor of from 1.1 to 1.47, depending on fermentation conditions.

Avruch et al, Can. J. Biochem 1976, 54(7), 657-665 and Xu et al, Biochem. Biophys. Res. Commun. 1988, 155(1), 509-517 describe that it is possible to detect, apart from zymosterol, also traces of cholesterol by specifically inhibiting C24-methyltransferase and also by a mutation in the gene locus *erg6* in *S. cerevisiae*.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the vector denoted pUG6-tHMG.

FIG. 2 illustrates the vector denoted pUG6-ERG1.

FIG. 3 illustrates the vector denoted pUG6-ERG11.

FIG. 4 illustrates the vectors denoted pFlat3 and pFlat1.

FIG. 5a illustrates the vectors denoted pFlat1-EBP and pFlat3-EBP.

FIG. 5b illustrates the vector denoted pFlat3-SC5D.

FIG. 5c illustrates the vector denoted pFlat1-Ebp.

FIG. 5d illustrates the vector denoted pFlat4-D24R.

FIG. 6 illustrates the vector denoted pFlat4.

FIG. 7 illustrates the vector denoted pFlat4-ERG4.

It is an object of the present invention to provide a method for preparing 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof, which method has advantageous properties such as a higher product yield.

We have found that this object is achieved by a method for preparing 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof, in which organisms are cultured which have, compared to the wild type, an increased activity of at least one of the activities selected from the group consisting of  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity and  $\Delta 24$ -reductase activity.

An increased activity compared to the wild type means, in the case of the starting organism not having said activity, that said activity is caused. In the case of the starting organism already having said activity, an increased activity compared to the wild type means an activity increased by a percentage.

$\Delta 8$ - $\Delta 7$ -Isomerase activity means the enzyme activity of a  $\Delta 8$ - $\Delta 7$ -isomerase, also referred to as  $\Delta 8$ - $\Delta 7$ -sterol isomerase.

A  $\Delta 8$ - $\Delta 7$ -isomerase means a protein which has the enzyme activity of converting zymosterol to cholesta-7,24-dienol.

Accordingly,  $\Delta 8$ - $\Delta 7$ -isomerase activity means the amount of zymosterol converted or the amount of cholesta-7,24-dienol formed by the protein  $\Delta 8$ - $\Delta 7$ -isomerase in a particular time.

In the case of an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity compared to the wild type, thus the amount of zymosterol converted or the amount of cholesta-7,24-dienol formed by the protein  $\Delta 8$ - $\Delta 7$ -isomerase in a particular time is increased in comparison with the wild type.

This increase in  $\Delta 8$ - $\Delta 7$ -isomerase activity is preferably at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably at least 100%, more preferably at least 300%, still more preferably at least 500%, in particular at least 600%, of the  $\Delta 8$ - $\Delta 7$ -isomerase activity of the wild type.

$\Delta 5$ -Desaturase activity means the enzyme activity of a  $\Delta 5$ -desaturase, also referred to as lathosterol 5-desaturase or sterol C5-desaturase.

A  $\Delta 5$ -desaturase means a protein which has the enzyme activity of converting cholesta-7,24-dienol to cholesta-5,7,24-trienol.

Accordingly,  $\Delta 5$ -desaturase activity means the amount of cholesta-7,24-dienol converted or the amount of cholesta-5,7,24-trienol formed by the protein  $\Delta 5$ -desaturase in a particular time.

In the case of an increased  $\Delta 5$ -desaturase activity compared to the wild type, thus the amount of cholesta-7,24-dienol converted or the amount of cholesta-5,7,24-trienol formed by the protein  $\Delta 5$ -desaturase in a particular time is increased in comparison with the wild type.

This increase in  $\Delta 5$ -desaturase activity is preferably at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably at least 100%, more preferably at least 300%, still more preferably at least 500%, in particular at least 600%, of the  $\Delta 5$ -desaturase activity of the wild type.

$\Delta 24$ -Reductase activity means the enzyme activity of a  $\Delta 24$ -reductase, also referred to as 24-dehydrocholesterol reductase.

A  $\Delta 24$ -reductase means a protein which has the enzyme activity of converting the double bond between C24 and C25 of cholesterol compounds to a single bond, for example converting cholesta-5,7,24-trienol to 7-dehydrocholesterol or zymosterol to lathosterol or cholesta-7,24-dienol to cholesta-7-enol.

Accordingly,  $\Delta 24$ -reductase activity means preferably the amount of cholesta-5,7,24-trienol converted or the amount of 7-dehydrocholesterol formed by the protein  $\Delta 24$ -reductase in a particular time.

In the case of an increased  $\Delta 24$ -reductase activity compared to the wild type, thus the amount of cholesta-5,7,24-trienol converted or the amount of 7-dehydrocholesterol formed by the protein  $\Delta 24$ -reductase in a particular time is increased in comparison with the wild type.

This increase in  $\Delta 24$ -reductase activity is preferably at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably at least 100%, more preferably at least 300%, still more preferably at least 500%, in particular at least 600%, of the  $\Delta 24$ -reductase activity of the wild type.

A wild type means the corresponding not genetically modified starting organism. Preferably and, in particular in those cases in which the organism or the wild type cannot be classified unambiguously, wild type means a reference organism for increasing the  $\Delta 8$ - $\Delta 7$ -isomerase activity, increasing the  $\Delta 5$ -desaturase activity, increasing the  $\Delta 24$ -reductase activity, reducing the C24-methyltransferase activity described below, reducing the  $\Delta 22$ -desaturase activity described below, increasing the HMG-CoA-reductase activity described below, increasing the lanosterol C14-demethylase activity described below, increasing the squalene-epoxidase activity described below, increasing the squalene-synthetase activity described below and increasing the sterol-acyltransferase activity described below and also for increasing the content of 7-dehydrocholesterol and/or of the biosynthetic intermediates and/or secondary products thereof. This reference organism is preferably the yeast strain *Saccharomyces cerevisiae* AH22.

In the method of the invention, organisms are cultured which, compared to the wild type, have an increased activity of at least one of the activities selected from the group consisting of  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity and  $\Delta 24$ -reductase activity.

In a preferred embodiment, organisms are cultured which, compared to the wild type, have an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity or  $\Delta 24$ -reductase activity.

In a particularly preferred embodiment of the method of the invention, the organisms have, compared to the wild type, an increased activity of at least two of the activities selected from the group consisting of  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity and  $\Delta 24$ -reductase activity.

Particularly preferred combinations are  $\Delta 8$ - $\Delta 7$ -isomerase activity and  $\Delta 5$ -desaturase activity, increased in comparison to the wild type,  $\Delta 8$ - $\Delta 7$ -isomerase activity and  $\Delta 24$ -reductase activity, increased in comparison to the wild type, and  $\Delta 5$ -desaturase activity and  $\Delta 24$ -reductase activity, increased in comparison with the wild type.

In a very particularly preferred embodiment of the method of the invention, the organisms have, compared to the wild type, an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity and  $\Delta 24$ -reductase activity.

The  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity and  $\Delta 24$ -reductase activity and also the HMG-CoA-reductase activity, lanosterol C14-demethylase activity, squalene-epoxidase activity, squalene-synthetase activity and sterol-

acyltransferase activity, which are described below, may be increased independently of one another in various ways, for example by eliminating inhibiting regulatory mechanisms at the expression and protein level or by increasing, compared to the wild type, gene expression of the corresponding nucleic acids, i.e. nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase,  $\Delta 5$ -desaturase,  $\Delta 24$ -reductase, HMG-CoA reductase, lanosterol C14-demethylase, squalene epoxidase, squalene synthetase or sterol acyltransferase.

Likewise, gene expression of the corresponding nucleic acid may be increased compared to the wild type in various ways, for example by inducing the appropriate genes by activators, i.e. by inducing the  $\Delta 8$ - $\Delta 7$ -isomerase gene, the  $\Delta 5$ -desaturase gene, the  $\Delta 24$ -reductase gene, the HMG-CoA-reductase gene, the lanosterol C14-demethylase gene, the squalene-epoxidase gene, the squalene-synthetase gene or the sterol-acyltransferase gene by activators, or by introducing one or more gene copies of the appropriate nucleic acids, i.e. by introducing one or more nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase,  $\Delta 5$ -desaturase,  $\Delta 24$ -reductase, HMG-CoA reductase, lanosterol C14-demethylase, squalene epoxidase, squalene synthetase or sterol acyltransferase into the organism.

Increasing the gene expression of a nucleic acid encoding a  $\Delta 8$ - $\Delta 7$ -isomerase,  $\Delta 5$ -desaturase,  $\Delta 24$ -reductase, HMG-CoA reductase, lanosterol C14-demethylase, squalene epoxidase, squalene synthetase or sterol acyltransferase means according to the invention also manipulation of the expression of endogenous  $\Delta 8$ - $\Delta 7$ -isomerases,  $\Delta 5$ -desaturases,  $\Delta 24$ -reductases, HMG-CoA reductases, lanosterol C14-demethylases, squalene epoxidases, squalene synthetases or sterol acyltransferases, which are intrinsic to the organism, in particular to the yeasts.

This may be achieved, for example, by modifying the promoter DNA sequence of genes coding for  $\Delta 8$ - $\Delta 7$ -isomerase,  $\Delta 5$ -desaturase,  $\Delta 24$ -reductase, HMG-CoA reductase, lanosterol C14-demethylase, squalene epoxidase, squalene synthetase or sterol acyltransferase. Such a modification which causes an increased rate of expression of the relevant gene may be carried out, for example, by deleting or inserting DNA sequences.

As described above, it is possible to modify expression of the endogenous  $\Delta 8$ - $\Delta 7$ -isomerase,  $\Delta 5$ -desaturase,  $\Delta 24$ -reductase, HMG-CoA reductase, lanosterol C14-demethylase, squalene epoxidase, squalene synthetase or sterol acyltransferase by applying exogenous stimuli. This may be carried out using particular physiological conditions, i.e. by applying foreign substances.

Furthermore, a modified or increased expression of endogenous  $\Delta 8$ - $\Delta 7$ -isomerase,  $\Delta 5$ -desaturase,  $\Delta 24$ -reductase, HMG-CoA reductase, lanosterol C14-demethylase, squalene epoxidase, squalene synthetase or sterol acyltransferase genes may be achieved by interaction of a regulatory protein which is not present in the untransformed organism with the promoter of said genes.

A regulator of this type may be a chimeric protein which consists of a DNA-binding domain and a transcriptional activator domain, as described, for example, in WO 96/06166.

In a preferred embodiment, the  $\Delta 8$ - $\Delta 7$ -isomerase activity is increased compared to the wild type by increasing the gene expression of a nucleic acid encoding a  $\Delta 8$ - $\Delta 7$ -isomerase.

In a further preferred embodiment, gene expression of a nucleic acid encoding a  $\Delta 8$ - $\Delta 7$ -isomerase is increased by introducing into the organism one or more nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase.

For this purpose, it is possible to use in principle any  $\Delta 8$ - $\Delta 7$ -isomerase gene, i.e. any nucleic acid encoding a  $\Delta 8$ - $\Delta 7$ -isomerase.

In the case of genomic  $\Delta 8$ - $\Delta 7$ -isomerase nucleic acid sequences from eukaryotic sources, which contain introns, preferably already processed nucleic acid sequences such as the corresponding cDNAs are to be used, if the host organism is unable to or cannot be enabled to express the appropriate  $\Delta 8$ - $\Delta 7$ -isomerase.

Examples of  $\Delta 8$ - $\Delta 7$ -isomerase genes are nucleic acids encoding a murine  $\Delta 8$ - $\Delta 7$ -isomerase (nucleic acid: Seq. ID. No. 1, protein: Seq. ID. No. 2) or a human  $\Delta 8$ - $\Delta 7$ -isomerase (nucleic acid: Seq. ID. No. 3, protein: Seq. ID. No. 4) (Braverman, N. et al., (1999): Mutations in the gene encoding

$3\beta$ -hydroxysteroid- $\Delta 8$ , $\Delta 7$ -isomerase cause X-linked dominant Conradi-Hunermann syndrome, Nat. Genet. 22(3), 291-294), or else nucleic acids encoding proteins which have the activity of a  $\Delta 8$ - $\Delta 7$ -isomerase, for example due to a broad substrate specificity, such as, for example, nucleic acids encoding a C8-isomerase *Saccharomyces cerevisiae* (ERG2) (Nucleic acid: Seq. ID. No. 5, protein: Seq. ID. No. 6) (Ashman, W. H. et al. (1991): Cloning and disruption of the yeast C-8 sterol isomerase gene. Lipids. August; 26(8): 628-32).

In this preferred embodiment, thus at least one further  $\Delta 8$ - $\Delta 7$ -isomerase gene is present in the transgenic organisms of the invention, compared to the wild type.

The number of  $\Delta 8$ - $\Delta 7$ -isomerase genes in the transgenic organisms of the invention is at least two, preferably more than two, particularly preferably more than three and very particularly preferably more than five.

All of the nucleic acids mentioned in the description may be, for example, an RNA sequence, DNA sequence or cDNA sequence.

Preferred  $\Delta 8$ - $\Delta 7$ -isomerase genes are nucleic acids encoding proteins which have a high substrate specificity for zymosterol. Therefore, preference is given in particular to  $\Delta 8$ - $\Delta 7$ -isomerase genes and to the corresponding  $\Delta 8$ - $\Delta 7$ -isomerases of mammals and to the functional equivalents thereof.

Accordingly, preference is given to using in the above-described method nucleic acids which encode proteins comprising the amino acid sequence SEQ.

ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which is at least 30%, preferably at least 50%, more preferably at least 70%, still more preferably at least 90%, most preferably at least 95%, identical at the amino acid level with the sequence SEQ. ID. NO. 2, and having the enzyme property of a  $\Delta 8$ - $\Delta 7$ -isomerase.

The sequence SEQ. ID. NO. 2 represents the amino acid sequence of *Mus musculus*  $\Delta 8$ - $\Delta 7$ -isomerase.

Further examples of  $\Delta 8$ - $\Delta 7$ -isomerases and  $\Delta 8$ - $\Delta 7$ -isomerase genes can readily be found, for example, for various organisms whose genomic sequence is known by comparing the homology of the amino acid sequences or the corresponding backtranslated nucleic acid sequences from databases with the Seq. ID. NO. 2.

The Homo sapiens  $\Delta 8$ - $\Delta 7$ -isomerase (Seq. ID. No. 4), for example, is 74% identical to the *Mus musculus*  $\Delta 8$ - $\Delta 7$ -isomerase (Seq. ID. No. 2).

Further examples of  $\Delta 8$ - $\Delta 7$ -isomerases and  $\Delta 8$ - $\Delta 7$ -isomerase genes can furthermore readily be found for various organisms whose genomic sequence is unknown, for

example starting from the sequence SEQ. ID. No. 1, by hybridization techniques and PCR techniques in a manner known per se.

The term "substitution" means in the description the replacement of one or more amino acids by one or more amino acids. Preference is given to carrying out "conservative" replacements in which the replacing amino acid has a similar property to that of the original amino acid, for example replacement of Glu by Asp, Gln by Asn, Val by Ile, Leu by Ile, Ser by Thr.

A deletion is the replacement of an amino acid by a direct bond. Preferred positions for deletions are the polypeptide termini and the linkages between the individual protein domains.

Insertions are introductions of amino acids into the polypeptide chain, with a direct bond formally being replaced by one or more amino acids.

Identity between two proteins means identity of the amino acids over the in each case entire length of the protein, in particular the identity which is calculated by comparison with the aid of the Lasergene software from DNASTAR Inc., Madison, Wis. (USA), using the Clustal method (Higgins D G, Sharp P M. Fast and sensitive multiple sequence alignments on a microcomputer. Comput Appl. Biosci. 1989 Apr. 5(2):151-1) and setting the following parameters: TABLE-US-00001 Multiple alignment parameter: Gap penalty 10 Gap length penalty 10 Pairwise alignment parameter: K-tuple 1 Gap penalty 3 Window 5 Diagonals saved 5

Accordingly, a protein which is at least 30% identical at the amino acid level with the sequence SEQ. ID. NO. 2 means a protein which is at least 30% identical when comparing its sequence with the sequence SEQ. ID. NO. 2, in particular according to the above program algorithm with the above set of parameters.

In a further, particularly preferred embodiment, the  $\Delta 8$ - $\Delta 7$ -isomerase activity is increased by introducing into organisms nucleic acids which encode proteins comprising the amino acid sequence of *Mus musculus*  $\Delta 8$ - $\Delta 7$ -isomerase (SEQ. ID. NO. 2).

Suitable nucleic acid sequences can be obtained, for example, by backtranslating the polypeptide sequence according to the genetic code.

Preference is given to using for this those codons which are frequently used according to the organism-specific codon usage. Said codon usage can readily be determined on the basis of computer analyses of other known genes of the organisms in question.

If the protein is to be expressed in yeast, for example, it is often advantageous to use the codon usage of yeast for backtranslation.

In a particularly preferred embodiment, a nucleic acid comprising the sequence SEQ. ID. NO. 1 is introduced into the organism.

The sequence SEQ. ID. NO. 1 represents the *Mus musculus* cDNA which encodes the  $\Delta 8$ - $\Delta 7$ -isomerase of the sequence SEQ ID NO. 2.

Furthermore, all of the  $\Delta 8$ - $\Delta 7$ -isomerase genes mentioned above can be prepared in a manner known per se by chemical synthesis from the nucleotide building blocks, for example by fragment condensation of individual overlapping complementary nucleic acid building blocks of the double helix. The chemical synthesis of oligonucleotides may be carried out, for example, in a known manner according to the phosphoramidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). Annealing of synthetic oligonucleotides and filling-in of gaps with the aid of the Klenow fragment of DNA polymerase and the

ligation reactions and also general cloning methods are described in Sambrook et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press.

In a preferred embodiment, the  $\Delta 5$ -desaturase activity is increased compared to the wild type by increasing the gene expression of a nucleic acid encoding a  $\Delta 5$ -desaturase.

In a further preferred embodiment, gene expression of a nucleic acid encoding a  $\Delta 5$ -desaturase is increased by introducing into the organism one or more nucleic acids encoding a  $\Delta 5$ -desaturase.

For this purpose, it is possible to use in principle any  $\Delta 5$ -desaturase gene, i.e. any nucleic acid encoding a  $\Delta 5$ -desaturase.

In the case of genomic  $\Delta 5$ -desaturase nucleic acid sequences from eukaryotic sources, which contain introns, preferably already processed nucleic acid sequences such as the corresponding cDNAs are to be used, if the host organism is unable to or cannot be enabled to express the appropriate  $\Delta 5$ -desaturase.

Examples of  $\Delta 5$ -desaturase genes are nucleic acids encoding a murine  $\Delta 5$ -desaturase (nucleic acid: Seq. ID. No. 7, protein: Seq. ID. No. 8) or a human  $\Delta 5$ -desaturase (nucleic acid: Seq. ID. No. 9, protein: Seq. ID. No. 10) (Nishi, S. et al., (2000): cDNA cloning of the mammalian sterol C5-desaturase and the expression in yeast mutant. Biochim. Biophys. Acta, 1490, (1-2), 106-108), or else nucleic acids encoding proteins which have the activity of a  $\Delta 5$ -desaturase, for example due to a broad substrate specificity, such as, for example, nucleic acids encoding a *Saccharomyces cerevisiae* C5-desaturase (ERG3) (nucleic acid: Seq. ID. No. 11, protein: Seq. ID. No. 12), (Arthington, B. A. et al. (1991): Cloning, disruption and sequence of the gene encoding yeast C-5 sterol desaturase. Gene. June 15; 102(1):39-44.).

In this preferred embodiment, thus at least one further  $\Delta 5$ -desaturase gene is present in the transgenic organisms of the invention, compared to the wild type.

The number of  $\Delta 5$ -desaturase genes in the transgenic organisms of the invention is at least two, preferably more than two, particularly preferably more than three and very particularly preferably more than five.

Preferred  $\Delta 5$ -desaturase genes are nucleic acids encoding proteins which have a high substrate specificity for cholesta-7,24-dienol. Therefore, preference is given in particular to  $\Delta 5$ -desaturase genes and to the corresponding  $\Delta 5$ -desaturases of mammals and to the functional equivalents thereof.

Accordingly, preference is given to using in the above-described method nucleic acids which encode proteins comprising the amino acid sequence SEQ. ID. NO. 8 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which is at least 30%, preferably at least 50%, more preferably at least 70%, still more preferably at least 90%, most preferably at least 95%, identical at the amino acid level with the sequence SEQ. ID. NO. 8, and having the enzyme property of a  $\Delta 5$ -desaturase.

The sequence SEQ. ID. NO. 8 represents the amino acid sequence of *Mus musculus*  $\Delta 5$ -desaturase.

Further examples of  $\Delta 5$ -desaturase and  $\Delta 5$ -desaturase genes can readily be found, for example, for various organisms whose genomic sequence is known by comparing the homology of the amino acid sequences or the corresponding backtranslated nucleic acid sequences from databases with the Seq ID. NO. 2.

The Homo sapiens  $\Delta 5$ -desaturase (Seq. ID. No. 10), for example, is 84% identical to *Mus musculus*  $\Delta 5$ -desaturase (Seq. ID. No. 8).

Further examples of  $\Delta 5$ -desaturases and  $\Delta 5$ -desaturase genes can furthermore readily be found for various organisms whose genomic sequence is unknown, for example starting from the sequence SEQ. ID. No. 7, by hybridization techniques and PCR techniques in a manner known per se.

Accordingly, a protein which is at least 30% identical at the amino acid level with the sequence SEQ. ID. NO. 8 means a protein which is at least 30% identical when comparing its sequence with the sequence SEQ. ID. NO. 8, in particular according to the above program algorithm with the above set of parameters.

In a further, particularly preferred embodiment, the  $\Delta 5$ -desaturase activity is increased by introducing into organisms nucleic acids which encode proteins comprising the amino acid sequence of *Mus musculus*  $\Delta 5$ -desaturase (SEQ. ID. NO. 8).

Suitable nucleic acid sequences can be obtained, for example, by backtranslating the polypeptide sequence according to the genetic code.

Preference is given to using for this those codons which are frequently used according to the organism-specific codon usage. Said codon usage can readily be determined on the basis of computer analyses of other known genes of the organisms in question.

If the protein is to be expressed in yeast, for example, it is often advantageous to use the codon usage of yeast for backtranslation.

In a particularly preferred embodiment, a nucleic acid comprising the sequence SEQ. ID. NO. 7 is introduced into the organism.

The sequence SEQ. ID. NO. 7 represents the *Mus musculus* cDNA which encodes the  $\Delta 5$ -desaturase of the sequence SEQ ID NO. 8.

Furthermore, all of the  $\Delta 5$ -desaturase genes mentioned above can be prepared in a manner known per se by chemical synthesis from the nucleotide building blocks, for example by fragment condensation of individual overlapping complementary nucleic acid building blocks of the double helix. The chemical synthesis of oligonucleotides may be carried out, for example, in a known manner according to the phosphoramidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). Annealing of synthetic oligonucleotides and filling-in of gaps with the aid of the Klenow fragment of DNA polymerase and the ligation reactions and also general cloning methods are described in Sambrook et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press.

In a preferred embodiment, the  $\Delta 24$ -reductase activity is increased compared to the wild type by increasing the gene expression of a nucleic acid encoding a  $\Delta 24$ -reductase.

In a further preferred embodiment, gene expression of a nucleic acid encoding a  $\Delta 24$ -reductase is increased by introducing into the organism one or more nucleic acids encoding a  $\Delta 24$ -reductase.

For this purpose, it is possible to use in principle any  $\Delta 24$ -reductase gene, i.e. any nucleic acid encoding a  $\Delta 24$ -reductase.

In the case of genomic  $\Delta 24$ -reductase nucleic acid sequences from eukaryotic sources, which contain introns, preferably already processed nucleic acid sequences such as the corresponding cDNAs are to be used, if the host organism is unable to or cannot be enabled to express the appropriate  $\Delta 24$ -reductase.

Examples of  $\Delta 24$ -reductase genes are nucleic acids encoding a murine  $\Delta 24$ -reductase (nucleic acid: Seq. ID. No. 13, protein: Seq. ID. No. 14) or a human  $\Delta 24$ -reductase (nucleic acid: Seq. ID. No. 15, protein: Seq. ID. No. 16)

(Waterham, H. R. et al.: Mutations in the 3 $\beta$ -Hydroxysterol  $\Delta$ 24-Reductase Gene Cause Desmosterolosis, an Autosomal Recessive Disorder of Cholesterol Biosynthesis, *Am. J. Hum. Genet.* 69 (4), 685-694 (2001)), or else nucleic acids encoding proteins which have the activity of a  $\Delta$ 24-reductase, for example due to a broad substrate specificity, such as, for example, nucleic acids encoding a *Saccharomyces cerevisiae*  $\Delta$ 24-reductase (ERG4) (nucleic acid: Seq. ID. No. 17, protein: Seq. ID. No. 18) (Lai, M. H. et al., (1994): The identification of a gene family in the *Saccharomyces cerevisiae* ergosterol biosynthesis pathway. *Gene*. March 11; 140(1):41-9).

In this preferred embodiment, thus at least one further  $\Delta$ 24-reductase gene is present in the transgenic organisms of the invention, compared to the wild type.

The number of  $\Delta$ 24-reductase genes in the transgenic organisms of the invention is at least two, preferably more than two, particularly preferably more than three and very particularly preferably more than five.

Preferred  $\Delta$ 24-reductase genes are nucleic acids encoding proteins which have a high substrate specificity for cholesterol-5,7,24-trienol. Therefore, preference is given in particular to  $\Delta$ 24-reductase genes and to the corresponding  $\Delta$ 24-reductase of mammals and to the functional equivalents thereof.

Accordingly, preference is given to using in the above-described method nucleic acids which encode proteins comprising the amino acid sequence SEQ. ID. NO. 14 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which is at least 30%, preferably at least 50%, more preferably at least 70%, still more preferably at least 90%, most preferably at least 95%, identical at the amino acid level with the sequence SEQ. ID. NO. 14, and having the enzyme property of a  $\Delta$ 24-reductase.

The sequence SEQ. ID. NO. 14 represents the amino acid sequence of *Mus musculus*  $\Delta$ 24-reductase.

Further examples of  $\Delta$ 24-reductases and  $\Delta$ 24-reductase genes can readily be found, for example, for various organisms whose genomic sequence is known by comparing the homology of the amino acid sequences or the corresponding backtranslated nucleic acid sequences from databases with the Seq ID. NO. 14.

The Homo sapiens  $\Delta$ 24-reductase (Seq. ID. No. 16), for example, is 96% identical to *Mus musculus*  $\Delta$ 24-reductase (Seq. ID. No. 14).

Further examples of  $\Delta$ 24-reductases and  $\Delta$ 24-reductase genes can furthermore readily be found for various organisms whose genomic sequence is unknown, for example starting from the sequence SEQ. ID. No. 13, by hybridization techniques and PCR techniques in a manner known per se.

Accordingly, a protein which is at least 30% identical at the amino acid level with the sequence SEQ. ID. NO. 14 means a protein which is at least 30% identical when comparing its sequence with the sequence SEQ. ID. NO. 14, in particular according to the above program algorithm with the above set of parameters.

In a further, particularly preferred embodiment, the  $\Delta$ 24-reductase activity is increased by introducing into organisms nucleic acids which encode proteins comprising the amino acid sequence of *Mus musculus*  $\Delta$ 24-reductase (SEQ. ID. NO. 14).

Suitable nucleic acid sequences can be obtained, for example, by backtranslating the polypeptide sequence according to the genetic code.

Preference is given to using for this those codons which are frequently used according to the organism-specific

codon usage. Said codon usage can readily be determined on the basis of computer analyses of other known genes of the organisms in question.

If the protein is to be expressed in yeast, for example, it is often advantageous to use the codon usage of yeast for backtranslation.

In a particularly preferred embodiment, a nucleic acid comprising the sequence SEQ. ID. NO. 13 is introduced into the organism.

The sequence SEQ. ID. NO. 13 represents the *Mus musculus* genomic DNA which encodes the  $\Delta$ 24-reductase of the sequence SEQ ID NO. 14.

Furthermore, all of the  $\Delta$ 24-reductase genes mentioned above can be prepared in a manner known per se by chemical synthesis from the nucleotide building blocks, for example by fragment condensation of individual overlapping complementary nucleic acid building blocks of the double helix. The chemical synthesis of oligonucleotides may be carried out, for example, in a known manner according to the phosphoramidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). Annealing of synthetic oligonucleotides and filling-in of gaps with the aid of the Klenow fragment of DNA polymerase and the ligation reactions and also general cloning methods are described in Sambrook et al. (1989), *Molecular cloning: A laboratory manual*, Cold Spring Harbor Laboratory Press.

In a further preferred embodiment of the method of the invention, organisms are cultured which have, compared to the wild type, a reduced activity of at least one of the activities selected from the group consisting of C24-methyltransferase activity and  $\Delta$ 22-desaturase activity in addition to the above-described genetic modifications.

In a further particularly preferred embodiment, organisms are cultured which have, compared to the wild type, a reduced C24-methyltransferase activity and a reduced  $\Delta$ 22-desaturase activity in addition to the above-described genetic modifications.

A reduced activity means both the reduced and the complete elimination of said activity. Reducing an activity therefore also comprises a reduction in the amount of the corresponding protein in the organism up to a complete absence of the corresponding protein, which can be tested, for example, via missing detectability of the corresponding enzyme activity or missing immunological detectability of the corresponding proteins.

A C24-methyltransferase activity means the enzyme activity of a C24-methyltransferase.

A C24-methyltransferase means a protein which has the enzyme activity of converting zymosterol to fecosterol (ergosta-8,24(28)dienol).

Accordingly, C24-methyltransferase activity means the amount of zymosterol converted or the amount of fecosterol formed by the protein C24-methyltransferase in a particular time.

In the case of a reduced C24-methyltransferase activity compared to the wild type, thus the amount of zymosterol converted or the amount of fecosterol formed by the protein C24-methyltransferase in a particular time is reduced in comparison with the wild type.

The C24-methyltransferase activity is reduced preferably to at least 90%, further preferably to at least 70%, further preferably to at least 50%, further preferably to at least 30%, more preferably to at least 10%, still more preferably to at least 5%, in particular to 0%, of the C24-methyltransferase activity of the wild type. Therefore, particular preference is given to eliminating the C24-methyltransferase activity in the organism.

$\Delta$ 22-desaturase activity means the enzyme activity of a  $\Delta$ 22-desaturase.

A  $\Delta$ 22-desaturase means a protein which has the enzyme activity of converting ergosta-5,7-dienol to ergosta-5,7,22,24-tetraen-3 $\beta$ -ol.

Accordingly,  $\Delta$ 22-desaturase activity means the amount of ergosta-5,7-dienol converted or the amount of ergosta-5,7,22,24-tetraen-3 $\beta$ -ol formed by the protein  $\Delta$ 22-desaturase in a particular time.

In the case of a reduced  $\Delta$ 22-desaturase activity compared to the wild type, thus the amount of ergosta-5,7-dienol converted or the amount of ergosta-5,7,22,24-tetraen-3 $\beta$ -ol formed by the protein  $\Delta$ 22-desaturase in a particular time is reduced in comparison with the wild type.

The  $\Delta$ 22-desaturase activity is reduced preferably to at least 90%, further preferably to at least 70%, further preferably to at least 50%, further preferably to at least 30%, more preferably to at least 10%, still more preferably to at least 5%, in particular to 0%, of the  $\Delta$ 22-desaturase activity of the wild type. Therefore, particular preference is given to eliminating the  $\Delta$ 22-desaturase activity in the organism.

The reduction in C24-methyltransferase activity and/or  $\Delta$ 22-desaturase activity may be carried out independently of one another by different cell-biological mechanisms, for example by inhibiting the corresponding activity at the protein level, for example by adding inhibitors of the corresponding enzymes or by reducing gene expression of the corresponding nucleic acids encoding a C24-methyltransferase or  $\Delta$ 22-desaturase, compared to the wild type.

In a particularly preferred embodiment of the method of the invention, the C24-methyltransferase activity and/or the  $\Delta$ 22-desaturase activity are reduced compared to the wild type by reducing the gene expression of the corresponding nucleic acids encoding a C24-methyltransferase or  $\Delta$ 22-desaturase.

Likewise, gene expression of the nucleic acids encoding a C24-methyltransferase or  $\Delta$ 22-desaturase may be reduced compared to the wild type in various ways, for example by

a) introducing nucleic acid sequences which can be transcribed to an antisense nucleic acid sequence which is capable of inhibiting the C24-methyltransferase activity and/or  $\Delta$ 22-desaturase activity, for example by inhibiting the expression of endogenous C24-methyltransferase and/or  $\Delta$ 22-desaturase activity,

b) overexpression of homologous C24-methyltransferase nucleic acid sequences and/or  $\Delta$ 22-desaturase nucleic acid sequences, which leads to cosuppression,

c) introducing nonsense mutations into the endogene by means of introducing RNA/DNA oligonucleotides into the organism,

d) introducing specific DNA-binding factors, for example factors of the zinc finger transcription factor type, which cause a reduction in gene expression or

e) generating knockout mutants, for example with the aid of T-DNA mutagenesis or homologous recombination.

In a preferred embodiment of the method of the invention, gene expression of the nucleic acids encoding a C24-methyltransferase or  $\Delta$ 22-desaturase is reduced by generating knockout mutants, particularly preferably by homologous recombination.

Therefore, preference is given to using an organism which has no functional C24-methyltransferase gene and/or  $\Delta$ 22-desaturase gene.

In a preferred embodiment, knockout mutants are generated, i.e. the C24-methyltransferase-gene target locus and/or the  $\Delta$ 22-desaturase-gene target locus are deleted with simultaneous integration of an expression cassette containing at

least one of the nucleic acids described above or below, which encode a protein whose activity is increased in comparison with the wild type, by homologous recombination.

For this purpose, it is possible to use nucleic acid constructs which, in addition to the expression cassettes described below which contain promoter, coding sequence and, where appropriate, terminator and in addition to a selection marker at the 3' and 5' ends, described below, contain nucleic acid sequences which are identical to nucleic acid sequences at the start and the end of the gene to be deleted.

After selection by recombinase systems, the selection marker may preferably be removed again, for example via IoxP signals at the 3' and 5' ends of the selection marker, using a Cre recombinase (Cre-IoxP system).

In the preferred organism *Saccharomyces cerevisiae*, the C24-methyltransferase gene is the gene ERG6 (SEQ. ID. NO. 19). SEQ. ID. NO. 20 represents the corresponding *Saccharomyces cerevisiae* C24-methyltransferase (Hardwick, K. G. et al.: SED6 is identical to ERG6, and encodes a putative methyltransferase required for ergosterol synthesis. Yeast. February; 10(2):265-9).

In the preferred organism *Saccharomyces cerevisiae*, the  $\Delta$ 22-desaturase gene is the gene ERG5 (SEQ. ID. NO. 21). SEQ. ID. NO. 22 represents the corresponding *Saccharomyces cerevisiae*  $\Delta$ 22-desaturase (Skaggs, B. A. et al: Cloning and characterization of the *Saccharomyces cerevisiae* C-22 sterol desaturase gene, encoding a second cytochrome P-450 involved in ergosterol biosynthesis, Gene. 1996 Feb. 22; 169(1):105-9).

In a further preferred embodiment of the method of the invention, organisms are cultured which have, in addition to the above-described modifications, an increased activity of at least one of the activities selected from the group consisting of HMG-CoA-reductase activity, lanosterol-C14-demethylase activity, squalene-epoxidase activity, squalene-synthetase activity and sterol-acyltransferase activity, compared to the wild type.

HMG-CoA-reductase activity means the enzyme activity of an HMG-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme-A reductase).

HMG-CoA reductase means a protein which has the enzyme activity of converting 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate.

Accordingly, HMG-CoA-reductase activity means the amount of 3-hydroxy-3-methylglutaryl-coenzyme A converted or the amount of mevalonate formed by the protein HMG-CoA reductase in a particular time.

In the case of an increased HMG-CoA-reductase activity compared to the wild type, thus the amount of 3-hydroxy-3-methylglutaryl-coenzyme A converted or the amount of mevalonate formed by the protein HMG-CoA reductase in a particular time is increased in comparison with the wild type.

This increase in HMG-CoA-reductase activity is preferably at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably at least 100%, more preferably at least 300%, still more preferably at least 500%, in particular at least 600%, of the HMG-CoA-reductase activity of the wild type.

Lanosterol C14-demethylase activity means the enzyme activity of a lanosterol C14-demethylase.

Lanosterol C14-demethylase means a protein which has the enzyme activity of converting lanosterol to 4,4-dimethylcholesta-8,14,24-trienol.

Accordingly, lanosterol C14-demethylase activity means the amount of lanosterol converted or the amount of 4,4-dimethylcholesta-8,14,24-trienol formed by the protein lanosterol C14-demethylase in a particular time.

In the case of an increased lanosterol C14-demethylase activity compared to the wild type, thus the amount of lanosterol converted or the amount of 4,4-dimethylcholesta-8,14,24-trienol formed by the protein lanosterol C14-demethylase in a particular time is increased in comparison with the wild type.

This increase in lanosterol C14-demethylase activity is preferably at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably at least 100%, more preferably at least 300%, still more preferably at least 500%, in particular at least 600%, of the lanosterol C14-demethylase activity of the wild type.

Squalene-epoxidase activity means the enzyme activity of a squalene epoxidase.

Squalene epoxidase means a protein which has the enzyme activity of converting squalene to squalene epoxide.

Accordingly, squalene-epoxidase activity means the amount of squalene converted or the amount of squalene epoxide formed by the protein squalene epoxidase in a particular time.

In the case of an increased squalene-epoxidase activity compared to the wild type, thus the amount of squalene converted or the amount of squalene epoxide formed by the protein squalene epoxidase in a particular time is increased in comparison with the wild type.

This increase in squalene-epoxidase activity is preferably at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably at least 100%, more preferably at least 300%, still more preferably at least 500%, in particular at least 600%, of the squalene-epoxidase activity of the wild type.

Squalene-synthetase activity means the enzyme activity of a squalene synthetase.

Squalene synthetase means a protein which has the enzyme activity of converting farnesyl-pyrophosphate to squalene.

Accordingly, squalene-synthetase activity means the amount of farnesyl-pyrophosphate converted or the amount of squalene formed by the protein squalene synthetase in a particular time.

In the case of an increased squalene-synthetase activity compared to the wild type, thus the amount of farnesyl-pyrophosphate converted or the amount of squalene formed by the protein squalene synthetase in a particular time is increased in comparison with the wild type.

This increase in squalene-synthetase activity is preferably at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably at least 100%, more preferably at least 300%, still more preferably at least 500%, in particular at least 600%, of the squalene-synthetase activity of the wild type.

Sterol-acyltransferase activity means the enzyme activity of a sterol acyltransferase.

Sterol acyltransferase means a protein which has the enzyme activity of converting 7-dehydrocholesterol to corresponding acetylated 7-dehydrocholesterol.

Accordingly, sterol-acyltransferase activity means the amount of 7-dehydrocholesterol converted or the amount of acetylated 7-dehydrocholesterol formed by the protein sterol acyltransferase in a particular time.

In the case of an increased sterol-acyltransferase activity compared to the wild type, thus the amount of 7-dehydrocholesterol converted or the amount of acetylated 7-dehy-

drocholesterol formed by the protein sterol acyltransferase in a particular time is increased in comparison with the wild type.

This increase in sterol-acyltransferase activity is preferably at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably at least 100%, more preferably at least 300%, still more preferably at least 500%, in particular at least 600%, of the sterol-acyltransferase activity of the wild type.

In a preferred embodiment, the HMG-CoA-reductase activity is increased compared to the wild type by increasing the gene expression of a nucleic acid encoding an HMG-CoA reductase.

In a particularly preferred embodiment of the method of the invention, gene expression of a nucleic acid encoding an HMG-CoA reductase is increased by introducing into the organism a nucleic acid construct comprising an HMG-CoA reductase-encoding nucleic acid whose expression in said organism is subject to a reduced regulation, in comparison with the wild type.

A reduced regulation in comparison with the wild type means a reduced regulation and, preferably, no regulation at the expression or protein level, in comparison with the above-defined wild type.

The reduced regulation may be achieved preferably by a promoter which is functionally linked with the coding sequence in the nucleic acid construct and which is subject to a reduced regulation in the organism, in comparison with the wild-type promoter.

For example, the medium ADH promoter in yeast is subject only to a reduced regulation and is therefore particularly preferred as promoter in the above-described nucleic acid construct.

This promoter fragment of the ADH12s promoter, also referred to as ADH1 hereinbelow, exhibits nearly constitutive expression (Ruohonen L, Penttila M, Keranen S. (1991) Optimization of Bacillus  $\alpha$ -amylase production by *Saccharomyces cerevisiae*. Yeast. May-June; 7(4):337-462; Lang C, Looman A C. (1995) Efficient expression and secretion of *Aspergillus niger* RH5344 polygalacturonase in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol. December; 44(1-2):147-56) so that transcriptional regulation no longer proceeds via intermediates of ergosterol biosynthesis.

Other preferred promoters with reduced regulation are constitutive promoters such as, for example, the yeast TEF1 promoter, the yeast GPD promoter or the yeast PGK promoter (Mumberg D, Muller R, Funk M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene. 1995 Apr. 14; 156(1): 119-22; Chen C Y, Oppermann H, Hitzeman R A. (1984) Homologous versus heterologous gene expression in the yeast, *Saccharomyces cerevisiae*. Nucleic Acids Res. December 11; 12(23):8951-70).

In a further preferred embodiment, reduced regulation can be achieved by using as an HMG-CoA reductase-encoding nucleic acid a nucleic acid whose expression in the organism is subject to a reduced regulation, in comparison with the orthologous nucleic acid intrinsic to said organism.

Particular preference is given to using as an HMG-CoA reductase-encoding nucleic acid a nucleic acid which encodes only the catalytic region of HMG-CoA reductase (truncated (t-) HMG-CoA reductase). This nucleic acid (t-HMG), described in EP 486 290 and WO 99/16886 encodes only the catalytically active part of HMG-CoA reductase, with the membrane domain responsible for regulation at the protein level missing. This nucleic acid is thus

subject to a reduced regulation, in particular in yeast, and leads to an increase in gene expression of HMG-CoA reductase.

In a particularly preferred embodiment, nucleic acids are introduced, preferably via the above-described nucleic acid construct, which encode proteins comprising the amino acid sequence SEQ. ID. NO. 24 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which is at least 30% identical at the amino acid level to the sequence SEQ ID. NO. 24, and having the enzyme property of an HMG-CoA reductase.

The sequence SEQ ID NO. 24 is the amino acid sequence of the truncated HMG-CoA reductase (t-HMG).

Further examples of HMG-CoA reductases and thus also of the t-HMG-CoA reductases reduced to the catalytic region or of the coding genes can readily be found, for example, for various organisms whose genomic sequence is known by comparing the homology of the amino acid sequences or of the corresponding backtranslated nucleic acid sequences from databases with the sequence SEQ ID. No. 24.

Further examples of HMG-CoA reductases and thus also of the t-HMG-CoA reductases reduced to the catalytic region and of the coding genes can furthermore readily be found for various organisms whose genomic sequence is unknown by hybridization techniques and PCR techniques in a manner known per se, for example starting from the sequence SEQ ID NO. 23.

Particular preference is given to using as a truncated HMG-CoA reductase-encoding nucleic acid a nucleic acid comprising the sequence SEQ ID NO. 23.

In a particularly preferred embodiment, the reduced regulation is achieved by using as an HMG-CoA reductase-encoding nucleic acid a nucleic acid whose expression in the organism is subject to a reduced regulation, in comparison with the orthologous nucleic acid intrinsic to said organism, and by using a promoter which is subject to a reduced regulation in said organism, in comparison with the wild-type promoter.

In a preferred embodiment, the lanosterol C14-demethylase activity is increased compared to the wild type by increasing the gene expression of a nucleic acid encoding a lanosterol C14-demethylase.

In a further preferred embodiment, gene expression of a nucleic acid encoding a lanosterol C14-demethylase is increased by introducing into the organism one or more nucleic acids encoding a lanosterol C14-demethylase.

For this purpose, it is possible to use in principle any lanosterol C14-demethylase gene (ERG11), i.e. any nucleic acids encoding a lanosterol C14-demethylase. In the case of genomic lanosterol C14-demethylase nucleic acid sequences from eukaryotic sources, which contain introns, already processed nucleic acid sequences such as the corresponding cDNAs are to be used preferably, if the host organism is unable to or cannot be enabled to express the appropriate lanosterol C14-demethylase.

Examples of lanosterol C14-demethylase genes are nucleic acids encoding a lanosterol C14-demethylase of *Saccharomyces cerevisiae* (Kalb V F, Loper J C, Dey C R, Woods C W, Sutter T R (1986) Isolation of a cytochrome P-450 structural gene from *Saccharomyces cerevisiae*. Gene 45(3):237-45), *Candida albicans* (Lamb D C, Kelly D E, Baldwin B C, Gozzo F, Boscott P, Richards W G, Kelly S L (1997) Differential inhibition of *Candida albicans* CYP51 with azole antifungal stereoisomers. FEMS Microbiol Lett 149(1):25-30), *Homo sapiens* (Stromstedt M, Rozman D, Waterman M R. (1996) The ubiquitously expressed human

CYP51 encodes lanosterol 14  $\alpha$ -demethylase, a cytochrome P450 whose expression is regulated by oxysterols. Arch Biochem Biophys 1996 May 1; 329(1):73-81c) or *Rattus norvegicus*, Aoyama Y, Funae Y, Noshiro M, Horiuchi T, Yoshida Y. (1994) Occurrence of a P450 showing high homology to yeast lanosterol 14-demethylase (P450 (14DM)) in the rat liver. Biochem Biophys Res Commun. June 30; 201(3):1320-6).

In this preferred embodiment, thus at least one further lanosterol C14-demethylase gene is present in the transgenic organisms of the invention, compared to the wild type.

The number of C14-demethylase genes in the transgenic organisms of the invention is at least two, preferably more than two, particularly preferably more than three and very particularly preferably more than five.

Preference is given to using in the above-described method nucleic acids which encode proteins comprising the amino acid sequence SEQ. ID. NO. 26 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which is at least 30%, preferably at least 50%, more preferably at least 70%, still more preferably at least 90%, most preferably at least 95%, identical at the amino acid level with the sequence SEQ. ID. NO. 26, and having the enzyme property of a lanosterol C14-demethylase.

The sequence SEQ. ID. NO. 26 represents the amino acid sequence of *Saccharomyces cerevisiae* lanosterol C14-demethylase.

Further examples of lanosterol C14-demethylases and lanosterol C14-demethylase genes can readily be found, for example, for various organisms whose genomic sequence is known by comparing the homology of the amino acid sequences or the corresponding backtranslated nucleic acid sequences from databases with the Seq ID. NO. 26.

Further examples of lanosterol C14-demethylases and lanosterol C14-demethylase genes can furthermore readily be found for various organisms whose genomic sequence is unknown, for example starting from the sequence SEQ. ID. No. 25, by hybridization techniques and PCR techniques in a manner known per se.

Accordingly, a protein which is at least 30% identical at the amino acid level with the sequence SEQ. ID. NO. 26 means a protein which is at least 30% identical when comparing its sequence with the sequence SEQ. ID. NO. 26, in particular according to the above program algorithm with the above set of parameters.

In another preferred embodiment, nucleic acids are introduced into organisms, which encode proteins comprising the amino acid sequence of *Saccharomyces cerevisiae* lanosterol C14-demethylase (SEQ. ID. NO. 26).

Suitable nucleic acid sequences can be obtained, for example, by backtranslating the polypeptide sequence according to the genetic code.

Preference is given to using for this those codons which are frequently used according to the organism-specific codon usage. Said codon usage can readily be determined on the basis of computer analyses of other known genes of the organisms in question.

If the protein is to be expressed in yeast, for example, it is often advantageous to use the codon usage of yeast for the backtranslation.

In a particularly preferred embodiment, a nucleic acid comprising the sequence SEQ. ID. NO. 25 is introduced into the organism.

The sequence SEQ. ID. NO. 25 represents the genomic DNA of *Saccharomyces cerevisiae* (ORF S0001049), which encodes the lanosterol C14-demethylase of the sequence SEQ ID NO. 26.

Furthermore, all of the lanosterol C14-demethylase genes mentioned above can be prepared in a manner known per se by chemical synthesis from the nucleotide building blocks, for example by fragment condensation of individual overlapping complementary nucleic acid building blocks of the double helix. The chemical synthesis of oligonucleotides may be carried out, for example, in a known manner according to the phosphoramidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). Annealing of synthetic oligonucleotides and filling-in of gaps with the aid of the Klenow fragment of DNA polymerase and the ligation reactions and also general cloning methods are described in Sambrook et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press.

In a preferred embodiment, the squalene-epoxidase activity is increased compared to the wild type by increasing the gene expression of a nucleic acid encoding a squalene epoxidase.

In a further preferred embodiment, gene expression of a nucleic acid encoding a squalene epoxidase is increased by introducing into the organism one or more nucleic acids encoding a squalene epoxidase.

For this purpose, it is possible to use in principle any squalene-epoxidase gene (ERG1), i.e. any nucleic acids encoding a squalene epoxidase. In the case of genomic squalene epoxidase nucleic acid sequences from eukaryotic sources, which contain introns, already processed nucleic acid sequences such as the corresponding cDNAs are to be used preferably, if the host organism is unable to or cannot be enabled to express the appropriate squalene epoxidase.

Examples of nucleic acids encoding a squalene epoxidase are nucleic acids encoding a squalene epoxidase of *Saccharomyces cerevisiae* (Jandrositz, A., et al (1991) The gene encoding squalene epoxidase from *Saccharomyces cerevisiae*: cloning and characterization. Gene 107:155-160, of *Mus musculus* (Kosuga K, Hata S, Osumi T, Sakakibara J, Ono T. (1995) Nucleotide sequence of a cDNA for mouse squalene epoxidase, Biochim Biophys Acta, February 21; 1260(3):345-8b), of *Rattus norvegicus* (Sakakibara J, Watanabe R, Kanai Y, Ono T. (1995) Molecular cloning and expression of rat squalene epoxidase. J Biol Chem January 6; 270(1):17-20c) or of *Homo sapiens* (Nakamura Y, Sakakibara J, Izumi T, Shibata A, Ono T. (1996) Transcriptional regulation of squalene epoxidase by sterols and inhibitors in HeLa cells., J. Biol. Chem. 1996, Apr. 5; 271(14):8053-6).

In this preferred embodiment, thus at least one further squalene epoxidase is present in the transgenic organisms of the invention, compared to the wild type.

The number of squalene-epoxidase genes in the transgenic organisms of the invention is at least two, preferably more than two, particularly preferably more than three and very particularly preferably more than five.

Preference is given to using in the above-described method nucleic acids which encode proteins comprising the amino acid sequence SEQ. ID. NO. 28 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which is at least 30%, preferably at least 50%, more preferably at least 70%, still more preferably at least 90%, most preferably at least 95%, identical at the amino acid level with the sequence SEQ. ID. NO. 28, and having the enzyme property of a squalene epoxidase.

The sequence SEQ. ID. NO. 28 represents the amino acid sequence of *Saccharomyces cerevisiae* squalene epoxidase.

Further examples of squalene epoxidases and squalene-epoxidase genes can readily be found, for example, for various organisms whose genomic sequence is known by comparing the homology of the amino acid sequences or the

corresponding backtranslated nucleic acid sequences from databases with the SeQ ID. NO. 28.

Further examples of squalene epoxidases and squalene-epoxidase genes can furthermore readily be found for various organisms whose genomic sequence is unknown, for example starting from the sequence SEQ. ID. No. 27, by hybridization techniques and PCR techniques in a manner known per se.

In another preferred embodiment, nucleic acids are introduced into organisms, which encode proteins comprising the amino acid sequence of *Saccharomyces cerevisiae* squalene epoxidase (SEQ. ID. NO. 28).

Suitable nucleic acid sequences can be obtained, for example, by backtranslating the polypeptide sequence according to the genetic code.

Preference is given to using for this those codons which are frequently used according to the organism-specific codon usage. Said codon usage can readily be determined on the basis of computer analyses of other known genes of the organisms in question.

If the protein is to be expressed in yeast, for example, it is often advantageous to use the codon usage of yeast for backtranslation.

In a particularly preferred embodiment, a nucleic acid comprising the sequence SEQ. ID. NO. 27 is introduced into the organism.

The sequence SEQ. ID. NO. 27 represents the genomic DNA of *Saccharomyces cerevisiae* (ORF YGR175C), which encodes the squalene epoxidase of the sequence SEQ ID NO. 28.

Furthermore, all of the squalene-epoxidase genes mentioned above can be prepared in a manner known per se by chemical synthesis from the nucleotide building blocks, for example by fragment condensation of individual overlapping complementary nucleic acid building blocks of the double helix. The chemical synthesis of oligonucleotides may be carried out, for example, in a known manner according to the phosphoramidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). Annealing of synthetic oligonucleotides and filling-in of gaps with the aid of the Klenow fragment of DNA polymerase and the ligation reactions and also general cloning methods are described in Sambrook et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press.

In a preferred embodiment, the squalene-synthetase activity is increased compared to the wild type by increasing the gene expression of a nucleic acid encoding a squalene synthetase.

In a further preferred embodiment, gene expression of a nucleic acid encoding a squalene synthetase is increased by introducing into the organism one or more nucleic acids encoding a squalene synthetase.

For this purpose, it is possible to use in principle any squalene-synthetase gene (ERG9), i.e. any nucleic acids encoding a squalene synthetase. In the case of genomic squalene synthetase nucleic acid sequences from eukaryotic sources, which contain introns, already processed nucleic acid sequences such as the corresponding cDNAs are to be used preferably, if the host organism is unable to or cannot be enabled to express the appropriate squalene synthetase.

Examples of nucleic acids encoding a squalene synthetase are nucleic acids encoding a *Saccharomyces cerevisiae* squalene synthetase (ERG9) (Jennings, S. M., (1991): Molecular cloning and characterization of the yeast gene for squalene synthetase. Proc Natl Acad Sci USA. July 15; 88(14):6038-42), nucleic acids encoding a *Botryococcus braunii* Okada squalene synthetase (Devarenne, T. P. et al.:

Molecular characterization of squalene synthetase from the green microalga *Botryococcus braunii*, raceB, Arch. Biochem. Biophys. 2000, Jan. 15, 373(2):307-17), nucleic acids encoding a Potato tuber squalene synthetase (Yoshioka H. et al.: cDNA cloning of sesquiterpene cyclase and squalene synthase, and expression of the genes in potato tuber infected with *Phytophthora infestans*, Plant. Cell. Physiol. 1999, September; 40(9):993-8) and nucleic acids encoding a *Glycyrrhiza glabra* squalene synthetase (Hayashi, H. et al.: Molecular cloning and characterization of two cDNAs for *Glycyrrhiza glabra* squalene synthase, Biol. Pharm. Bull. 1999, September; 22(9):947-50).

In this preferred embodiment, thus at least one further squalene-synthetase gene is present in the transgenic organisms of the invention, compared to the wild type.

The number of squalene-synthetase genes in the transgenic organisms of the invention is at least two, preferably more than two, particularly preferably more than three and very particularly preferably more than five.

Preference is given to using in the above-described method nucleic acids which encode proteins comprising the amino acid sequence SEQ. ID. NO. 30 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which is at least 30%, preferably at least 50%, more preferably at least 70%, still more preferably at least 90%, most preferably at least 95%, identical at the amino acid level with the sequence SEQ. ID. NO. 30, and having the enzyme property of a squalene synthetase.

The sequence SEQ. ID. NO. 30 represents the amino acid sequence of *Saccharomyces cerevisiae* squalene synthetase (ERG9).

Further examples of squalene synthetases and squalene-synthetase genes can readily be found, for example, for various organisms whose genomic sequence is known by comparing the homology of the amino acid sequences or the corresponding backtranslated nucleic acid sequences from databases with the SEQ. ID. NO. 30.

Further examples of squalene synthetases and squalene-synthetase genes can furthermore readily be found for various organisms whose genomic sequence is unknown, for example starting from the sequence SEQ. ID. No. 29, by hybridization techniques and PCR techniques in a manner known per se.

In another preferred embodiment, nucleic acids are introduced into organisms, which encode proteins comprising the amino acid sequence of *Saccharomyces cerevisiae* squalene synthetase (SEQ. ID. NO. 30).

Suitable nucleic acid sequences can be obtained, for example, by backtranslating the polypeptide sequence according to the genetic code.

Preference is given to using for these codons which are frequently used according to the organism-specific codon usage. Said codon usage can readily be determined on the basis of computer analyses of other known genes of the organisms in question.

If the protein is to be expressed in yeast, for example, it is often advantageous to use the codon usage of yeast for the backtranslation.

In a particularly preferred embodiment, a nucleic acid comprising the sequence SEQ. ID. NO. 29 is introduced into the organism.

The sequence SEQ. ID. NO. 29 represents the genomic DNA of *Saccharomyces cerevisiae* (ORF YHR190W), which encodes the squalene synthetase of the sequence SEQ. ID. NO. 30.

Furthermore, all of the squalene-synthetase genes mentioned above can be prepared in a manner known per se by

chemical synthesis from the nucleotide building blocks, for example by fragment condensation of individual overlapping complementary nucleic acid building blocks of the double helix. The chemical synthesis of oligonucleotides may be carried out, for example, in a known manner according to the phosphoramidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). Annealing of synthetic oligonucleotides and filling-in of gaps with the aid of the Klenow fragment of DNA polymerase and the ligation reactions and also general cloning methods are described in Sambrook et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press.

In a preferred embodiment, the sterol-acyltransferase activity is increased compared to the wild type by increasing the gene expression of a nucleic acid encoding a sterol acyltransferase.

In a further preferred embodiment, gene expression of a nucleic acid encoding a sterol acyltransferase is increased by introducing into the organism one or more nucleic acids encoding a sterol acyltransferase.

For this purpose, it is possible to use in principle any sterol-acyltransferase gene (SAT1 or SAT2), i.e. any nucleic acids encoding a sterol acyltransferase.

In the case of genomic sterol acyltransferase nucleic acid sequences from eukaryotic sources, which contain introns, already processed nucleic acid sequences such as the corresponding cDNAs are to be used preferably, if the host organism is unable to or cannot be enabled to express the appropriate sterol acyltransferase.

Examples of nucleic acids encoding a sterol acyltransferase are nucleic acids encoding a *Saccharomyces cerevisiae* sterol acyltransferase (SAT1) or (SAT2) (Yang, H.: Sterol esterification in yeast: a two-gene process. Science. 1996 May 31; 272(5266):1353-6), a further nucleic acid encoding a further *Saccharomyces cerevisiae* sterol acyltransferase (J. Biol. Chem. 1996, September 27; 271(39):24157-63), nucleic acids encoding a human sterol acyltransferase (Chang, C. C. et al., Molecular cloning and functional expression of human acyl-coenzyme A:cholesterol acyltransferase cDNA in mutant Chinese hamster ovary cells, J. Biol. Chem. 1993, Oct. 5; 268(28):20747-55) and nucleic acids encoding a murine sterol acyltransferase (Uelmen, P. J.: Tissue-specific expression and cholesterol regulation of acylcoenzyme A:cholesterol acyltransferase (ACAT) in mice. Molecular cloning of mouse ACAT cDNA, chromosomal localization, and regulation of ACAT in vivo and in vitro, J. Biol. Chem. 1995 Nov. 3; 270(44):26192-201).

In this preferred embodiment, thus at least one further sterol-acyltransferase gene is present in the transgenic organisms of the invention, compared to the wild type.

The number of sterol-acyltransferase genes in the transgenic organisms of the invention is at least two, preferably more than two, particularly preferably more than three and very particularly preferably more than five.

Preference is given to using in the above-described method nucleic acids which encode proteins comprising the amino acid sequence SEQ. ID. NO. 32 or SEQ. ID. NO. 50 or a sequence derived from these sequences by substitution, insertion or deletion of amino acids, which is at least 30%, preferably at least 50%, more preferably at least 70%, still more preferably at least 90%, most preferably at least 95%, identical at the amino acid level with the sequence SEQ. ID. NO. 32 or SEQ. ID. NO. 50, and having the enzyme property of a sterol acyltransferase.

The sequence SEQ. ID. NO. 32 represents the amino acid sequence of *Saccharomyces cerevisiae* sterol acyltransferase SAT1.

The sequence SEQ. ID. NO. 50 represents the amino acid sequence *Saccharomyces cerevisiae* sterol acyltransferase SAT2.

SAT 1 and SAT2 differ from one another by a different substrate specificity.

Further examples of sterol acyltransferases and sterol-acyltransferase genes can readily be found, for example, for various organisms whose genomic sequence is known by comparing the homology of the amino acid sequences or the corresponding backtranslated nucleic acid sequences from databases with the SeQ ID. NO. 32 or 50.

Further examples of sterol acyltransferase and sterol-acyltransferase genes can furthermore readily be found for various organisms whose genomic sequence is unknown, for example starting from the sequence SEQ. ID. No. 31 or 49, by hybridization techniques and PCR techniques in a manner known per se.

In another preferred embodiment, nucleic acids are introduced into organisms, which encode proteins comprising the amino acid sequence of *Saccharomyces cerevisiae* sterol acyltransferase SAT1 (SEQ. ID. NO. 32) or *Saccharomyces cerevisiae* sterol acyltransferase SAT2 (SEQ. ID. NO. 50).

Suitable nucleic acid sequences can be obtained, for example, by backtranslating the polypeptide sequence according to the genetic code.

Preference is given to using for this those codons which are frequently used according to the organism-specific codon usage. Said codon usage can readily be determined on the basis of computer analyses of other known genes of the organisms in question.

If the protein is to be expressed in yeast, for example, it is often advantageous to use the codon usage of yeast for the backtranslation.

In a particularly preferred embodiment, a nucleic acid comprising the sequence SEQ. ID. NO. 31 or 49 is introduced into the organism.

The sequence SEQ. ID. NO. 31 represents the genomic DNA of *Saccharomyces cerevisiae* (ORF YNR019W), which encodes the sterol acyltransferase SAT1 of the sequence SEQ ID NO. 32.

The sequence SEQ. ID. NO. 49 represents the genomic DNA of *Saccharomyces cerevisiae* (ORF YCR048W), which encodes the sterol acyltransferase SAT2 of the sequence SEQ ID NO. 50.

Furthermore, all of the sterol-acyltransferase genes mentioned above can be prepared in a manner known per se by chemical synthesis from the nucleotide building blocks, for example by fragment condensation of individual overlapping complementary nucleic acid building blocks of the double helix. The chemical synthesis of oligonucleotides may be carried out, for example, in a known manner according to the phosphoramidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). Annealing of synthetic oligonucleotides and filling-in of gaps with the aid of the Klenow fragment of DNA polymerase and the ligation reactions and also general cloning methods are described in Sambrook et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press.

According to the invention, organisms mean, for example, bacteria, in particular bacteria of the genus *Bacillus*, *Escherichia coli*, *Lactobacillus* spec. or *Streptomyces* spec., for example yeasts, in particular yeasts of the genus *Saccharomyces cerevisiae*, *Pichia pastoris* or *Klyveromyces* spec.

for example fungi, in particular fungi of the genus *Aspergillus* spec., *Penicillium* spec. or *Dictyostelium* spec.

and also, for example, insect cell lines, which are capable, either as wild type or owing to previous genetic modification, of producing zymosterol and/or the biosynthetic intermediates and/or secondary products thereof.

5 Particularly preferred organisms are yeasts, in particular those of the species *Saccharomyces cerevisiae*, in particular the yeast strains *Saccharomyces cerevisiae* AH22, *Saccharomyces cerevisiae* GRF, *Saccharomyces cerevisiae* DBY747 and *Saccharomyces cerevisiae* BY4741.

10 In the case of yeasts as organisms or genetically modified organisms, it is possible, as mentioned above, to increase at least one of the activities selected from the group consisting of  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity and  $\Delta 24$ -reductase activity by overexpressing the corresponding nucleic acids.

The overexpression may be carried out both homologically by introducing nucleic acids intrinsic to yeast and heterologously by introducing nucleic acids from other organisms, in particular mammals, or natural or artificial variants derived therefrom into the yeast. Preference is given to using mammalian genes in yeasts, since these genes have a better substrate specificity with respect to 7-dehydrocholesterol.

25 The  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity,  $\Delta 24$ -reductase activity, C24-methyltransferase activity,  $\Delta 22$ -desaturase activity, HMG-CoA-reductase activity, lanosterol-C14-demethylase activity, squalene-epoxidase activity, squalene-synthetase activity and sterol-acyltransferase activity of the genetically modified organism of the invention and of the reference organism is determined under the following conditions:

The activity of HMG-CoA reductase is determined as described in Th. Polakowski, Molekularbiologische Beeinflussung des Ergosterolstoffwechsels der Hefe *Saccharomyces cerevisiae* [influencing the ergosterol metabolism of the yeast *Saccharomyces cerevisiae* by molecular biological means], Shaker-Verlag, Aachen 1999, ISBN 3-8265-6211-9, beschrieben.

35 According to this,  $10^9$  yeast cells of a 48 h culture are harvested by centrifugation (3500.times.g, 5 min) and washed in 2 ml of buffer I (100 mM potassium phosphate buffer, pH 7.0). The cell pellet is taken up in 500  $\mu$ l of buffer 1 (cytosolic proteins) or 2 (100 mM potassium phosphate buffer pH 7.0; 1% Triton X-100) (total proteins), and 1  $\mu$ l of 500 mM PMSF in isopropanol is added. 500  $\mu$ l of glass beads (d=0.5 mm) are added to the cells and the cells are disrupted by vortexing 5.times. for one minute each. The liquid between the glass beads is transferred to a new Eppendorf vessel. Cell debris and membrane components are removed by centrifugation (14000.times.g; 15 min).

50 The supernatant is transferred to a new Eppendorf vessel and represents the protein fraction.

The activity of HMG-CoA reductase is determined by measuring NADPH+H<sup>+</sup> consumption during the reduction of 3-hydroxy-3-methylglutaryl-CoA which is added as substrate.

In a 1000  $\mu$ l assay mixture, 20  $\mu$ l of yeast protein isolate are combined with 910  $\mu$ l of buffer I; 50  $\mu$ l of 0.1 M DTT and 10  $\mu$ l of 16 mM NADPH+H<sup>+</sup>. The mixture is adjusted to 30.degree. C. and measured in a spectrophotometer at 340 nm for 7.5 min. The decrease in NADPH, which is measured over this period, is the rate of degradation without addition of substrate and is taken into account as background.

65 Subsequently, substrate (10  $\mu$ l of 30 mM HMG-CoA) is added, and measurement continues for another 7.5 min. The HMG-CoA-reductase activity is calculated by determining the specific rate of NADPH degradation.

The activity of lanosterol C14-demethylase is determined as described in Omura, T and Sato, R. (1964) The carbon monoxide binding pigment in liver microsomes. *J. Biol. Chem.* 239, 2370-2378. In this assay, the amount of P450 enzyme as holoenzyme with bound heme can be semi-quantified. The (active) holoenzyme (with heme) can be reduced by CO and only the CO-reduced enzyme has an absorption maximum at 450 nm. Thus the absorption maximum at 450 nm is a measure for lanosterol C14-demethylase activity.

The activity is determined by diluting a microsomal fraction (4-10 mg/ml protein in 100 mM potassium phosphate buffer) 1:4 so that the protein concentration used in the assay is 2 mg/ml. The assay is carried out directly in a cuvette.

A spatula tipful of dithionite ( $S_2O_4Na_2$ ) is added to the microsomes. The baseline is recorded in the 380-500 nm region in a spectrophotometer.

Subsequently, approx. 20-30 CO bubbles are passed through the sample. The absorption is then measured in the same region. The absorption level at 450 nm corresponds to the amount of P450 enzyme in the assay mixture.

The activity of squalene epoxidase is determined as described in Leber R, Landl K, Zinser E, Ahorn H, Spok A, Kohlwein S D, Turnowsky F, Daum G. (1998) Dual localization of squalene epoxidase, Erg1p, in yeast reflects a relationship between the endoplasmic reticulum and lipid particles. *Mol. Biol. Cell.* 1998, February; 9(2):375-86.

In this method, a total volume of 500  $\mu$ l contains from 0.35 to 0.7 mg of microsomal protein or from 3.5 to 75  $\mu$ g of lipid-particle protein in 100 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 mM FAD, 3 mM NADPH, 0.1 mM squalene 2,3-epoxidase cyclase inhibitor U18666A, 32  $\mu$ M [ $^3H$ ] squalene dispersed in 0.005% Tween 80.

The assay is carried out at 30.degree. C. After 10 minutes of pretreatment, the reaction is started by adding squalene and stopped after 15, 30 or 45 min by lipid extraction with 3 ml of chloroform/methanol (2:1 vol/vol) and 750  $\mu$ l of 0.035%  $MgCl_2$ .

The lipids are dried under nitrogen and redissolved in 0.5 ml of chloroform/methanol (2:1 vol/vol). For thin layer chromatography, portions are applied to a Silica Gel 60 plate (0.2 mm) and fractionated using chloroform as eluent. The positions containing [ $^3H$ ]2,3-oxidosqualene and [ $^3H$ ] squalene were scraped off and quantified in a scintillation counter.

The  $\Delta 8$ - $\Delta 7$ -isomerase activity is determined, with a slight modification, as described in Silve S. et al.: Emopamil-binding Protein, a Mammalian Protein That Binds a Series of Structurally Diverse Neuroprotective Agents, Exhibits 8-7 Sterol Isomerase Activity in Yeast. *J Biol Chem* 1996 Sep. 13; 271(37):22434-40:

Microsomes prepared from a culture volume of 10 ml are incubated in the presence of 75  $\mu$ M cholesta-8-en-3-ol at 30.degree. C. for 3 h. The sterols are then extracted with 4 times 5 ml of hexane and purified. Aliquots are analyzed by means of GC/MS.

The  $\Delta 5$ -desaturase activity is determined, with slight modification, as described in Nishi, S. et al. (2000): cDNA cloning of the mammalian sterol C5-desaturase and the expression in yeast mutant. *Biochim. Biophys. Acta* 1490(1-2), 106-108:

Microsomes prepared from a culture volume of 10 ml are incubated in the presence of 75  $\mu$ M lathosterol and 2 mM NADH at 30.degree. C. for 3 h. The sterols are then extracted with 4 times 5 ml of hexane and purified. Aliquots are analyzed by means of GC/MS.

The  $\Delta 24$ -reductase activity can be determined as described below:

Microsomes prepared from a culture volume of 10 ml are incubated in the presence of 75  $\mu$ M cholesta-5,7,24-trienol at 30.degree. C. for 3 h. The sterols are then extracted with 4 times 5 ml of hexane and purified. Aliquots are analyzed by means of GC/MS.

The C24-methyltransferase activity can be determined as described below:

80% of the protein Erg6p (C24-methyltransferase) are detectable in lipid particles in the yeast (Athenstaedt K, Zweytick D, Jandrositz A, Kohlwein S D, Daum G: Identification and characterization of major lipid particle proteins of the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* 1999 October; 181(20):6441-8). The enzyme activity is determined by preparing lipid particles from a culture volume (48 h) of 100 ml (according to a method described in Athensstaedt K, Zweytick D, Jandrositz A, Kohlwein S D, Daum G: Identification and characterization of major lipid particle proteins of the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* 1999 October; 181(20):6441-8).

The protein content is determined by a Biorad enzyme assay and 3 mg of protein are used in a volume of 500  $\mu$ l for each assay mixture. 50  $\mu$ M [methyl- $^3H_3$ ]-S-adenosylmethionine and 50  $\mu$ M zymosterol are added to the assay mixture which is then incubated at 35.degree. C. for 10 min. Subsequently, the same volume (500  $\mu$ l) of chloroform/methanol (4:1) is added and the sterols are then extracted.

The proportion of zymosterol with incorporated [methyl- $^3H_3$ ]-S-adenosylmethionine can be determined by means of scintillation measurement, since chloroform/methanol extraction extracts only lipid-soluble substances. For quantification, the radioactive decays are likewise determined for 50  $\mu$ M [methyl- $^3H_3$ ]-S-adenosylmethionine by means of scintillation measurement.

This method is a modification of the method described in Nes WD, Guo D, Zhou W.: Substrate-based inhibitors of the (S)-adenosyl-L-methionine: $\Delta 24(25)$ -to  $\Delta 24(28)$ -sterol methyl transferase from *Saccharomyces cerevisiae*, *Arch. Biochem. Biophys.* 1997 Jun. 1; 342(1):68-81.

The activity of  $\Delta 22$ -desaturase (ERG5p) can be determined as described below:

Various concentrations of Ergosta-5,7-dienol, purified from *S. cerevisiae* erg5 mutants (Parks et al, 1985. Yeast sterols.yeast mutants as tools for the study of sterol metabolism. *Methods Enzymol.* 111:333-346) and 50  $\mu$ g of dilaurylphosphatidylcholine are mixed and treated with ultrasound until a white suspension is formed. Prepared microsomes are added (1 ml)(3 mg/ml protein). NADPH (1 mM final concentration) is added to the assay mixture to start the enzyme reaction. The mixture is incubated at 37.degree. C. for 20 min. The reaction is stopped by adding 3 ml of methanol and sterols are hydrolyzed by adding 2 ml of 60% (wt/vol) KOH in water. The mixture is incubated at 90.degree. C. for 2 h. After cooling, the mixture is extracted three times with 5 ml of hexane and concentrated in a rotary evaporator. Subsequently, the sterols are silylated with bis(trimethylsilyl)trifluoroacetamide (50  $\mu$ l in 50  $\mu$ l toluene) at 60.degree. C. for 1 h. The sterols are analyzed by gas chromatography-mass spectrometry (GC-MS) (for example Model VG 12-250 gas chromatograph-mass spectrometer; VG Biotech, Manchester, United Kingdom). The resultant  $\Delta 22$ -desaturated intermediate can be identified depending on the amount of substrate used. Microsomes which are not incubated with substrate serve as reference.

This method is a modification of the method described in Lamb et al: Purification, reconstitution, and inhibition of

cytochrome P-450 sterol  $\Delta 22$ -desaturase from the pathogenic fungus *Candida glabrata*. *Antimicrob Agents Chemother.* 1999 July; 43(7):1725-8.

The squalene-synthetase activity can be determined as described below:

The assays contain 50 mM MOPS, pH 7.2, 10 mM  $MgCl_2$ , 1% (v/v) Tween-80, 10% (v/v) 2-propanol, 1 mM DTT, 1 mg/mL BSA, NADPH, FPP (or PSPP) and microsomes (protein content 3 mg) in a total volume of 200  $\mu$ l in glass tubes. The reaction mixtures containing the radioactive substrate [ $1-^3H$ ]FPP (15-30 mCi/ $\mu$ mol) are incubated at 30.degree. C. for 30 min and one volume of 1:1 (v/v) 40% aqueous KOH:methanol is added to the suspension mixture. Liquid NaCl is added to saturate the solution and 2 ml of naphtha containing 0.5% (v/v) squalene are likewise added.

The suspension is vortexed for 30 s. In each case 1 ml of the naphtha layer is applied to a packed 0.5.times.6 cm aluminum column (80-200 mesh, Fisher) using a Pasteur pipette. The column has been pre-equilibrated with 2 ml of naphtha containing 0.5% (v/v) squalene. The column is then eluted with 5.times.1 ml of toluene containing 0.5% (v/v) squalene. Squalene radioactivity is measured in Cytoscint (ICN) scintillation cocktail in a scintillation counter (Beckman).

This method is a modification of the method described in Radisky et al., *Biochemistry.* 2000 Feb. 22; 39(7):1748-60, Zhang et al. (1993) *Arch. Biochem. Biophys.* 304, 133-143 and Poulter, C. D. et al. (1989) *J. Am. Chem. Soc.* 111, 3734-3739.

The sterol-acyltransferase activity can be determined as described below:

A 200 ml main culture is inoculated at 1% strength from a 20 ml preculture which has been incubated for two days and is incubated in complete medium overnight. The cells are harvested and then washed in two volumes of HP buffer (100 mM potassium phosphate buffer, pH 7.4; 0.5 mM EDTA; 1 mM glutathione; 20  $\mu$ M leupeptin; 64  $\mu$ M benzamide; 2 mM PMSF) and resuspended in HP buffer.

After adding 1 g of glass beads, the cells are disrupted by vortexing 8 times for one minute each. The supernatant is ultracentrifuged at 105000.times.g. The pellet is taken up in 1 ml of ACAT buffer (100 mM potassium phosphate buffer pH7,4; 1 mM glutathione).

The enzyme assay is carried out in a volume of 500  $\mu$ l. The substrate ergosterol is taken up in 62.5 ml of 0.5.times.s.ACAT buffer with vigorous vortexing. 250  $\mu$ l of this solution are used as substrate in the assay. To this, 20  $\mu$ l of protein extract, 50  $\mu$ l of water and 130  $\mu$ l of 0.5.times.ACAT buffer are added.

The mixture is incubated at 37.degree. C. for 15 min. Subsequently, 50  $\mu$ l of  $14C$ -oleoyl-CoA (600000 dpm) are added and the reaction is stopped after one minute by adding 4 ml of chloroform/methanol (2:1). To this, 500  $\mu$ l of  $H_2O$  are added. The phases are separated by briefly centrifuging the suspension at 2000.times.g. The lower phase is evaporated to dryness in a pear-shaped flask and redissolved in 100  $\mu$ l of chloroform/methanol (4:1) and applied to a TLC plate (silica gel 60 F254). The TLC is carried out using petroleum ether/diethyl ether/acetic acid 90:10:1 as eluent. The spots of the steryl ester fractions are cut out and the number of radioactive decays is determined in a scintillation column. The enzyme activity can be determined via the amount of sterile ester-bound  $14C$ -oleoyl-CoA molecules.

In a preferred embodiment of the method of the invention 7-dehydrocholesterol and/or the biosynthetic intermediates and/or intermediates thereof are prepared by culturing

organisms, in particular yeasts, which have, compared to the wild type, an increased activity of at least one of the activities selected from the group consisting of  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity and  $\Delta 24$ -reductase activity and which have additionally a reduced activity of at least one of the activities selected from the group consisting of C24-methyltransferase activity and  $\Delta 22$ -desaturase activity and which have additionally an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity and an increased squalene-epoxidase activity.

In other preferred embodiments of the method of the invention, 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof are prepared by culturing organisms, in particular yeasts, which have, compared to the wild type,

- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity,
- an increased  $\Delta 5$ -desaturase activity,
- an increased  $\Delta 24$ -reductase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity and an increased  $\Delta 5$ -desaturase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity and an increased  $\Delta 24$ -reductase activity,
- an increased  $\Delta 5$ -desaturase activity and an increased  $\Delta 24$ -reductase activity, an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity and an increased  $\Delta 24$ -reductase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity and a reduced C24-methyltransferase activity,
- an increased  $\Delta 5$ -desaturase activity and a reduced C24-methyltransferase activity,
- an increased  $\Delta 24$ -reductase activity and a reduced C24-methyltransferase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity and a reduced C24-methyltransferase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 24$ -reductase activity and a reduced C24-methyltransferase activity,
- an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity and a reduced C24-methyltransferase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity and a reduced C24-methyltransferase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity and a reduced  $\Delta 22$ -desaturase activity,
- an increased  $\Delta 5$ -desaturase activity and a reduced  $\Delta 22$ -desaturase activity,
- an increased  $\Delta 24$ -reductase activity and a reduced  $\Delta 22$ -desaturase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity and a reduced  $\Delta 22$ -desaturase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 24$ -reductase activity and a reduced  $\Delta 22$ -desaturase activity,
- an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity and a reduced  $\Delta 22$ -desaturase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity and a reduced  $\Delta 22$ -desaturase activity,
- an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, a reduced  $\Delta 22$ -desaturase activity and a reduced C24-ethyltransferase activity,
- an increased  $\Delta 5$ -desaturase activity, a reduced  $\Delta 22$ -desaturase activity and a reduced C24-methyltransferase activity,





an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity and a reduced  $\Delta 22$ -desaturase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 5$ -desaturase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity,

an increased lanosterol-C14-demethylase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity,

an increased lanosterol-C14-demethylase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity,

an increased lanosterol-C14-demethylase activity, an increased squalene-epoxidase activity and a reduced C24-methyltransferase activity, or

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity, an increased squalene-epoxidase activity and a reduced C24-methyltransferase activity.

In further particularly preferred embodiments of the method of the invention, 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof are prepared by culturing organisms, in particular yeasts, which have, compared to the wild type, an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity and an increased squalene-epoxidase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity,

an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity, an increased squalene-epoxidase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity,

an increased lanosterol-C14-demethylase activity, an increased squalene-epoxidase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity,

an increased lanosterol-C14-demethylase activity, an increased squalene-epoxidase activity, an increased squalene-synthetase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity,

an increased lanosterol-C14-demethylase activity, an increased squalene-epoxidase activity, an increased sterol-acyltransferase activity and a reduced C24-methyltransferase activity,

an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity, an increased  $\Delta 5$ -desaturase activity, an increased  $\Delta 24$ -reductase activity, a reduced  $\Delta 22$ -desaturase activity, an increased HMG-CoA-reductase activity, an increased lanosterol-C14-demethylase activity, an increased squalene-epoxidase activity, an increased squalene-synthetase activity, an increased sterol-acyltransferase activity and a reduced C24-methyltransferase activity.

Biosynthetic 7-dehydrocholesterol intermediates mean all compounds which appear as intermediates during 7-dehydrocholesterol biosynthesis in the organism used, preferably the compounds mevalonate, farnesyl pyrophosphate, geraniol pyrophosphate, squalene epoxide, 4-dimethylcholesta-8, 14,24-trienol, 4,4-dimethylzymosterol, squalene, farnesol, geraniol, lanosterol, zymosterol, lathosterol, cholesta-7,24-dienol and cholesta-5,7,24-trienol.

Biosynthetic secondary products of zymosterol mean all compounds which can be derived biosynthetically from 7-dehydrocholesterol in the organism used, i.e. for which 7-dehydrocholesterol appears as an intermediate. These may be compounds which the organism used produces naturally from 7-dehydrocholesterol, such as, for example, cholesterol or vitamin D<sub>3</sub> in mammals. However, they also mean compounds which can be produced in the organism from 7-dehydrocholesterol only by introducing genes and enzyme activities of other organisms for which the starting organism has no orthologous gene.

It is possible, for example, to prepare secondary products from 7-dehydrocholesterol, which are naturally present only in mammals, by introducing mammalian genes into yeast:

Introducing a human or murine nucleic acid encoding a human or murine  $\Delta$ -7-reductase enables the yeast to produce cholesterol.

Under UV irradiation, vitamin D<sub>3</sub> (cholecalciferol) is produced from 7-dehydrocholesterol via provitamin D<sub>3</sub> by rearrangement.

Therefore, the biosynthetic secondary products of 7-dehydrocholesterol mean in particular provitamin D<sub>3</sub>, vitamin D<sub>3</sub> (cholecalciferol) and/or cholesterol.

Preferred biosynthetic secondary products are provitamin D<sub>3</sub> and in particular vitamin D<sub>3</sub>.

The compounds prepared in the method of the invention may be used in biotransformations, chemical reactions and for therapeutic purposes, for example for producing vitamin D<sub>3</sub> from 7-dehydrocholesterol via UV irradiation, or for producing steroid hormones via biotransformation starting from cholesta-7,24-dienol or cholesta-5,7,24-trienol.

In the inventive method for preparing 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof, the step of culturing the genetically modified organisms, also referred to as transgenic organisms hereinbelow, is preferably followed by harvesting said organisms and isolating 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof from said organisms.

The organisms are harvested in a manner known per se and appropriate for the particular organism. Microorganisms such as bacteria, mosses, yeasts and fungi or plant cells which are cultured in liquid media by fermentation may be removed, for example, by centrifugation, decanting or filtration.

7-Dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof are isolated from the harvested biomass together or each compound is harvested separately in a manner known per se, for example by extraction and, where appropriate, further chemical or physical purification processes such as, for example, precipitation methods, crystallography, thermal separation methods such as rectification methods or physical separation methods such as, for example, chromatography.

The transgenic organisms, in particular yeasts, are preferably prepared either by transforming the starting organisms, in particular yeasts, with a nucleic acid construct containing at least one nucleic acid selected from the group consisting of nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase, nucleic acids encoding a  $\Delta 5$ -desaturase and nucleic acids encoding a  $\Delta 24$ -reductase which are functionally linked with one or more regulatory signals ensuring transcription and translation in organisms. In this embodiment, the transgenic organisms are prepared using a nucleic acid construct.

In a particularly preferred embodiment, the above-described nucleic acid construct additionally contains at least one nucleic acid selected from the group consisting of nucleic acids encoding an HMG-CoA-reductase activity, nucleic acids encoding a lanosterol-C14-demethylase, nucleic acids encoding a squalene epoxidase, nucleic acids encoding a squalene synthetase and nucleic acids encoding a sterol acyltransferase which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms.

However, the transgenic organisms may also preferably be prepared by transforming the starting organisms, in particular yeasts, with at least one nucleic acid construct selected from the group consisting of nucleic acid constructs containing nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase, nucleic acid construct containing nucleic acids encoding a  $\Delta 5$ -desaturase and nucleic acid construct containing nucleic acids encoding a  $\Delta 24$ -reductase which nucleic acids are in each case functionally linked to one or more regulatory signals ensuring transcription and translation in organisms. In this embodiment, the transgenic organisms are prepared using individual nucleic acid constructs or a combination of nucleic acid constructs.

In a particularly preferred embodiment, the above-described combination of nucleic acid constructs additionally comprises at least one nucleic acid construct selected from

the group consisting of nucleic acid construct containing nucleic acids encoding an HMG-CoA-reductase activity, nucleic acid construct containing nucleic acids encoding a lanosterol-C14-demethylase, nucleic acid construct containing nucleic acids encoding a squalene epoxidase, nucleic acid construct containing nucleic acids encoding a squalene synthetase and nucleic acid construct containing nucleic acids encoding a sterol acyltransferase which nucleic acids are in each case functionally linked to one or more regulatory signals ensuring transcription and translation in organisms.

Nucleic acid constructs in which the encoding nucleic acid sequence is functionally linked to one or more regulatory signals ensuring transcription and translation in organisms, in particular in yeasts, are also referred to as expression cassettes hereinbelow.

Examples of nucleic acid constructs containing said expression cassette are vectors and plasmids.

Accordingly, the invention further relates to nucleic acid constructs, in particular nucleic acid constructs functioning as expression cassettes, which contain at least one nucleic acid selected from the group consisting of nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase, nucleic acids encoding a  $\Delta 5$ -desaturase and nucleic acids encoding a  $\Delta 24$ -reductase which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms.

In a preferred embodiment, said nucleic acid construct additionally comprises at least one nucleic acid selected from the group consisting of nucleic acids encoding an HMG-CoA-reductase activity, nucleic acids encoding a lanosterol-C14-demethylase, nucleic acids encoding a squalene epoxidase, nucleic acids encoding a squalene synthetase and nucleic acids encoding a sterol acyltransferase which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms.

As an alternative, it is also possible to prepare the transgenic organisms of the invention by transformation with individual nucleic acid constructs or with a combination of nucleic acid constructs, said combination comprising at least one nucleic acid construct selected from the groups A to C

A nucleic acid construct comprising nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase, which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms,

B nucleic acid construct comprising nucleic acids encoding a  $\Delta 5$ -desaturase, which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms and

C nucleic acid construct comprising nucleic acids encoding a  $\Delta 24$ -reductase, which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms, and at least one nucleic acid construct selected from the groups D to H

D nucleic acid construct comprising nucleic acids encoding an HMG-CoA reductase, which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms, E nucleic acid construct comprising nucleic acids encoding a lanosterol C14-demethylase, which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms, F nucleic acid construct comprising nucleic acids encoding a squalene epoxidase, which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms, G nucleic acid construct comprising nucleic acids encoding a squalene synthetase, which are functionally linked to one or more regulatory signals ensuring

ing transcription and translation in organisms, H nucleic acid construct comprising nucleic acids encoding a sterol acyltransferase, which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms.

The regulatory signals preferably contain one or more promoters which ensure transcription and translation in organisms, in particular in yeasts.

The expression cassettes include regulatory signals, i.e. regulatory nucleic acid sequences, which control expression of the coding sequence in the host cell. According to a preferred embodiment, an expression cassette comprises upstream, i.e. at the 5' end of the coding sequence, a promoter and downstream, i.e. at the 3' end, a terminator and, where appropriate, further regulatory elements which are operatively linked to the coding sequence for at least one of the above-described genes located in between.

Operative linkage means the sequential arrangement of promoter, coding sequence, where appropriate, terminator and, where appropriate, further regulatory elements in such a way that each of the regulatory elements can properly carry out its function in the expression of the coding sequence.

The preferred nucleic acid constructs, expression cassettes and plasmids for yeasts and fungi and methods for preparing transgenic yeasts and also the transgenic yeasts themselves are described by way of example below.

A suitable promoter of the expression cassette is in principle any promoter which is able to control the expression of foreign genes in organisms, in particular in yeasts.

Preference is given to using in particular a promoter which is subject to reduced regulation in yeast, such as, for example, the medium ADH promoter.

This promoter fragment of the ADH12s promoter, also referred to as ADH1 hereinbelow, exhibits nearly constitutive expression (Ruohonen L, Penttila M, Keranen S. (1991) Optimization of *Bacillus*  $\alpha$ -amylase production by *Saccharomyces cerevisiae*. *Yeast*. May-June; 7(4):337-462; Lang C, Looman A C. (1995) Efficient expression and secretion of *Aspergillus niger* RH5344 polygalacturonase in *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol*. December; 44(1-2):147-56) so that transcriptional regulation no longer proceeds via intermediates of ergosterol biosynthesis.

Other preferred promoters with reduced regulation are constitutive promoters such as, for example, the yeast TEF1 promoter, the yeast GPD promoter or the yeast PGK promoter (Mumberg D, Muller R, Funk M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene*. 1995 Apr. 14; 156(1): 119-22; Chen C Y, Oppermann H, Hitzeman R A. (1984) Homologous versus heterologous gene expression in the yeast, *Saccharomyces cerevisiae*. *Nucleic Acids Res*. December 11; 12(23):8951-70).

The expression cassette may also contain inducible promoters, in particular a chemically inducible promoter which can be used to control expression of the nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase,  $\Delta 5$ -desaturase,  $\Delta 24$ -reductase, HMG-CoA-reductase, lanosterol-C14-demethylase, squalene epoxidase, squalene synthetase or sterol acyltransferase in the organism at a particular time.

Promoters of this kind, such as, for example, the yeast *Cup1* promoter (Etcheverry T. (1990) Induced expression using yeast copper metallothionein promoter. *Methods Enzymol*. 1990; 185:319-29.), the yeast *Gall-10* promoter (Ronicke V, Graulich W, Mumberg D, Muller R, Funk M. (1997) Use of conditional promoters for expression of heterologous proteins in *Saccharomyces cerevisiae*, *Methods Enzymol*. 283:313-22) or the yeast *Pho5* promoter

(Bajwa W, Rudolph H, Hinnen A. (1987) *PHO5* upstream sequences confer phosphate control on the constitutive *PHO3* gene. *Yeast*. 1987 March; 3(1):33-42), may be used, for example.

A suitable terminator of the expression cassette is in principle any terminator which is able to control the expression of foreign genes in organisms, in particular in yeasts.

Preference is given to the tryptophan terminator of yeasts (*TRP1* terminator).

An expression cassette is preferably prepared by fusing a suitable promoter with the above-described nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase,  $\Delta 5$ -desaturase,  $\Delta 24$ -reductase, HMG-CoA-reductase, lanosterol-C14-demethylase, squalene epoxidase, squalene synthetase or sterol acyltransferase and, where appropriate, a terminator according to common recombination and cloning techniques as described, for example, in T. Maniatis, E. F. Fritsch and J. Sambrook, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989) and in T. J. Silhavy, M. L. Berman and L. W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1984) and in Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley-Interscience (1987).

The nucleic acids of the invention may be prepared synthetically or obtained naturally or may contain a mixture of synthetic and natural nucleic acid components and may also comprise various heterologous gene sections of various organisms.

As described above, preference is given to synthetic nucleotide sequences with codons which are preferred by yeasts. These codons which are preferred by yeasts may be determined from codons which have the highest frequency in proteins and which are expressed in most of the interesting yeast species.

When preparing an expression cassette, it is possible to manipulate various DNA fragments in order to obtain a nucleotide sequence which expediently can be read in the correct direction and is provided with a correct reading frame. The DNA fragments may be linked to one another by attaching adaptors or linkers to said fragments.

Expediently, the promoter and terminator regions may be provided in the direction of transcription with a linker or polylinker which contains one or more restriction sites for inserting this sequence. Normally, the linker has from 1 to 10, mostly from 1 to 8, preferably from 2 to 6, restriction sites. Generally, the linker is, within the regulatory regions, less than 100 bp, frequently less than 60 bp, but at least 5 bp, in length. The promoter may be both native or homologous and non-native or heterologous to the host organism. The expression cassette preferably includes in the 5'-3' direction of transcription the promoter, a coding nucleic acid sequence or a nucleic acid construct and a region for transcriptional termination. Various termination regions can be exchanged with one another randomly.

It is furthermore possible to use manipulations which provide appropriate restriction cleavage sites or which remove excess DNA or restriction cleavage sites. In those cases for which insertions, deletions or substitutions such as, for example, transitions and transversions are suitable, in vitro mutagenesis, primer repair, restriction or ligation can be used.

In suitable manipulations such as, for example, restriction, "chewing-back" or filling-in of protruding ends to form "blunt ends", complementary fragment ends may be provided for ligation.

The invention further relates to the use of the above-described nucleic acids, the above-described nucleic acid constructs or the above-described proteins for preparing transgenic organisms, in particular yeasts.

Preferably, said transgenic organisms, in particular yeasts, have an increased content of 7-dehydrocholesterol and/or of the biosynthetic intermediates and/or secondary products thereof compared to the wild type.

Therefore, the invention further relates to the use of the above-described nucleic acids or the nucleic acid constructs of the invention for increasing the content of 7-dehydrocholesterol and/or of the biosynthetic intermediates and/or secondary products thereof in organisms.

The above-described proteins and nucleic acids may be used for producing 7-dehydrocholesterol and/or the biosynthetic intermediates and/or secondary products thereof in transgenic organisms.

The transfer of foreign genes into the genome of an organism, in particular of yeast, is referred to as transformation.

For this purpose, methods known per se can be used for transformation, in particular in yeasts.

Examples of suitable methods for transforming yeasts are the LiAC method as described in Schiestl R H, Gietz R D. (1989) High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier, *Curr Genet.* December; 16 (5-6):339-46, electroporation as described in Manivasakam P, Schiestl R H. (1993) High efficiency transformation of *Saccharomyces cerevisiae* by electroporation. *Nucleic Acids Res.* September 11; 21(18):4414-5, and the preparation of protoplasts, as described in Morgan A J. (1983) Yeast strain improvement by protoplast fusion and transformation, *Experientia Suppl.* 46:155-66

The construct to be expressed is preferably cloned into a vector, in particular into plasmids which are suitable for transforming yeasts, such as, for example, the vector systems Yep24 (Naumovski L, Friedberg E C (1982) Molecular cloning of eucaryotic genes required for excision repair of UV-irradiated DNA: isolation and partial characterization of the RAD<sub>3</sub> gene of *Saccharomyces cerevisiae*. *J Bacteriol* October; 152(1):323-31), Yep13 (Broach J R, Strathern J N, Hicks J B. (1979) Transformation in yeast: development of a hybrid cloning vector and isolation of the CAN1 gene. *Gene.* 1979 December; 8(1):121-33), the pRS series of vectors (Centromer and Episomal) (Sikorski R S, Hieter P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics.* May; 122(1):19-27) and the vector systems YCp19 or pYEXBX.

Accordingly, the invention furthermore relates to vectors, in particular plasmids, which comprise the above-described nucleic acids, nucleic acid constructs or expression cassettes.

The invention further relates to a method for preparing genetically modified organisms by functionally introducing an above-described nucleic acid or an above-described nucleic acid construct into the starting organism.

The invention further relates to said genetically modified organisms, the genetic modification increasing at least one of the activities selected from the group consisting of  $\Delta 8$ - $\Delta 7$ -isomerase activity,  $\Delta 5$ -desaturase activity and  $\Delta 24$ -reductase activity, compared to a wild type.

Preferably, at least one of the activities is increased by increasing the gene expression of at least one nucleic acid selected from the group consisting of nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase, nucleic acids encoding a  $\Delta 5$ -desaturase and nucleic acids encoding a  $\Delta 24$ -reductase.

Preferably, gene expression of the above-described nucleic acids is increased by increasing in the organism the copy number of the nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase, encoding a  $\Delta 5$ -desaturase and/or encoding a  $\Delta 24$ -reductase.

Accordingly, the invention preferably relates to an above-described genetically modified organism which contains two or more nucleic acids encoding a  $\Delta 8$ - $\Delta 7$ -isomerase and/or two or more nucleic acids encoding a  $\Delta 5$ -desaturase and/or two or more nucleic acids encoding a  $\Delta 24$ -reductase.

In a preferred embodiment, the genetically modified organism has, compared to the wild type, in addition to the above-described genetic modifications a reduced activity of at least one of the activities selected from the group consisting of C24-methyltransferase activity and  $\Delta 22$ -desaturase activity.

The reduction of at least one of the activities is preferably caused by reducing, compared to the wild type, gene expression of at least one nucleic acid selected from the group consisting of nucleic acids encoding a C24-methyltransferase and nucleic acids encoding a  $\Delta 22$ -desaturase.

A particularly preferred genetically modified organism has, apart from the above-described genetic modifications, no functional C24-methyltransferase gene and/or  $\Delta 22$ -desaturase gene.

Particular preference is given to above-mentioned genetically modified organisms in which the genetic modification additionally increases at least one of the activities selected from the group consisting of HMG-CoA-reductase activity, lanosterol-C14-demethylase activity, squalene-epoxidase activity, squalene-synthetase activity and sterol-acyltransferase activity compared to a wild type.

Preferably, at least one of these activities is increased, as mentioned above, by increasing, compared to the wild type, gene expression of at least one nucleic acid selected from the group consisting of nucleic acids encoding an HMG-CoA-reductase activity, nucleic acids encoding a lanosterol-C14-demethylase, nucleic acids encoding a squalene epoxidase, nucleic acids encoding a squalene synthetase and nucleic acids encoding a sterol acyltransferase.

Preferably, gene expression of at least one nucleic acid selected from the group consisting of nucleic acids encoding an HMG-CoA-reductase activity, nucleic acids encoding a lanosterol-C14-demethylase, nucleic acids encoding a squalene epoxidase, nucleic acids encoding a squalene synthetase and nucleic acids encoding a sterol acyltransferase is increased compared to the wild type by increasing in the organism the copy number of at least one nucleic acid selected from the group consisting of nucleic acids encoding an HMG-CoA-reductase activity, nucleic acids encoding a lanosterol-C14-demethylase, nucleic acids encoding a squalene epoxidase, nucleic acids encoding a squalene synthetase and nucleic acids encoding a sterol acyltransferase.

Accordingly, the invention preferably relates to an above-described genetically modified organism which contains two or more of at least one nucleic acid selected from the group consisting of nucleic acids encoding an HMG-CoA-reductase activity, nucleic acids encoding a lanosterol-C14-demethylase, nucleic acids encoding a squalene epoxidase, nucleic acids encoding a squalene synthetase and nucleic acids encoding a sterol acyltransferase.

In particular, the invention preferably relates to a genetically modified organism which contains, in addition to the above-described genetic modifications, two or more nucleic acids encoding an HMG-CoA-reductase and/or two or more nucleic acids encoding a lanosterol-C14-demethylase and/or two or more nucleic acids encoding a squalene epoxidase

and/or two or more nucleic acids encoding a squalene synthetase and/or two or more nucleic acids encoding a sterol acyltransferase.

The above-described genetically modified organisms have, compared to the wild type, an increased content of 7-dehydrocholesterol and/or of the biosynthetic intermediates and/or secondary products thereof.

Accordingly, the invention relates to an above-described genetically modified organism which, compared to the wild type, has an increased content of 7-dehydrocholesterol and/or of the biosynthetic intermediates and/or secondary products thereof.

Preferred genetically modified organisms are yeasts or fungi which have been genetically modified according to the invention, in particular yeasts which have been genetically modified according to the invention, in particular the yeast species *Saccharomyces cerevisiae* which has been genetically modified according to the invention, in particular the genetically modified yeast strains *Saccharomyces cerevisiae* AH22, *Saccharomyces cerevisiae* GRF, *Saccharomyces cerevisiae* DBY747 and *Saccharomyces cerevisiae* BY4741.

In the scope of the present invention, increasing the content of 7-dehydrocholesterol and/or of the biosynthetic intermediates and/or secondary products thereof preferably means the artificially acquired ability to produce biosynthetically an increased amount of at least one of these compounds mentioned above in the genetically modified organism compared to the genetically unmodified organism.

Accordingly, as mentioned at the beginning, wild type preferably means the genetically unmodified organism, but in particular the reference organism mentioned above.

An increased content of 7-dehydrocholesterol and/or of the biosynthetic intermediates and/or secondary products thereof in comparison with the wild type means in particular the increase in the content of at least one of the abovementioned compounds in the organism by at least 50%, preferably 100%, more preferably 200%, particularly preferably 400%, in comparison with the wild type.

The content of at least one of the mentioned compounds is preferably determined according to analytical methods known per se and preferably refers to those compartments of the organism, in which sterols are produced.

The invention is illustrated by the following examples but is not limited to them:

## I. GENERAL EXPERIMENTAL CONDITIONS

### 1. Restriction

Restriction of the plasmids (1 to 10 µg) was carried out in 30 µl reaction mixtures. For this purpose, the DNA was taken up in 24 µl of H<sub>2</sub>O and admixed with 3 µl of the appropriate buffer, 1 ml of BSA (bovine serum albumin) and 2 µl of enzyme. The enzyme concentration was 1 unit/µl or 5 units/µl, depending on the amount of DNA. In some cases, 1 µl of RNase was added to the reaction mixture in order to degrade the tRNA. The restriction mixture was incubated at 37.degree. C. for 2 hours. The restriction was monitored using a minigel.

### 2. Gel Electrophoreses

The gel electrophoreses were carried out in minigel or wide minigel apparatuses. The minigels (approx. 20 ml, 8 pockets) and the wide minigels (50 ml, 15 or 30 pockets) consisted of 1% strength agarose in TAE. The running buffer used was 1.times.TAE.

After adding 3 µl of stop solution, the samples (10 µl) were applied. A-DNA cut with HindIII (bands at: 23.1 kb; 9.4 kb; 6.6 kb; 4.4 kb; 2.3 kb; 2.0 kb; 0.6 kb) served as

standard. For fractionation, a voltage of 80 V was applied for 45 to 60 min. Thereafter, the gel was stained in ethidium bromide solution and documented under UV light using the INTAS video documentation system or photographed using an orange filter.

### 3. Gel Elution

The desired fragments were isolated by means of gel elution. The restriction mixture was applied to several pockets of a minigel and fractionated. Only λ-HindIII and a "sacrifice lane" were stained in ethidium bromide solution, examined under UV light, and the desired fragment was marked. This prevented the DNA of the remaining pockets from being damaged by ethidium bromide and UV light. Putting the stained and unstained gel slices side by side made it possible to excise the desired fragment from the unstained gel slice on the basis of the marking. The agarose slice with the fragment to be isolated was introduced into a dialysis tube, sealed in air-bubble-free together with a small amount of TAE buffer and introduced into the BioRad minigel apparatus. The running buffer was 1.times.TAE and the voltage was 100 V for 40 min. Afterward, the polarity was switched for 2 min in order to redissolve DNA sticking to the dialysis tube. The buffer in the dialysis tube, which contained the DNA fragments, was transferred to reaction vessels and subjected to ethanol precipitation. For this purpose, 1/10 volume of 3M sodium acetate, tRNA (1 µl per 50 µl of solution) and 2.5 volumes of ice-cold 96% strength ethanol were added to the DNA solution. The mixture was incubated at -20.degree. C. for 30 min and then removed by centrifugation at 12 000 rpm, 4.degree. C., 30 min. The DNA pellet was dried and taken up in 10 to 50 µl of H<sub>2</sub>O (depending on the amount of DNA).

### 4. Klenow Treatment

The Klenow treatment fills in protruding ends of DNA fragments, resulting in blunt ends. Per 1 µg of DNA, the following reaction mixture was pipetted: DNA.times. .times. pellet+.times. 11.times. µ.times. .times. 1.times. .times. H 2.times. 0+.times. 1.5.times. .times. 10.times. Klenow.times. .times. buffer+.times. 1.times. µ.times. .times. 1.times. .times. 0.1.times. .times. M.times. .times.DTT+.times. 1.times. µ.times. .times. I.times. .times. nucleotide.times. .times. (dNTP.times. .times. 2.times. .times. mM)+.times. 1.times. µ.times. .times. I.times. .times. Klenow.times. .times. polymerase.times. .times. (1.times. .times. unit/µ.times. .times. I) 25

The DNA should be from an ethanol precipitation, in order to prevent contaminations from inhibiting the Klenow polymerase. The reaction mixture was incubated at 37.degree. C. for 30 min, and the reaction was stopped by incubating for another 5 min at 70.degree. C. The DNA was recovered from the reaction mixture by ethanol precipitation and taken up in 10 µl of H<sub>2</sub>O.

### 5. Ligation

The DNA fragments to be ligated were combined. The final volume of 13.1 µl contained approx. 0.5 µl of DNA with a vector/insert ratio of 1:5. The sample was incubated at 70.degree. C. for 45 seconds, cooled to room temperature (approx. 3 min) and then incubated on ice for 10 min. The ligation buffers were then added: 2.6 µl of 500 mM Tris-HCl pH 7.5 and 1.3 µl of 100 mM MgCl<sub>2</sub>, followed by incubation on ice for a further 10 min. After adding 1 µl of 500 mM DTT and 1 µl of 10 mM ATP and another 10 min on ice, 1 µl of ligase (1 unit/pl) was added. The whole treatment should be carried out as free from vibrations as possible so that adjoining DNA ends are not separated again. The ligation was carried out at 14.degree. C. over night.

6. Transformation of *E. Coli*

Competent *Escherichia coli* (*E. coli*) NM522 cells were transformed with the DNA of the ligation mixture. A reaction mixture containing 50 µg of the pScL3 plasmids and a reaction mixture without DNA were run as positive control and zero control, respectively. For each transformation mixture, 100 µl of 8% PEG solution, 10 µl of DNA and 200 µl of competent cells (*E. coli* NM522) were pipetted into a benchtop-centrifuge tube. The reaction mixtures were put on ice for 30 min and agitated occasionally.

Then the heat shock was carried out: 1 min at 42.degree. C. For regeneration, 1 ml of LB medium was added to the cells and the suspension was incubated on a shaker at 37.degree. C. for 90 min. In each case, 100 µl of the undiluted reaction mixtures, a 1:10 dilution and a 1:100 dilution were plated on LB+ampicillin plates and incubated at 37.degree. C. over night.

7. Plasmid Isolation from *E. Coli* (Miniprep)

*E. coli* colonies were grown in 1.5 ml of LB+ampicillin medium in benchtop-centrifuge tubes at 37.degree. C. and 120 rpm over night. On the next day, the cells were removed by centrifugation at 5000 rpm and 4.degree. C. for 5 min and the pellet was taken up in 50 µl of TE buffer. 100 µl of 0.2 N NaOH, 1% SDS solution were added to and mixed with each reaction mixture, and the mixture was put on ice for 5 min (lysis of the cells). Then, 400 µl of Na acetate/NaCl solution (230 µl of H<sub>2</sub>O, 130 µl of 3 M sodium acetate, 40 µl of 5M NaCl) were added, the reaction mixture was mixed and put on ice for a further 15 min (protein precipitation). After centrifugation at 11 000 rpm for 15 minutes, the supernatant containing the plasmid DNA was transferred to an Eppendorf vessel. If the supernatant was not completely clear, centrifugation was repeated. 360 µl of ice-cold isopropanol were added to the supernatant and the reaction mixture was incubated at -20.degree. C. for 30 min (DNA precipitation). The DNA was removed by centrifugation (15 min, 12 000 rpm, 4.degree. C.), the supernatant was discarded, the pellet was washed in 100 µl of ice-cold 96% strength ethanol, incubated at -20.degree. C. for 15 min and again removed by centrifugation (15 min, 12 000 rpm, 4.degree. C.). The pellet was dried in a Speed Vac and then taken up in 100 µl of H<sub>2</sub>O. The plasmid DNA was characterized by restriction analysis. For this purpose, 10 µl of each reaction mixture were restriction-digested and fractionated gel-electrophoretically in a wide minigel (see above).

8. Plasmid Preparation from *E. Coli* (Maxiprep)

In order to isolate larger amounts of plasmid DNA, the maxiprep method was carried out. Two flasks with 100 ml of LB+ampicillin medium were inoculated with a colony or with 100 µl of a frozen culture which carries the plasmid to be isolated and incubated at 37.degree. C. and 120 rpm over night. On the next day, the culture (200 ml) was transferred to a GSA beaker and centrifuged at 4000 rpm (2600.times.g) for 10 min. The cell pellet was taken up in 6 ml of TE buffer. The cell wall was digested by adding 1.2 ml of lysozyme solution (20 mg/ml of TE buffer) and incubated at room temperature for 10 min. Subsequently, the cells were lysed with 12 ml of a 0.2 N NaOH, 1% SDS solution, followed by incubation at room temperature for another 5 min. The proteins were precipitated by adding 9 ml of a cooled 3 M sodium acetate solution (pH 4.8) and incubation on ice for 15 minutes. After centrifugation (GSA: 13 000 rpm (27 500.times.g), 20 min, 4.degree. C.), the supernatant containing the DNA was transferred to a new GSA beaker and the DNA was precipitated with 15 ml of ice-cold isopropanol and incubation at -20.degree. C. for 30 min. The DNA pellet was washed in 5 ml of ice-cold ethanol and dried in air

(approx. 30-60 min). Thereafter, it was taken up in 1 ml of H<sub>2</sub>O. The plasmid was checked by restriction analysis. The concentration was determined by applying dilutions to a minigel. The salt content was reduced by microdialysis (pore size 0.025 µm) for 30-60 minutes.

## 9. Transformation of Yeast

For the transformation of yeast, a preculture of the strain *Saccharomyces cerevisiae* AH22 was prepared. A flask containing 20 ml of YE medium was inoculated with 100 µl of the frozen culture and incubated at 28.degree. C. and 120 rpm over night. The main culture was carried out under the same conditions in flasks containing 100 ml of YE medium which was inoculated with 10 µl, 20 µl or 50 µl of the preculture.

## 9.1 Preparation of Competent Cells

On the next day, the cells in the flasks were counted by means of a Thoma chamber and the flask containing from 3-5.times.10<sup>7</sup> cells/ml was chosen for the subsequent procedure. The cells were harvested by centrifugation (GSA: 5000 rpm (4000.times.g) 10 min). The cell pellet was taken up in 10 ml of TE buffer and distributed into two benchtop-centrifuged tubes (5 ml each). The cells were removed by centrifugation at 6000 rpm for 3 min and then washed twice with in each case 5 ml of TE buffer. The cell pellet was then taken up in 330 µl of lithium acetate buffer per 10<sup>9</sup> cells, transferred to a sterile 50 ml Erlenmeyer flask and agitated at 28.degree. C. for one hour. As a result, the cells were competent for transformation.

## 9.2 Transformation

For each transformation mixture, 15 µl of herring sperm DNA (10 mg/ml), 10 µl of the DNA to be transformed (approx. 0.5 µg) and 330 µl of competent cells were pipetted into a benchtop-centrifuged tube and incubated at 28.degree. C. for 30 min (without agitation). Then, 700 µl of 50% PEG 6000 were added and the suspension was incubated at 28.degree. C. for another hour, without agitation. This was followed by a heat shock at 42.degree. C. for 5 min. 100 µl of the suspension were plated on selection medium (YNB, Difco) in order to select for leucine prototrophy. In the case of selection for G418 resistance, the cells are regenerated after the heat shock (see under 9.3 Regeneration phase).

## 9.3 Regeneration Phase

Since the selection marker is the resistance to G418, the cells needed time to express the resistance gene. 4 ml of YE medium were added to the transformation mixtures which were then incubated on the shaker (120 rpm) at 28.degree. C. over night. On the next day, the cells were removed by centrifugation (6000 rpm, 3 min), taken up in 1 ml YE medium, and 100 µl or 200 µl thereof were plated on YE+G418 plates. The plates were incubated at 28.degree. C. for several days.

## 10. PCR Reaction Conditions

The reaction conditions for the polymerase chain reaction must be optimized in each individual case and do not apply absolutely to each reaction mixture. Thus it is possible, inter alia, to vary the amount of DNA used, the salt concentrations and the melting temperature. For our task, it proved advantageous to combine in an Eppendorf vessel which was suitable for use in a thermocycler the following substances: 5 µl of Super buffer, 811 of dNTPs (0.625 µM each), 5' primer, 3' primer and 0.2 µg of template DNA, dissolved in enough water so as to result in a total volume of 50 µl for the PCR reaction mixture, were added to 2 µl of (=0.1 U) Super Taq polymerase. The reaction mixture was briefly centri-

fused and overlaid with a drop of oil. Between 37 and 40 cycles were chosen for amplification.

## II. EXAMPLES

### Example 1

Expression and overexpression of a truncated HMG-CoA reductase, a squalene epoxidase (ERG1) and/or a lanosterol-C14-demethylase (ERG11), partially with deletion of ERG5 and ERG6 in *S. cerevisiae* GRF18 and GRFura3, respectively.

#### 1.1 Preparation of the Plasmids pFlat1 and pFlat3 and pFlat4

The expression vector pFlat3 was prepared by linearizing the plasmid YEp24 (Naumovski L, Friedberg E C (1982) Molecular cloning of eucaryotic genes required for excision repair of UV-irradiated DNA: isolation and partial characterization of the RAD<sub>3</sub> gene of *Saccharomyces cerevisiae*. J Bacteriol October; 152(1):323-31) via restriction with SphI and a 900 bp SphI fragment of the vector pPT2B (Lang C, Looman A C. (1995) Efficient expression and secretion of *Aspergillus niger* RH5344 polygalacturonase in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol. December; 44(1-2): 147-56) which contains the ADH1 promoter and the TRP1 terminator of the yeast *Saccharomyces cerevisiae* and a multiple-cloning site of the vector pUC19 (Yanisch-Perron C, Vieira J, Messing J. (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13 mp18 and pUC19 vectors. Gene. 1985; 33(1): 103-19.) was integrated.

The multiple-cloning site was extended by a polylinker containing the restriction sites NotI and XhoI. The polylinker was integrated via the Sall cleavage site of the vector. The resulting plasmid is denoted pFlat1.

The vector pFlat3 was prepared by linearizing the vector pFlat1 by the enzyme NcoI and blunt-ending it by means of Klenow treatment. This was followed by integrating a BamHI fragment which had been blunt-ended by means of Klenow-polymerase treatment and which contains the yeast LEU2 gene and originates from the plasmid YDpL (Berben, G., Dumont, J., Gilliquet, V., Bolle, P. A. and Hilger F. (1991) The YDp Plasm ids: a Uniform Set of Vectors Bearing Versatile Disruption Cassettes for *Saccharomyces cerevisiae*. Yeast 7: 475-477.).

The vector pFlat4 was prepared by linearizing the vector pFlat1 by the enzyme NcoI and blunt-ending it by means of Klenow treatment. This was followed by integrating a BamHI fragment which had been blunt-ended by means of Klenow-polymerase treatment and which contains the yeast HIS3 gene and originates from plasmid YDpH (Berben et al., 1991).

#### 1.2 Integration of ERG1, ERG11, ERG4, ERG2 or ERG3 or of the Δ24-Reductase Gene into the Vectors pFlat1, pFlat3 and pFlat4

First, a NotI restriction cleavage site was inserted at the 5'-coding side of the genes ERG1, ERG11, ERG4, Δ24-reductase, ERG2 or ERG3 and an XhoI restriction cleavage site was inserted at the 3'-coding side of said genes by means of PCR and the corresponding coding regions were amplified. Subsequently, the amplicons were treated with the restriction enzymes NotI and XhoI. The plasmids pFlat1, pFlat3 and pFlat4 were treated in parallel with enzymes NotI and XhoI. The cleaved amplicons were then integrated into the cleaved plasmids via ligation using T4 ligase. FIG. 7 depicts as an example the plasmid pFLAT-3-ERG4.

Primer sequences for cloning ERG1, ERG11, ERG2, ERG3, ERG4, Δ24-reductase: TABLE-US-00002 Primer ERG1-5' (SEQ. ID. No. 51): CTGCGGCCGC ATCATGTCTG CTGTAAACGT TGC Primer ERG1-3' (SEQ. ID. No. 52): TTCTCGAGTT AACCAATCAA CTCACCAAAC Primer ERG11-5' (SEQ. ID. No. 53): CTGCGGCCGCAG-GATGTCTGCTACCAAGTCAATCG Primer ERG11-3' (SEQ. ID. No. 54):

ATCTCGAGCTTAGATCTTTTGTCTGGATTCTC  
 10 Primer ERG2-5' (SEQ. ID. No. 55): CTGCGGCCGCAC-CATGAAGTTTTTCCCCT CC Primer ERG2-3' (SEQ. ID. No. 56): TTCTCGAGTTAGAACTTTTGTTTTG-CAACAAG Primer ERG3-5' (SEQ. ID. No. 57): CTGCGGCCGCAATATGGATTGGTCTTAGAAGTCG Primer ERG3-3' (SEQ. ID. No. 58): AACTCGAGTCAGTTGT-TCTTCTTGGTATTTG Primer ERG4-5' (SEQ. ID. No. 59): CTGCGGCCGCACTATGGCAAAGGATAATAGTGAG  
 15 Primer ERG4-3' (SEQ. ID. No. 60): TTCTCGAGCTA-GAAAACATAAGGAATAAAGAC Primer Δ24R-5' (SEQ. ID. No. 47): CTGCGGCCGCAAGATGGAGCCCGGT-GTCGC Primer Δ24R-3' (SEQ. ID. No. 48) AACTC-GAGTCAGTGCCTTGCCGCCTTGC  
 20 1.3 Preparation of the Integration Vectors pUG6-tHMG, pUG6-ERG1, pUG6-ERG11 1.3.1 pUG6-tHMG

The DNA sequence for the expression cassette composed of ADH1-promoter-tHMG-tryptophan-terminator was isolated from the vector YepH2 (Polakowski, T., Stahl, U., Lang, C. (1998): Overexpression of a cytosolic HMG-CoA reductase in yeast leads to squalene accumulation. Appl. Microbiol. Biotechnol. 49: 66-71) by restriction with the enzymes EcoRV and Bsp68I (NruI) by using standard methods. The DNA fragment obtained was cloned with blunt ends into the EcoRV cleavage site of the vector pUG6 (Guldener, U et al. (1996): A new efficient gene disruption cassette for repeated use in budding yeast, Nucleic Acids Res. July 1; 24(13):2519-24), resulting in the vector denoted pUG6-tHMG (FIG. 1).

#### 1.3.2 pUG6-ERG1

The DNA sequence for the expression cassette composed of ADH1-promoter-ERG1-tryptophan-terminator was isolated from the vector pFlat3-ERG1 by restriction with the enzymes NheI and Bsp68I (NruI), using standard methods. After Klenow treatment, the DNA fragment obtained was cloned with blunt ends into the EcoRV cleavage site of the vector pUG6 (Guldener, U et al. (1996): A new efficient gene disruption cassette for repeated use in budding yeast, Nucleic Acids Res. July 1; 24(13):2519-24), resulting in the vector denoted pUG6-ERG1 (FIG. 2).

#### 1.3.3 pUG6-ERG11

The DNA sequence for the expression cassette composed of ADH1-promoter-ERG11-tryptophan-terminator was isolated from the vector pFlat3-ERG11 by restriction with the enzymes EcoRV and Bsp68I (NruI) using standard methods. The DNA fragment obtained was cloned with blunt ends into the EcoRV cleavage site of the vector pUG6 (Guldener, U et al. (1996): A new efficient gene disruption cassette for repeated use in budding yeast, Nucleic Acids Res. July 1; 24(13):2519-24), resulting in the vector denoted pUG6-ERG11 (FIG. 3).

#### 1.4. Integrative Transformation of the Expression Cassettes into the Yeast Strains GRF or GRFura3

After plasmid isolation, fragments of the vectors pUG6-tHMG, pUG6-ERG1 and pUG6-ERG11 were amplified by means of PCR in such a way that the resulting fragments consist of the following components: IoxP-kanMX-loxP-ADH1 promoter-target gene-tryptophan terminator, with

target gene meaning tHMG, ERG1 and, ERG11 and kanMX respectively, meaning a kanamycin-resistance gene.

The selected primers were oligonucleotide sequences which contain in the annealing region the sequences beyond the cassettes to be amplified of the vector pUG6-target gene and which contain at the 5' and 3' protruding ends in each case 40 base pairs of the 5' or 3' sequence of the integration locus. This ensures that on the one hand the entire fragment, including KanMX and target gene, is amplified and, on the other hand, this fragment can then be transformed into yeast and be integrated by homologous recombination into the target gene locus of the yeast. Depending on the target gene locus in the yeast, the following oligonucleotide sequences were used as primers:

For integration at the URA3 gene locus: TABLE-US-00003 For integration at the URA3 gene locus: URA3-Crelox-5' (SEQ. ID. No. 33): 5'-ATGTCGAAAG CTA-CATATAA GGAACGTGCT GCATCTCATC CCAG-CTGAAG CTTCGTACGC-3' URA3-Crelox-3' (SEQ. ID. No. 34): 5'-TTAGTTTTGC TGGCCGCATC TTCTCAA-ATA TGCTTCCCAG GCATAGGCCA CTAGTGGATC TG-3' For integration at the LEU2 gene locus: LEU2-Crelox-5' (SEQ. ID. No. 35): 5'-GAAIACTCAG GTATCG-TAAG ATGCAAGAGT TCGAATCTCT CCAGCTGAAG CTTCGTACGC-3' LEU2-Crelox-3' (SEQ. ID. No. 36): 5'-TCTACCCTAT GAACATAATC CATTITGTAA TTTC-GTGTGC GCATAGGCCA CTAGTGGATC TG-3'

For integration at the HIS3 gene locus: TABLE-US-00004 HIS3-Crelox-5' (SEQ. ID. No. 37): 5'-ATGACA-GAGC AGAAACCCCT AGTAAAGCGT ATTACAAATG CCAGCTGAAG CTTCGTACGC-3' HIS3-Crelox-3' (SEQ. ID. No. 38): 5'-CTACATAAGA ACACTTTGG TGGAGGGAAC ATCGTTGGTA GCATAGGCCA CTAGTG-GATC TG-3'

For integration at the ERG6 gene locus: TABLE-US-00005 ERG6-Crelox-5' (SEQ. ID. No. 39): 5'-ATGAGT-GAAA CAGAATTGAG AAAAAGACAG GCCCAAT-TCA CCAGCTGAAG CTTCGTACGC-3' ERG6-Crelox-3' (SEQ. ID. No. 40): 5'-TTATTGAGTT GCTTCTTGGG AAGTTTGGGA GGGGGTTTCG GCATAGGCCA CTAG-TGGATC TG-3'

For integration at the ERG5 gene locus: TABLE-US-00006 ERG5-Crelox-5' (SEQ. ID. No. 41): 5'-ATGAGT-TCTG TCGCAGAAAA TATAATACAA CATGCCACTC CCAGCTGAAG CTTCGTACGC-3' ERG5-Crelox-3' (SEQ. ID. No. 42): 5'-TTATTCTGAAG ACTTCTCCAG TA-ATTGGGTC TCTCTTTTTG GCATAGGCCA CTAGTG-GATC TG-3'

The resistance to Geneticin (G418) served as selection marker. The resulting strains contained a copy of the particular target gene (tHMG, ERG1 or ERG11) under the control of the ADH promoter and the tryptophan terminator. At the same time, it was possible to delete the particular gene of the target locus by integrating the expression cassette. In

order to subsequently remove again the gene for G418 resistance, the resultant yeast strain was transformed with the cre recombinase-containing vector pSH47 (Guldener U, Heck S, Fielder T, Beinhauer J, Hegemann J H. (1996) A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res. July 1; 24(13):2519-24). This vector caused the expression of cre recombinase in the yeast, and, as a consequence, the sequence region within the two loxP sequences was removed by recombination, and this in turn resulted in only one of the two loxP sequences and the ADH1 promoter-target gene-tryptophan terminator expression cassette remaining in the target gene locus.

As a consequence, the yeast strain loses its G418 resistance again and is therefore suitable for integrating or removing further genes by means of this "cre-lox" system into or from said yeast strain. The vector pSH47 can then be removed selectively by cultivation on FOA medium.

Thus it is possible to integrate a plurality of target genes successively into the yeast strain under the control of the ADH1 promoter and tryptophan terminator at various target loci.

First, a target gene is integrated at the URA3 locus or a ura3 strain is used in order to render the yeast strain uracil-auxotrophic, since the vector pSH47 contains a URA3 gene for selection of uracil-prototrophic strains. FIG. 4 shows an example of the method.

This method produced the yeast integration and deletion strains listed in Table 1, with, in a manner known per se, the gene in lower-case letters representing a deletion and the gene in capital letters representing an integration.

TABLE 1

No.	Strain name	Modification No. Strain name compared to GRF yeast strain
I	GRFtH1	ura3, tHMG:leu2
II	GRFtH1e1	ERG1:ura3, tHMG:leu2
III	GRFtH1E11	ura3, tHMG:leu2, ERG11:his3
IV	GRFtH1E1E11	ERG1:ura3, tHMG:leu2, ERG11:his3
V	GRFtH1E1E11erg5erg6	ura3, tHMG:leu2, ERG1:erg6, ERG11:erg5
VI	GRFtH1erg5erg6	ura3, tHMG:leu2, erg5, erg6

The yeast strains were cultured in a culture volume of 20 ml in WMVIII medium at 28.degree. C. and 160 rpm for 48 hours. Subsequently, 500 µl of this preculture were transferred to a 50 ml main culture of the same medium and cultured in a baffled flask at 28.degree. C. and 160 rpm for 3 days.

After 3 days, the sterols and squalene were extracted (Parks L W, Bottema C D, Rodriguez R J, Lewis T A. (1985) Yeast sterols: yeast mutants as tools for the study of sterol metabolism. Methods Enzymol. 1985; 111:333-46.) and analyzed by means of gas chromatography. The following values were obtained (see Table 2).

		Content of sterols 1 to 11 in [peak area/gTS]										
No.	Strain name	1	2	3	4	5	6	7	8	9	10	11
I	GRFtH1	9.9	0.8	0.3	1.2	1.1	1.0	0.0	0.0	0.0	0.0	4.7
II	GRFtH1E1	6.8	1.9	0.4	1.5	2.2	2.1	0.0	0.0	0.0	0.0	6.9
III	GRFtH1E11	9.9	0.4	0.7	2.3	1.9	1.9	0.0	0.0	0.0	0.0	5.0
IV	GRFtH1E1E11	6.0	1.2	0.9	3.0	2.3	2.2	0.0	0.0	0.0	0.0	7.2

-continued

No.	Strain name	Content of sterols 1 to 11 in [peak area/gTS]										
		1	2	3	4	5	6	7	8	9	10	11
V	GRFtH1E1E11 erg5erg6	5.8	0.8	0.4	23.1	0.0	0.0	0.0	0.0	11.8	0.0	0.0
VI	GRFtH1erg5erg6	9.9	0.8	0.3	12.6	0.0	0.0	0.0	0.0	7.1	0.0	0.0

1 = Squalene  
 2 = Lanosterol  
 3 = Dimethylzymosterol  
 4 = Zymosterol  
 5 = Fecosterol  
 6 = Episterol  
 7 = Cholesta-7,24-dienol  
 8 = Cholesta-8-enol  
 9 = Cholesta-5,7,24-trienol  
 10 = 7-Dehydrocholesterol  
 11 = Ergosterol

## Example 2

Expression of the Heterologous Gene Encoding a  $\Delta 8$ - $\Delta 7$ -Isomerase (Ebp) from Mice (*Mus musculus*) in Yeast

The cDNA sequence of *Mus musculus*  $\Delta 8$ - $\Delta 7$ -isomerase (Moebius, F. F., Soellner, K. E. M., Fiechter, B., Huck, C. W., Bonn, G., Glossmann, H. (1999): Histidine77, Glutamic Acid123, Threonine126, Asparagine194, and Tryptophan197 of Human Emopamil Protein Are Required for in Vivo Sterol  $\Delta 8$ - $\Delta 7$  Isomerisation. *Biochem. 38*, 1119-1127) was amplified by PCR from the cDNA clone IMAGp998A22757 (Host: *E. coli* DH10B) of the Deutsches Ressourcenzentrum für Genomforschung [German resource center for genome research] GmbH (Berlin).

The primers used here are the DNA oligomers Ebp-5' (SEQ. ID. No. 43) and Ebp-3' (SEQ. ID. No. 44). The DNA fragment obtained was treated with restriction enzymes NotI and XhoI and then integrated into the vectors pFlat3 and pFlat1 (FIG. 4) which likewise been treated with the enzymes NotI and XhoI beforehand by means of a ligase

20 reaction. The resulting vectors pFlat1-EBP and pFlat3-EBP (FIG. 5a) contain the EBP gene under the control of the ADH promoter and the tryptophan terminator.

The expression vector pFlat3-EBP was then transformed into the yeast strains I to VI of Table 1 from Example 1 and also into the GRFura3 strain. The yeast strains obtained in this way were then cultured in a culture volume of 20 ml in WMVIII medium at 28.degree. C. and 160 rpm for 48 hours. Subsequently, 500  $\mu$ l of this preculture were transferred to a 50 ml main culture of the same medium and cultured in a baffled flask at 28.degree. C. and 160 rpm for 3 days.

30 The sterols were extracted after 3 days and analyzed by means of gas chromatography, as described in Example 1. The influence of the expression of a *Mus musculus*  $\Delta 8$ - $\Delta 7$ -isomerase in combination with the expression of the transcriptionally deregulated intrinsic yeast genes tHMG and/or ERG1 and/or ERG11 and/or deletion of the intrinsic yeast genes ERG6 and ERG5 is listed in Table 3. The abbreviations have the following meanings: -=decrease; 0=no change; /=not present; +, ++, +++, ++++=concentrated to highly concentrated.

No.	Strain name	Influence of the genetic modifications on the sterol content compared to the GRF yeast strain										
		1	2	3	4	5	6	7	8	9	10	11
VII	GRFtH1 pFlat3-Ebp	0	0	0	0	0	0	/	/	/	/	0
VIII	GRFtH1E1 pFlat3-Ebp	0	0	0	-	0	0	+	/	/	/	0
IX	GRFtH1E11 pFlat3- Ebp	0	0	0	-	0	0	+	/	/	/	0
X	GRFtH1E1E11 pFlat3- Ebp	0	0	0	-	0	0	+	/	/	/	0
XI	GRFtH1E1E11erg5erg6 pFlat3-Ebp	0	0	0	-	/	/	+	/	++	/	/
XII	GRFtH1erg5erg6 pFlat3-Ebp	0	0	0	-	/	/	+	/	+	/	/

1 = Squalene  
 2 = Lanosterol  
 3 = Dimethylzymosterol  
 4 = Zymosterol  
 5 = Fecosterol  
 6 = Episterol  
 7 = Cholesta-7,24-dienol  
 8 = Cholesta-8-enol  
 9 = Cholesta-5,7,24-trienol  
 10 = 7-Dehydrocholesterol  
 11 = Ergosterol

Expression of the Heterologous Gene Encoding a  $\Delta 5$ -Desaturase (Sc5d) from Mice (*Mus musculus*) in Yeast

The cDNA sequence of *Mus musculus*  $\Delta 5$ -desaturase (Nishi, S., Hideaki, N., Ishibashi, T. (2000): cDNA cloning of the mammalian sterol C5-desaturase and the expression in yeast mutant. *Biochim. Biophys. A* 1490, 106-108) was amplified by PCR from the cDNA clone IMAGp998K144618 (Host: *E. coli* DH10B) of the Deutsches Ressourcenzentrum für Genomforschung [German resource center for genome research] GmbH (Berlin). The primers used here are the DNA oligomers Sc5d-5' (SEQ. ID. No. 45) and Sc5d-3' (SEQ. ID. No. 46). The DNA fragment obtained was treated with restriction enzymes NotI and XhoI and then integrated into the vector pFlat3 (FIG. 4) which likewise had been treated with the enzymes NotI and XhoI beforehand, by means of a ligase reaction. The resulting vector pFlat3-SC5D (FIG. 5b) contains the SC5D gene under the control of the ADH promoter and the tryptophan terminator.

The expression vector pFlat3-SC5D was then transformed into the yeast strains I to VI of Table 1 from Example 1 and also into the GRFura3 strain. The yeast strains obtained in this way were then cultured in a culture volume of 20 ml in WMVIII medium at 28.degree. C. and 160 rpm for 48 hours. Subsequently, 500  $\mu$ l of this preculture were transferred to a 50 ml main culture of the same medium and cultured in a baffled flask at 28.degree. C. and 160 rpm for 3 days.

The sterols were extracted after 3 days and analyzed by means of gas chromatography, as described in Example 1. The influence of the expression of a *Mus musculus*  $\Delta 5$ -desaturase in combination with the expression of the transcriptionally deregulated intrinsic yeast genes tHMG and/or ERG1 and/or ERG11 and/or deletion of the intrinsic yeast genes ERG6 and ERG5 is listed in Table 4. The abbreviations have the following meanings: -=decrease; 0=no change; /=not present; +, ++, +++, ++++=concentrated to highly concentrated.

Expression of the Heterologous Gene Encoding a  $\Delta 24$ -Reductase (D24R) from Mice (*Mus musculus*) in Yeast

The cDNA sequence of *Mus musculus*  $\Delta 24$ -reductase (Waterham, H. R., Koster, J., Romeijn, G. J., Hennekam, R. C., Vreken, P., Andersson, H. C., FitzPatrick, D. R., Kelley, R. I. and Wanders, R. J., Mutations in the 3 $\beta$ -Hydroxysterol  $\Delta 24$ -Reductase Gene Cause Desmosterolosis, an Autosomal Recessive Disorder of Cholesterol Biosynthesis, *Am. J. Hum. Genet.* 69 (4), 685-694 (2001)) was amplified by PCR from the cDNA clone IMAGp998K179532 (Host: *E. coli* DH10B) of the Deutsches Ressourcenzentrum für Genomforschung [German resource center for genome research] GmbH (Berlin).

The primers used here are the DNA oligomers D24R-5' (SEQ. ID. No. 47) and D24R-3' (SEQ. ID. No. 48). The DNA fragment obtained was treated with restriction enzymes NotI and XhoI and then integrated into the vector pFlat4 (FIG. 6) which likewise had been treated with the enzymes NotI and XhoI beforehand, by means of a ligase reaction. The resulting vector pFlat4-D24R (FIG. 5d) contains the D24R gene under the control of the ADH1 promoter and the tryptophan terminator.

The expression vector pFlat4-D24R was then transformed into the yeast strains I to VI of Table 1 from Example 1 and also into the GRFura3 strain. The yeast strains obtained in this way were then cultured in a culture volume of 20 ml in WMVIII medium at 28.degree. C. and 160 rpm for 48 hours. Subsequently, 500  $\mu$ l of this preculture were transferred to a 50 ml main culture of the same medium and cultured in a baffled flask at 28.degree. C. and 160 rpm for 3 days.

The sterols were extracted after 3 days and analyzed by means of gas chromatography, as described in Example 1. The influence of the expression of a *Mus musculus*  $\Delta 24$ -reductase in combination with the expression of the transcriptionally deregulated intrinsic yeast genes tHMG and/or ERG1 and/or ERG11 and/or deletion of the intrinsic yeast genes ERG6 and ERG5 is listed in Table 5. The abbreviations have the following meanings: -=decrease; 0=no change; /=not present; +, ++, +++, ++++=concentrated to highly concentrated.

TABLE 4

No.	Strain name	Influence of the genetic modifications on the sterol content compared to the GRF yeast strain										
		1	2	3	4	5	6	7	8	9	10	11
XIII	GRFtH1 pFlat3-Sc5d	0	0	0	0	0	0	/	/	/	/	0
XIV	GRFtH1E1 pFlat3-Sc5d	0	0	0	-	0	0	/	/	+	/	0
XV	GRFtH1E11 pFlat3-Sc5d	0	0	0	-	0	0	/	/	+	/	0
XVI	GRFtH1E1E11 pFlat3-Sc5d	0	0	0	-	0	0	/	/	+	/	0
XVII	GRFtH1E1E11erg5erg6 pFlat3-Sc5d	0	-	0	--	/	/	/	/	+++	+	/
XVIII	GRFtH1erg5erg6 pFlat3-Sc5d	0	0	0	--	/	/	/	/	++	/	/

1 = Squalene

2 = Lanosterol

3 = Dimethylzymosterol

4 = Zymosterol

5 = Fecosterol

6 = Episterol

7 = Cholesta-7,24-dienol

8 = Cholesta-8-enol

9 = Cholesta-5,7,24-trienol

10 = 7-Dehydrocholesterol

11 = Ergosterol

TABLE 5

No.	Strain name	Influence of the genetic modifications on the sterol content compared to the GRF yeast strain										
		1	2	3	4	5	6	7	8	9	10	11
XIX	GRFtH1 pFlat4-D24R	0	0	0	0	0	0	/	/	/	/	0
XX	GRFtH1E1 pFlat4-D24R	0	-	-	-	0	0	/	/	/	+	0
XXI	GRFtH1E11 pFlat4-D24R	0	0	0	-	0	0	/	+	/	+	0
XXII	GRFtH1E1E11 pFlat4-D24R	0	0	0	-	0	0	/	+	/	+	0
XXIII	GRFtH1E1E11erg5erg6 pFlat4-D24R	0	-	-	--	/	/	0	+	+	+++	/
XXIV	GRFtH1erg5erg6 pFlat4-D24R	0	-	-	-	/	/	0	+	+	++	/

- 1 = Squalene
- 2 = Lanosterol
- 3 = Dimethylzymosterol
- 4 = Zymosterol
- 5 = Fecosterol
- 6 = Episterol
- 7 = Cholesta-7,24-dienol
- 8 = Cholesta-8-enol
- 9 = Cholesta-5,7,24 trienol
- 10 = 7-Dehydrocholesterol
- 11 = Ergosterol

Example 5

Coexpression of the Heterologous Genes Encoding a  $\Delta 8-\Delta 7$ -Isomerase (Ebp) from Mice (*Mus musculus*) and a C5-Desaturase (Sc5d) from Mice (*Mus musculus*) in Yeast

The expression vectors pFlat1-EBP (from Example 2) and pFlat3-SC5D (from Example 3) were transformed into the yeast strains I to VI of Table 1 of Example 1 and also into the GRFura3 strain. The yeast strains obtained in this way were then cultured in a culture volume of 20 ml in WMVIII medium at 28.degree. C. and 160 rpm for 48 hours. Subsequently, 500  $\mu$ l of this preculture were transferred to a 50 ml

main culture of the same medium and cultured in a baffled flask at 28.degree. C. and 160 rpm for 3 days.

The sterols were extracted after 3 days and analyzed by means of gas chromatography, as described in Example 1. The influence of the expression of a  $\Delta 8-\Delta 7$ -isomerase and a *Mus musculus* C5-desaturase in combination with the expression of the transcriptionally deregulated intrinsic yeast genes tHMG and/or ERG1 and/or ERG11 and/or deletion of the intrinsic yeast genes ERG6 and ERG5 is listed in Table 6. The abbreviations have the following meanings: -=decrease; 0=no change; /=not present; +, ++, +++, +++++=concentrated to highly concentrated.

TABLE 6

No.	Strain name	Influence of the genetic modifications on the sterol content compared to the GRF yeast strain										
		1	2	3	4	5	6	7	8	9	10	11
VVX	GRFtH1 pFlat3-Ebp/pFlat1-Sc5d	0	0	0	-	0	0	/	/	+	/	0
XXVI	GRFtH1E1 pFlat3-Ebp/pFlat1-Sc5d	0	-	0	--	0	0	/	/	+	/	0
XXVII	GRFtH1E11 pFlat3-Ebp/pFlat1-Sc5d	0	0	0	--	0	0	/	/	+	/	0
XXVIII	GRFtH1E1E11 pFlat3-Ebp/pFlat1-Sc5d	0	-	-	--	0	0	/	/	++	/	0
XXIX	GRFtH1E1E11erg5erg6 pFlat3-Ebp/pFlat1-Sc5d	0	-	0	--	/	/	/	/	+++	+	/
XXX	GRFtH1erg5erg6 pFlat3-Ebp/pFlat1-Sc5d	0	0	0	-	/	/	/	/	++	+	/

- 1 = Squalene
- 2 = Lanosterol
- 3 = Dimethylzymosterol
- 4 = Zymosterol
- 5 = Fecosterol
- 6 = Episterol
- 7 = Cholesta-7,24-dienol
- 8 = Cholesta-8-enol
- 9 = Cholesta-5,7,24 trienol
- 10 = 7-Dehydrocholesterol
- 11 = Ergosterol

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Example 6

Coexpression of the Heterologous Genes Encoding a Δ8-Δ7-Isomerase (Ebp) from Mice (*Mus musculus*) Encod- ing a C5-Desaturase (Sc5d) from Mice (*Mus musculus*) and a Δ24-Reductase from Mice (*Mus musculus*) in Yeast

The expression vectors pFlat1-EBP (from Example 2) and pFlat3-SC5D (from Example 3) and pFlat4-D24R (from Example 4) were transformed into the yeast strains I to VI of Table 1 of Example 1 and also into the GRFura3 strain. The yeast strains obtained in this way were then cultured in a culture volume of 20 ml in WMVIII medium at 28.degree. C. and 160 rpm for 48 hours. Subsequently, 500 μl of this

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preculture were transferred to a 50 ml main culture of the same medium and cultured in a baffled flask at 28.degree. C. and 160 rpm for 3 days.

The sterols were extracted after 3 days and analyzed by means of gas chromatography, as described in Example 1. The influence of the expression of a Δ8-Δ7-isomerase, a *Mus musculus* C5-desaturase and a *Mus musculus* Δ24-reductase in combination with the expression of the transcriptionally deregulated intrinsic yeast genes tHMG and/or ERG1 and/or ERG11 and/or deletion of the intrinsic yeast genes ERG6 and ERG5 is listed in Table 7. The abbreviations have the following meanings: --decrease; 0=no change; /=not present; ++, +++, ++++=concentrated to highly concentrated.

TABLE 7

No.	Strain name	Influence of the genetic modifications on the sterol content compared to the GRF yeast strain											
		1	2	3	4	5	6	7	8	9	10	11	
XXXI	GRFtH1 pFlat3-Ebp/pFlat1-Sc5d/pFlat4-D24R	0	0	0	-	0	0	/	/	/	+	0	
XXXII	GRFtH1E1 pFlat3-Ebp/pFlat1-Sc5d/pFlat4-D24R	0	-	0	--	0	0	/	/	/	+	0	
XXXIII	GRFtH1E11 pFlat3-Ebp/pFlat1-Sc5d/pFlat4-D24R	0	0	0	--	0	0	/	/	/	+	0	
XXXIV	GRFtH1E1E11 pFlat3-Ebp/pFlat1-Sc5d/pFlat4-D24R	0	-	-	--	0	0	/	/	/	+	0	
XXXV	GRFtH1E1E11erg5erg6 pFlat3-Ebp/pFlat1-Sc5d/pFlat4-D24R	0	-	0	---	/	/	/	/	/	+	++++	/
XXXVI	GRFtH1erg5erg6 pFlat3-Ebp/pFlat1-Sc5d/pFlat4-D24R	0	0	0	-	/	/	/	/	/	++	+++	/

- 1 = Squalene
- 2 = Lanosterol
- 3 = Dimethyl zymosterol
- 4 = Zymosterol
- 5 = Fecosterol
- 6 = Episterol
- 7 = Cholesta-7,24-dienol
- 8 = Cholesta-8-enol
- 9 = Cholesta-5,7,24 trienol
- 10 = 7-Dehydrocholesterol
- 11 = Ergosterol

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 60

<210> SEQ ID NO 1  
 <211> LENGTH: 693  
 <212> TYPE: DNA  
 <213> ORGANISM: Mus musculus  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(693)

<400> SEQUENCE: 1

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atg acc aac aat acg gtc ccc ttg cac ccg tac tgg ccc agg cac ctg      48
Met Thr Thr Asn Thr Val Pro Leu His Pro Tyr Trp Pro Arg His Leu
    1             5             10             15

aag ctg gac aac ttc gtg cct aat gac ctc ccg act tcg cat atc ctg      96
Lys Leu Asp Asn Phe Val Pro Asn Asp Leu Pro Thr Ser His Ile Leu
    20             25             30

gtt ggc ctc ttc tcc atc tct ggg ggc cta att gtg atc acg tgg ctg     144
    
```

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Val	Gly	Leu	Phe	Ser	Ile	Ser	Gly	Gly	Leu	Ile	Val	Ile	Thr	Trp	Leu	
		35					40					45				
ttg	tct	agc	cga	gct	tcc	gtc	gtc	cca	ctt	gga	gct	ggg	cgg	cga	ctg	192
Leu	Ser	Ser	Arg	Ala	Ser	Val	Val	Pro	Leu	Gly	Ala	Gly	Arg	Arg	Leu	
	50					55				60						
gcc	ttg	tgc	tgg	ttt	gct	gtg	tgt	acc	ttc	att	cac	ctt	gtg	atc	gag	240
Ala	Leu	Cys	Trp	Phe	Ala	Val	Cys	Thr	Phe	Ile	His	Leu	Val	Ile	Glu	
	65				70					75					80	
ggc	tgg	ttc	tct	ctc	tac	aat	ggc	atc	ctt	tta	gaa	gac	caa	gcc	ttc	288
Gly	Trp	Phe	Ser	Leu	Tyr	Asn	Gly	Ile	Leu	Leu	Glu	Asp	Gln	Ala	Phe	
				85					90					95		
tta	tcc	caa	ctc	tgg	aaa	gag	tat	tcc	aag	gga	gat	agc	cga	tat	atc	336
Leu	Ser	Gln	Leu	Trp	Lys	Glu	Tyr	Ser	Lys	Gly	Asp	Ser	Arg	Tyr	Ile	
		100						105						110		
ctt	agt	gac	agc	ttc	gtc	gtc	tgt	atg	gag	act	gtc	aca	gct	tgt	ctc	384
Leu	Ser	Asp	Ser	Phe	Val	Val	Cys	Met	Glu	Thr	Val	Thr	Ala	Cys	Leu	
		115					120							125		
tgg	gga	cca	ctc	agc	cta	tgg	gta	gtg	att	gcc	ttt	ctc	cgc	caa	cag	432
Trp	Gly	Pro	Leu	Ser	Leu	Trp	Val	Val	Ile	Ala	Phe	Leu	Arg	Gln	Gln	
	130					135					140					
ccc	ttc	cgc	ttt	gtc	cta	cag	ctt	gtg	gtg	tct	atg	ggc	cag	ata	tac	480
Pro	Phe	Arg	Phe	Val	Leu	Gln	Leu	Val	Val	Ser	Met	Gly	Gln	Ile	Tyr	
	145				150					155					160	
ggg	gat	gtg	ctg	tac	ttc	ctg	aca	gag	cta	cac	gaa	gga	ctc	cag	cat	528
Gly	Asp	Val	Leu	Tyr	Phe	Leu	Thr	Glu	Leu	His	Glu	Gly	Leu	Gln	His	
			165						170					175		
ggg	gag	ata	ggc	cac	ccc	gtt	tat	ttc	tgg	ttc	tat	ttt	gtt	ttc	ctg	576
Gly	Glu	Ile	Gly	His	Pro	Val	Tyr	Phe	Trp	Phe	Tyr	Phe	Val	Phe	Leu	
			180					185						190		
aat	gct	gta	tgg	ttg	gtg	ata	cca	agc	atc	ctt	gtg	ctt	gat	gcc	ata	624
Asn	Ala	Val	Trp	Leu	Val	Ile	Pro	Ser	Ile	Leu	Val	Leu	Asp	Ala	Ile	
		195					200							205		
aag	cat	ctc	act	agt	gcc	cag	agc	gtg	ctg	gac	agc	aaa	gtc	atg	aaa	672
Lys	His	Leu	Thr	Ser	Ala	Gln	Ser	Val	Leu	Asp	Ser	Lys	Val	Met	Lys	
		210				215								220		
att	aag	agc	aag	cat	aac	taa										693
Ile	Lys	Ser	Lys	His	Asn											
		225			230											

<210> SEQ ID NO 2  
 <211> LENGTH: 230  
 <212> TYPE: PRT  
 <213> ORGANISM: Mus musculus

<400> SEQUENCE: 2

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



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gga gag ctg ggc cac cct ctc tac ttc tgg ttt tac ttt gtc ttc atg 576  
 Gly Glu Leu Gly His Pro Leu Tyr Phe Trp Phe Tyr Phe Val Phe Met  
                   180                  185                  190

aat gcc ctg tgg ctg gtg ctg cct gga gtc ctt gtg ctt gat gct gtg 624  
 Asn Ala Leu Trp Leu Val Leu Pro Gly Val Leu Val Leu Asp Ala Val  
                   195                  200                  205

aag cac ctc act cat gcc cag agc acg ctg gat gcc aag gcc aca aaa 672  
 Lys His Leu Thr His Ala Gln Ser Thr Leu Asp Ala Lys Ala Thr Lys  
                   210                  215                  220

gcc aag agc aag aag aac tga 693  
 Ala Lys Ser Lys Lys Asn  
 225                  230

<210> SEQ ID NO 4

<211> LENGTH: 230

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Thr Thr Asn Ala Gly Pro Leu His Pro Tyr Trp Pro Gln His Leu  
 1                  5                  10                  15

Arg Leu Asp Asn Phe Val Pro Asn Asp Arg Pro Thr Trp His Ile Leu  
                   20                  25                  30

Ala Gly Leu Phe Ser Val Thr Gly Val Leu Val Val Thr Thr Trp Leu  
                   35                  40                  45

Leu Ser Gly Arg Ala Ala Val Val Pro Leu Gly Thr Trp Arg Arg Leu  
                   50                  55                  60

Ser Leu Cys Trp Phe Ala Val Cys Gly Phe Ile His Leu Val Ile Glu  
 65                  70                  75                  80

Gly Trp Phe Val Leu Tyr Tyr Glu Asp Leu Leu Gly Asp Gln Ala Phe  
                   85                  90                  95

Leu Ser Gln Leu Trp Lys Glu Tyr Ala Lys Gly Asp Ser Arg Tyr Ile  
                   100                  105                  110

Leu Gly Asp Asn Phe Thr Val Cys Met Glu Thr Ile Thr Ala Cys Leu  
                   115                  120                  125

Trp Gly Pro Leu Ser Leu Trp Val Val Ile Ala Phe Leu Arg Gln His  
 130                  135                  140

Pro Leu Arg Phe Ile Leu Gln Leu Val Val Ser Val Gly Gln Ile Tyr  
 145                  150                  155                  160

Gly Asp Val Leu Tyr Phe Leu Thr Glu His Arg Asp Gly Phe Gln His  
                   165                  170                  175

Gly Glu Leu Gly His Pro Leu Tyr Phe Trp Phe Tyr Phe Val Phe Met  
                   180                  185                  190

Asn Ala Leu Trp Leu Val Leu Pro Gly Val Leu Val Leu Asp Ala Val  
                   195                  200                  205

Lys His Leu Thr His Ala Gln Ser Thr Leu Asp Ala Lys Ala Thr Lys  
 210                  215                  220

Ala Lys Ser Lys Lys Asn  
 225                  230

<210> SEQ ID NO 5

<211> LENGTH: 669

<212> TYPE: DNA

<213> ORGANISM: Saccharomyces cerevisiae

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(669)

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&lt;400&gt; SEQUENCE: 5

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atg aag ttt ttc cca ctc ctt ttg ttg att ggt gtt gta ggc tac att    48
Met Lys Phe Phe Pro Leu Leu Leu Leu Ile Gly Val Val Gly Tyr Ile
  1           5           10           15

atg aac gta ttg ttc act acc tgg ttg cca acc aat tac atg ttc gat    96
Met Asn Val Leu Phe Thr Thr Trp Leu Pro Thr Asn Tyr Met Phe Asp
          20           25           30

cca aaa act ttg aac gaa ata tgt aac tcg gtg att agc aaa cac aac    144
Pro Lys Thr Leu Asn Glu Ile Cys Asn Ser Val Ile Ser Lys His Asn
          35           40           45

gca gca gaa ggt tta tcc act gaa gac ctg tta cag gat gtc aga gac    192
Ala Ala Glu Gly Leu Ser Thr Glu Asp Leu Leu Gln Asp Val Arg Asp
          50           55           60

gca ctt gcc tct cat tac ggg gac gaa tac atc aac agg tac gtc aaa    240
Ala Leu Ala Ser His Tyr Gly Asp Glu Tyr Ile Asn Arg Tyr Val Lys
          65           70           75           80

gaa gaa tgg gtc ttc aac aat gct ggt ggt gcg atg ggc caa atg atc    288
Glu Glu Trp Val Phe Asn Asn Ala Gly Gly Ala Met Gly Gln Met Ile
          85           90           95

atc cta cac gct tcc gta tcc gag tac tta att cta ttc gga acc gct    336
Ile Leu His Ala Ser Val Ser Glu Tyr Leu Ile Leu Phe Gly Thr Ala
          100          105          110

gtt ggt act gaa ggg cac aca ggt gtt cac ttt gct gac gac tat ttt    384
Val Gly Thr Glu Gly His Thr Gly Val His Phe Ala Asp Asp Tyr Phe
          115          120          125

acc atc tta cat ggt acg caa atc gca gca ttg cca tat gcc act gaa    432
Thr Ile Leu His Gly Thr Gln Ile Ala Ala Leu Pro Tyr Ala Thr Glu
          130          135          140

gcc gaa gtt tac act cct ggt atg act cat cac ttg aag aag gga tac    480
Ala Glu Val Tyr Thr Pro Gly Met Thr His His Leu Lys Lys Gly Tyr
          145          150          155          160

gcc aag caa tac agc atg cca ggt ggt tcc ttt gcc ctt gaa ttg gct    528
Ala Lys Gln Tyr Ser Met Pro Gly Gly Ser Phe Ala Leu Glu Leu Ala
          165          170          175

caa ggc tgg att cca tgt atg ttg cca ttc ggg ttt ttg gac act ttc    576
Gln Gly Trp Ile Pro Cys Met Leu Pro Phe Gly Phe Leu Asp Thr Phe
          180          185          190

tcc agt act ctt gat tta tac act cta tat aga act gtc tac ctg act    624
Ser Ser Thr Leu Asp Leu Tyr Thr Leu Tyr Arg Thr Val Tyr Leu Thr
          195          200          205

gcc agg gac atg ggt aag aac ttg ttg caa aac aaa aag ttc taa    669
Ala Arg Asp Met Gly Lys Asn Leu Leu Gln Asn Lys Lys Phe
          210          215          220

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&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 222

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Saccharomyces cerevisiae*

&lt;400&gt; SEQUENCE: 6

```

Met Lys Phe Phe Pro Leu Leu Leu Leu Ile Gly Val Val Gly Tyr Ile
  1           5           10           15

Met Asn Val Leu Phe Thr Thr Trp Leu Pro Thr Asn Tyr Met Phe Asp
          20           25           30

Pro Lys Thr Leu Asn Glu Ile Cys Asn Ser Val Ile Ser Lys His Asn
          35           40           45

Ala Ala Glu Gly Leu Ser Thr Glu Asp Leu Leu Gln Asp Val Arg Asp
          50           55           60

Ala Leu Ala Ser His Tyr Gly Asp Glu Tyr Ile Asn Arg Tyr Val Lys

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65	70	75	80
Glu Glu Trp Val Phe Asn Asn Ala Gly Gly Ala Met Gly Gln Met Ile	85	90	95
Ile Leu His Ala Ser Val Ser Glu Tyr Leu Ile Leu Phe Gly Thr Ala	100	105	110
Val Gly Thr Glu Gly His Thr Gly Val His Phe Ala Asp Asp Tyr Phe	115	120	125
Thr Ile Leu His Gly Thr Gln Ile Ala Ala Leu Pro Tyr Ala Thr Glu	130	135	140
Ala Glu Val Tyr Thr Pro Gly Met Thr His His Leu Lys Lys Gly Tyr	145	150	155
Ala Lys Gln Tyr Ser Met Pro Gly Gly Ser Phe Ala Leu Glu Leu Ala	165	170	175
Gln Gly Trp Ile Pro Cys Met Leu Pro Phe Gly Phe Leu Asp Thr Phe	180	185	190
Ser Ser Thr Leu Asp Leu Tyr Thr Leu Tyr Arg Thr Val Tyr Leu Thr	195	200	205
Ala Arg Asp Met Gly Lys Asn Leu Leu Gln Asn Lys Lys Phe	210	215	220
<p>&lt;210&gt; SEQ ID NO 7                  &lt;211&gt; LENGTH: 900                  &lt;212&gt; TYPE: DNA                  &lt;213&gt; ORGANISM: Mus musculus                  &lt;220&gt; FEATURE:                  &lt;221&gt; NAME/KEY: CDS                  &lt;222&gt; LOCATION: (1)..(900)</p>			
<p>&lt;400&gt; SEQUENCE: 7</p>			
atg gac ctg gtt ctc agt gcc gcc gat tac tac ttc ttc act ccg tat			48
Met Asp Leu Val Leu Ser Ala Ala Asp Tyr Tyr Phe Phe Thr Pro Tyr	1	5	10
gta tat cca gcc acg tgg ccc gag gac aac atc atc cga caa act att			96
Val Tyr Pro Ala Thr Trp Pro Glu Asp Asn Ile Ile Arg Gln Thr Ile	20	25	30
agc ctc ctg att gtc aca aac ctg ggt gct tac att ctc tac ttc ttc			144
Ser Leu Leu Ile Val Thr Asn Leu Gly Ala Tyr Ile Leu Tyr Phe Phe	35	40	45
tgt gca acc ctc agc tat tat ttt gtc tat gat cat tcc tta atg aaa			192
Cys Ala Thr Leu Ser Tyr Tyr Phe Val Tyr Asp His Ser Leu Met Lys	50	55	60
cac cca cag ttt tta aag aac caa gtc tgc cgt gag atc gtg ttc act			240
His Pro Gln Phe Leu Lys Asn Gln Val Ser Arg Glu Ile Val Phe Thr	65	70	75
gtc aag tct ttg cct tgg atc agc atc ccc acc gtc tca cta ttc ctg			288
Val Lys Ser Leu Pro Trp Ile Ser Ile Pro Thr Val Ser Leu Phe Leu	85	90	95
ctg gag ctg agg ggt tac agc aaa ctc tac gat gac atc gga gac ttt			336
Leu Glu Leu Arg Gly Tyr Ser Lys Leu Tyr Asp Asp Ile Gly Asp Phe	100	105	110
cca aat ggc tgg att cat ctc atg gtt agc gtc gta tcc ttc ctc ttt			384
Pro Asn Gly Trp Ile His Leu Met Val Ser Val Val Ser Phe Leu Phe	115	120	125
ttc aca gac atg ttg atc tac agg att cat agg ggc ctg cac cac aga			432
Phe Thr Asp Met Leu Ile Tyr Arg Ile His Arg Gly Leu His His Arg	130	135	140
ctg gtc tac aag cgc ata cat aaa cca cat cat att tgg aag atc ccc			480
Leu Val Tyr Lys Arg Ile His Lys Pro His His Ile Trp Lys Ile Pro	145	150	155

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acg ccg ttt gca agt cat gct ttt cac cct gtg gac ggc ttc ctt cag      528
Thr Pro Phe Ala Ser His Ala Phe His Pro Val Asp Gly Phe Leu Gln
                165                      170                      175

agt ctg cct tac cat ata tac ccc ttt gtc ttt cca ctg cac aag gtg      576
Ser Leu Pro Tyr His Ile Tyr Pro Phe Val Phe Pro Leu His Lys Val
                180                      185                      190

gtc tac tta ggt tta tat gtc ttg gtt aat gtc tgg aca att tct att      624
Val Tyr Leu Gly Leu Tyr Val Leu Val Asn Val Trp Thr Ile Ser Ile
                195                      200                      205

cat gat ggt gat ttt cgg gtt ccc cag atc tta agg cca ttt att aac      672
His Asp Gly Asp Phe Arg Val Pro Gln Ile Leu Arg Pro Phe Ile Asn
                210                      215                      220

ggg tca gct cac cac aca gac cac cac atg ttc ttt gac tat aac tat      720
Gly Ser Ala His His Thr Asp His His Met Phe Phe Asp Tyr Asn Tyr
                225                      230                      235                      240

gga cag tat ttc aca ttg tgg gat aga att gga ggc tct ttt aaa cat      768
Gly Gln Tyr Phe Thr Leu Trp Asp Arg Ile Gly Gly Ser Phe Lys His
                245                      250                      255

cct tcc tct ttt gaa ggg aaa gga cca cat agt tac gtg aag aac atg      816
Pro Ser Ser Phe Glu Gly Lys Gly Pro His Ser Tyr Val Lys Asn Met
                260                      265                      270

aca gaa aaa gaa tct aac agc ttt gca gaa aac ggc tgt aaa ggc aaa      864
Thr Glu Lys Glu Ser Asn Ser Ser Phe Ala Glu Asn Gly Cys Lys Gly Lys
                275                      280                      285

aaa gta agc aat gga gag ttt aca aag aat aag tag      900
Lys Val Ser Asn Gly Glu Phe Thr Lys Asn Lys
                290                      295
    
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<210> SEQ ID NO 8
<211> LENGTH: 299
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
    
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<400> SEQUENCE: 8

```

Met Asp Leu Val Leu Ser Ala Ala Asp Tyr Tyr Phe Phe Thr Pro Tyr
1                5                10                15

Val Tyr Pro Ala Thr Trp Pro Glu Asp Asn Ile Ile Arg Gln Thr Ile
                20                25                30

Ser Leu Leu Ile Val Thr Asn Leu Gly Ala Tyr Ile Leu Tyr Phe Phe
35                40                45

Cys Ala Thr Leu Ser Tyr Tyr Phe Val Tyr Asp His Ser Leu Met Lys
50                55                60

His Pro Gln Phe Leu Lys Asn Gln Val Ser Arg Glu Ile Val Phe Thr
65                70                75                80

Val Lys Ser Leu Pro Trp Ile Ser Ile Pro Thr Val Ser Leu Phe Leu
85                90                95

Leu Glu Leu Arg Gly Tyr Ser Lys Leu Tyr Asp Asp Ile Gly Asp Phe
100               105               110

Pro Asn Gly Trp Ile His Leu Met Val Ser Val Val Ser Phe Leu Phe
115               120               125

Phe Thr Asp Met Leu Ile Tyr Arg Ile His Arg Gly Leu His His Arg
130               135               140

Leu Val Tyr Lys Arg Ile His Lys Pro His His Ile Trp Lys Ile Pro
145               150               155               160

Thr Pro Phe Ala Ser His Ala Phe His Pro Val Asp Gly Phe Leu Gln
165               170               175

Ser Leu Pro Tyr His Ile Tyr Pro Phe Val Phe Pro Leu His Lys Val
    
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180				185				190								
Val	Tyr	Leu	Gly	Leu	Tyr	Val	Leu	Val	Asn	Val	Trp	Thr	Ile	Ser	Ile	
		195					200					205				
His	Asp	Gly	Asp	Phe	Arg	Val	Pro	Gln	Ile	Leu	Arg	Pro	Phe	Ile	Asn	
	210					215					220					
Gly	Ser	Ala	His	His	Thr	Asp	His	His	Met	Phe	Phe	Asp	Tyr	Asn	Tyr	
	225				230					235					240	
Gly	Gln	Tyr	Phe	Thr	Leu	Trp	Asp	Arg	Ile	Gly	Gly	Ser	Phe	Lys	His	
				245					250					255		
Pro	Ser	Ser	Phe	Glu	Gly	Lys	Gly	Pro	His	Ser	Tyr	Val	Lys	Asn	Met	
			260					265						270		
Thr	Glu	Lys	Glu	Ser	Asn	Ser	Phe	Ala	Glu	Asn	Gly	Cys	Lys	Gly	Lys	
		275					280					285				
Lys	Val	Ser	Asn	Gly	Glu	Phe	Thr	Lys	Asn	Lys						
	290					295										
<210> SEQ ID NO 9																
<211> LENGTH: 900																
<212> TYPE: DNA																
<213> ORGANISM: Homo sapiens																
<220> FEATURE:																
<221> NAME/KEY: CDS																
<222> LOCATION: (1)..(900)																
<400> SEQUENCE: 9																
atg	gat	ctt	gta	ctc	cgt	gtt	gca	gat	tac	tat	ttt	ttt	aca	cca	tac	48
Met	Asp	Leu	Val	Leu	Arg	Val	Ala	Asp	Tyr	Tyr	Phe	Phe	Thr	Pro	Tyr	
	1				5				10					15		
gtg	tat	cca	gcc	aca	tgg	cca	gaa	gat	gac	atc	ttc	cga	caa	gct	att	96
Val	Tyr	Pro	Ala	Thr	Trp	Pro	Glu	Asp	Asp	Ile	Phe	Arg	Gln	Ala	Ile	
			20					25					30			
agt	ctt	ctg	att	gta	aca	aat	gtt	ggt	gct	tac	atc	ctt	tat	ttc	ttc	144
Ser	Leu	Leu	Ile	Val	Thr	Asn	Val	Gly	Ala	Tyr	Ile	Leu	Tyr	Phe	Phe	
		35					40					45				
tgt	gca	aca	ctg	agc	tat	tat	ttt	gtc	ttc	gat	cat	gca	tta	atg	aaa	192
Cys	Ala	Thr	Leu	Ser	Tyr	Tyr	Phe	Val	Phe	Asp	His	Ala	Leu	Met	Lys	
	50					55				60						
cat	cca	caa	ttt	tta	aag	aat	caa	gtc	cgt	cga	gag	att	aag	ttt	act	240
His	Pro	Gln	Phe	Leu	Lys	Asn	Gln	Val	Arg	Arg	Glu	Ile	Lys	Phe	Thr	
	65				70					75				80		
gtc	cag	gca	ttg	cca	tgg	ata	agt	att	ctt	act	gtt	gca	ctg	ttc	ttg	288
Val	Gln	Ala	Leu	Pro	Trp	Ile	Ser	Ile	Leu	Thr	Val	Ala	Leu	Phe	Leu	
				85					90				95			
ctg	gag	ata	aga	ggt	tac	agc	aaa	tta	cat	gat	gac	cta	gga	gag	ttt	336
Leu	Glu	Ile	Arg	Gly	Tyr	Ser	Lys	Leu	His	Asp	Asp	Leu	Gly	Glu	Phe	
			100					105					110			
cca	tat	gga	ttg	ttt	gaa	ctt	gtc	ggt	agt	ata	ata	tct	ttc	ctc	ttt	384
Pro	Tyr	Gly	Leu	Phe	Glu	Leu	Val	Val	Ser	Ile	Ile	Ser	Phe	Leu	Phe	
		115					120					125				
ttc	act	gac	atg	ttc	atc	tac	tgg	att	cac	aga	ggc	ctt	cat	cat	aga	432
Phe	Thr	Asp	Met	Phe	Ile	Tyr	Trp	Ile	His	Arg	Gly	Leu	His	His	Arg	
	130					135					140					
ctg	gta	tat	aag	cgc	cta	cat	aaa	cct	cac	cat	att	tgg	aag	att	cct	480
Leu	Val	Tyr	Lys	Arg	Leu	His	Lys	Pro	His	His	Ile	Trp	Lys	Ile	Pro	
	145				150					155				160		
act	cca	ttt	gca	agt	cat	gct	ttt	cac	cct	att	gat	ggc	ttt	ctt	cag	528
Thr	Pro	Phe	Ala	Ser	His	Ala	Phe	His	Pro	Ile	Asp	Gly	Phe	Leu	Gln	
			165						170				175			
agt	cta	cct	tac	cat	ata	tac	cct	ttt	atc	ttt	cca	tta	cac	aag	gtg	576

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Ser Leu Pro Tyr His Ile Tyr Pro Phe Ile Phe Pro Leu His Lys Val  
 180 185 190

ggt tat tta agt ctg tac atc ttg gtt aat atc tgg aca att tcc att 624  
 Val Tyr Leu Ser Leu Tyr Ile Leu Val Asn Ile Trp Thr Ile Ser Ile  
 195 200 205

cat gac ggt gat ttt cgt gtc ccc caa atc tta cag cca ttt att aat 672  
 His Asp Gly Asp Phe Arg Val Pro Gln Ile Leu Gln Pro Phe Ile Asn  
 210 215 220

ggc tca gct cat cat aca gac cac cat atg ttc ttt gac tat aat tat 720  
 Gly Ser Ala His His Thr Asp His His Met Phe Phe Asp Tyr Asn Tyr  
 225 230 235 240

gga caa tat ttc act ttg tgg gat agg att ggc ggc tca ttc aaa aat 768  
 Gly Gln Tyr Phe Thr Leu Trp Asp Arg Ile Gly Gly Ser Phe Lys Asn  
 245 250 255

cct tca tcc ttt gag ggg aag gga ccg ctc agt tat gtg aag gag atg 816  
 Pro Ser Ser Phe Glu Gly Lys Gly Pro Leu Ser Tyr Val Lys Glu Met  
 260 265 270

aca gag gga aag cgc agc agc cct tca gga aat ggc tgt aag aat gaa 864  
 Thr Glu Gly Lys Arg Ser Ser Pro Ser Gly Asn Gly Cys Lys Asn Glu  
 275 280 285

aaa tta ttc aat gga gag ttt aca aag act gaa tag 900  
 Lys Leu Phe Asn Gly Glu Phe Thr Lys Thr Glu  
 290 295

<210> SEQ ID NO 10  
 <211> LENGTH: 299  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Met Asp Leu Val Leu Arg Val Ala Asp Tyr Tyr Phe Phe Thr Pro Tyr  
 1 5 10 15

Val Tyr Pro Ala Thr Trp Pro Glu Asp Asp Ile Phe Arg Gln Ala Ile  
 20 25 30

Ser Leu Leu Ile Val Thr Asn Val Gly Ala Tyr Ile Leu Tyr Phe Phe  
 35 40 45

Cys Ala Thr Leu Ser Tyr Tyr Phe Val Phe Asp His Ala Leu Met Lys  
 50 55 60

His Pro Gln Phe Leu Lys Asn Gln Val Arg Arg Glu Ile Lys Phe Thr  
 65 70 75 80

Val Gln Ala Leu Pro Trp Ile Ser Ile Leu Thr Val Ala Leu Phe Leu  
 85 90 95

Leu Glu Ile Arg Gly Tyr Ser Lys Leu His Asp Asp Leu Gly Glu Phe  
 100 105 110

Pro Tyr Gly Leu Phe Glu Leu Val Val Ser Ile Ile Ser Phe Leu Phe  
 115 120 125

Phe Thr Asp Met Phe Ile Tyr Trp Ile His Arg Gly Leu His His Arg  
 130 135 140

Leu Val Tyr Lys Arg Leu His Lys Pro His His Ile Trp Lys Ile Pro  
 145 150 155 160

Thr Pro Phe Ala Ser His Ala Phe His Pro Ile Asp Gly Phe Leu Gln  
 165 170 175

Ser Leu Pro Tyr His Ile Tyr Pro Phe Ile Phe Pro Leu His Lys Val  
 180 185 190

Val Tyr Leu Ser Leu Tyr Ile Leu Val Asn Ile Trp Thr Ile Ser Ile  
 195 200 205

His Asp Gly Asp Phe Arg Val Pro Gln Ile Leu Gln Pro Phe Ile Asn

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210	215	220	
Gly Ser Ala His His Thr Asp His His Met Phe Phe Asp Tyr Asn Tyr			
225	230	235	240
Gly Gln Tyr Phe Thr Leu Trp Asp Arg Ile Gly Gly Ser Phe Lys Asn			
	245	250	255
Pro Ser Ser Phe Glu Gly Lys Gly Pro Leu Ser Tyr Val Lys Glu Met			
	260	265	270
Thr Glu Gly Lys Arg Ser Ser Pro Ser Gly Asn Gly Cys Lys Asn Glu			
	275	280	285
Lys Leu Phe Asn Gly Glu Phe Thr Lys Thr Glu			
290	295		
<210> SEQ ID NO 11			
<211> LENGTH: 1098			
<212> TYPE: DNA			
<213> ORGANISM: <i>Saccharomyces cerevisiae</i>			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(1098)			
<400> SEQUENCE: 11			
atg gat ttg gtc tta gaa gtc gct gac cat tat gtc tta gac gac ttg			48
Met Asp Leu Val Leu Glu Val Ala Asp His Tyr Val Leu Asp Asp Leu			
1	5	10	15
tac gct aaa gtt ctg ccc gct tcg ttg gca gct aat att cct gtc aag			96
Tyr Ala Lys Val Leu Pro Ala Ser Leu Ala Ala Asn Ile Pro Val Lys			
	20	25	30
tgg cag aaa ttg cta ggg ttg aac agt ggg ttc agc aat tct acg att			144
Trp Gln Lys Leu Leu Gly Leu Asn Ser Gly Phe Ser Asn Ser Thr Ile			
	35	40	45
ttg cag gag act ttg aac tcc aag aat gcc gtc aaa gaa tgt aga agg			192
Leu Gln Glu Thr Leu Asn Ser Lys Asn Ala Val Lys Glu Cys Arg Arg			
	50	55	60
ttc tac ggg cag gtg cca ttc ctg ttt gat atg tcg acg acg tct ttt			240
Phe Tyr Gly Gln Val Pro Phe Leu Phe Asp Met Ser Thr Thr Ser Phe			
	65	70	75
gca tcg cta ttg cct cgt tcc agc atc ttg aga gaa ttc ctc tca cta			288
Ala Ser Leu Leu Pro Arg Ser Ser Ile Leu Arg Glu Phe Leu Ser Leu			
	85	90	95
tgg gtt att gtt acg atc ttt ggt tta cta ctt tac tta ttc acg gct			336
Trp Val Ile Val Thr Ile Phe Gly Leu Leu Leu Tyr Leu Phe Thr Ala			
	100	105	110
agt ctc agc tac gtg ttt gtg ttt gac aag tcg att ttc aac cat cct			384
Ser Leu Ser Tyr Val Phe Val Phe Asp Lys Ser Ile Phe Asn His Pro			
	115	120	125
cgt tac ttg aaa aac caa atg gca atg gaa atc aag ttg gca gtc agt			432
Arg Tyr Leu Lys Asn Gln Met Ala Met Glu Ile Lys Leu Ala Val Ser			
	130	135	140
gct atc cca tgg atg tcg atg ttg acc gtt cca tgg ttt gtt atg gaa			480
Ala Ile Pro Trp Met Ser Met Leu Thr Val Pro Trp Phe Val Met Glu			
	145	150	155
ttg aac ggc cat tct aaa cta tac atg aag att gat tat gaa aac cac			528
Leu Asn Gly His Ser Lys Leu Tyr Met Lys Ile Asp Tyr Glu Asn His			
	165	170	175
ggt gta agg aag ctc att atc gag tac ttc act ttc atc ttt ttc act			576
Gly Val Arg Lys Leu Ile Ile Glu Tyr Phe Thr Phe Ile Phe Thr			
	180	185	190
gat tgc ggt gtg tat tta gcg cac aga tgg ttg cat tgg cca agg gtc			624
Asp Cys Gly Val Tyr Leu Ala His Arg Trp Leu His Trp Pro Arg Val			
	195	200	205



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

<210> SEQ ID NO 13  
 <211> LENGTH: 1557  
 <212> TYPE: DNA  
 <213> ORGANISM: Mus musculus  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1557)

<400> SEQUENCE: 13

atg gag ccc gcc gtg tcg ctg gcc gtg tgc gcg ctg ctc ttt ctg ctc	48
Met Glu Pro Ala Val Ser Leu Ala Val Cys Ala Leu Leu Phe Leu Leu	
1 5 10 15	
tgg gtg cga gtg aag ggg ttg gag ttc gtt ctc atc cac cag cgc tgg	96
Trp Val Arg Val Lys Gly Leu Glu Phe Val Leu Ile His Gln Arg Trp	
20 25 30	
gtg ttc gtg tgc ctc ttc ttg ctg ccg ctc tcg ctc atc ttc gat atc	144
Val Phe Val Cys Leu Phe Leu Leu Pro Leu Ser Leu Ile Phe Asp Ile	
35 40 45	
tac tac tac gtg cgc gcc tgg gtg gtg ttc aag ctg agc agt gcg ccg	192
Tyr Tyr Tyr Val Arg Ala Trp Val Val Phe Lys Leu Ser Ser Ala Pro	
50 55 60	
cgc ctg cac gag cag cgc gtg cgg gac atc cag aaa cag gtc cgg gaa	240
Arg Leu His Glu Gln Arg Val Arg Asp Ile Gln Lys Gln Val Arg Glu	
65 70 75 80	
tgg aag gaa cag gcc agt aag acc ttc atg tgc acg ggg cgc cca ggc	288
Trp Lys Glu Gln Gly Ser Lys Thr Phe Met Cys Thr Gly Arg Pro Gly	
85 90 95	
tgg ctc act gtc tcg ctg cga gtc gga aag tac aag aag acc cat aag	336
Trp Leu Thr Val Ser Leu Arg Val Gly Lys Tyr Lys Lys Thr His Lys	
100 105 110	
aac atc atg atc aac ctg atg gac atc ctg gag gtg gac acc aag aaa	384

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Asn	Ile	Met	Ile	Asn	Leu	Met	Asp	Ile	Leu	Glu	Val	Asp	Thr	Lys	Lys	
		115					120					125				
cag	att	ggt	cga	gtg	gag	ccc	ttg	gtg	tct	atg	ggt	cag	gtg	aca	gct	432
Gln	Ile	Val	Arg	Val	Glu	Pro	Leu	Val	Ser	Met	Gly	Gln	Val	Thr	Ala	
		130				135					140					
ttg	ctg	aac	tcc	att	ggc	tgg	acc	ctg	cct	gtg	ttg	cct	gag	ctt	gat	480
Leu	Leu	Asn	Ser	Ile	Gly	Trp	Thr	Leu	Pro	Val	Leu	Pro	Glu	Leu	Asp	
		145			150					155					160	
gac	ctc	aca	gtg	ggg	ggc	ctg	atc	atg	ggc	aca	ggc	atc	gag	tca	tcg	528
Asp	Leu	Thr	Val	Gly	Gly	Leu	Ile	Met	Gly	Thr	Gly	Ile	Glu	Ser	Ser	
				165					170					175		
tcc	cac	aag	tat	ggc	ctg	ttc	caa	cac	att	tgc	act	gcc	tac	gag	ctg	576
Ser	His	Lys	Tyr	Gly	Leu	Phe	Gln	His	Ile	Cys	Thr	Ala	Tyr	Glu	Leu	
			180					185					190			
atc	ctg	gca	gac	ggc	agc	ttt	gtg	cgc	tgc	aca	ccg	tct	gaa	aac	tca	624
Ile	Leu	Ala	Asp	Gly	Ser	Phe	Val	Arg	Cys	Thr	Pro	Ser	Glu	Asn	Ser	
		195				200						205				
gac	ctg	ttc	tat	gcc	gtg	ccc	tgg	tcc	tgt	ggg	acc	ctg	ggc	ttc	ctg	672
Asp	Leu	Phe	Tyr	Ala	Val	Pro	Trp	Ser	Cys	Gly	Thr	Leu	Gly	Phe	Leu	
		210				215					220					
gtg	gct	gcc	gag	atc	cgg	atc	atc	ccg	gcc	aag	aag	tat	gtc	aag	ctg	720
Val	Ala	Ala	Glu	Ile	Arg	Ile	Ile	Pro	Ala	Lys	Lys	Tyr	Val	Lys	Leu	
		225			230					235					240	
cgg	ttt	gag	cct	ggt	cgg	ggc	ctg	gag	gcc	atc	tgt	gaa	aaa	ttc	acc	768
Arg	Phe	Glu	Pro	Val	Arg	Gly	Leu	Glu	Ala	Ile	Cys	Glu	Lys	Phe	Thr	
			245						250					255		
cgc	gag	tcc	cag	cgg	ctg	gag	aac	cac	ttc	gtg	gaa	ggg	ttg	ctg	tac	816
Arg	Glu	Ser	Gln	Arg	Leu	Glu	Asn	His	Phe	Val	Glu	Gly	Leu	Leu	Tyr	
			260					265					270			
tcc	ctg	gat	gag	gct	gtg	gct	gtc	atc	atg	aca	ggg	gtc	atg	acg	gac	864
Ser	Leu	Asp	Glu	Ala	Val	Ala	Val	Ile	Met	Thr	Gly	Val	Met	Thr	Asp	
		275					280						285			
gac	gta	gag	tcc	agc	aag	ctg	aat	agc	att	ggc	agt	tac	tac	aag	ccc	912
Asp	Val	Glu	Ser	Ser	Lys	Leu	Asn	Ser	Ile	Gly	Ser	Tyr	Tyr	Lys	Pro	
		290				295					300					
tgg	ttc	ttc	aag	cat	gtg	gag	aac	tac	ctg	aag	aca	aac	cgg	gag	ggc	960
Trp	Phe	Phe	Lys	His	Val	Glu	Asn	Tyr	Leu	Lys	Thr	Asn	Arg	Glu	Gly	
		305			310					315				320		
ctc	gaa	tac	att	ccc	ctg	aga	cac	tac	tac	cac	cga	cac	acg	cgc	agc	1008
Leu	Glu	Tyr	Ile	Pro	Leu	Arg	His	Tyr	Tyr	His	Arg	His	Thr	Arg	Ser	
				325					330					335		
atc	ttc	tgg	gag	ctc	cag	gac	atc	atc	cct	ttc	ggc	aac	aac	ccc	atc	1056
Ile	Phe	Trp	Glu	Leu	Gln	Asp	Ile	Ile	Pro	Phe	Gly	Asn	Asn	Pro	Ile	
			340					345						350		
ttc	cgc	tac	ctc	ttc	ggc	tgg	atg	gtg	cct	ccc	aag	atc	tcc	ctc	ctg	1104
Phe	Arg	Tyr	Leu	Phe	Gly	Trp	Met	Val	Pro	Pro	Lys	Ile	Ser	Leu	Leu	
			355					360					365			
aag	ctg	acc	cag	ggc	gag	acg	cta	cgc	aag	ctg	tac	gag	cag	cac	cac	1152
Lys	Leu	Thr	Gln	Gly	Glu	Thr	Leu	Arg	Lys	Leu	Tyr	Glu	Gln	His	His	
			370					375						380		
gtg	gtg	cag	gac	atg	ctg	gtg	ccc	atg	aag	tgc	atg	tca	cag	gcc	ctg	1200
Val	Val	Gln	Asp	Met	Leu	Val	Pro	Met	Lys	Cys	Met	Ser	Gln	Ala	Leu	
					385					390				400		
cat	acc	ttc	caa	aat	gac	atc	cac	gtc	tac	ccc	atc	tgg	ctg	tgc	cca	1248
His	Thr	Phe	Gln	Asn	Asp	Ile	His	Val	Tyr	Pro	Ile	Trp	Leu	Cys	Pro	
				405					410					415		
ttc	atc	ctg	ccc	agc	cag	cca	gga	cta	gtg	cat	ccc	aag	gga	gat	gaa	1296
Phe	Ile	Leu	Pro	Ser	Gln	Pro	Gly	Leu	Val	His	Pro	Lys	Gly	Asp	Glu	
			420						425					430		

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gca gag ctc tac gtg gac atc ggg gca tac ggg gag cca cgt gtg aag 1344
Ala Glu Leu Tyr Val Asp Ile Gly Ala Tyr Gly Glu Pro Arg Val Lys
      435                440                445

cac ttc gag gcc agg tcc tgc atg agg cag ctg gag aag ttt gtg cgg 1392
His Phe Glu Ala Arg Ser Cys Met Arg Gln Leu Glu Lys Phe Val Arg
      450                455                460

agt gtg cac ggg ttc caa atg tta tac gcc gat tgc tat atg aac cgc 1440
Ser Val His Gly Phe Gln Met Leu Tyr Ala Asp Cys Tyr Met Asn Arg
      465                470                475                480

gag gaa ttc tgg gag atg ttc gat ggc tcc ttg tac cac aag ctg cgc 1488
Glu Glu Phe Trp Glu Met Phe Asp Gly Ser Leu Tyr His Lys Leu Arg
      485                490                495

aag cag ctg ggc tgc cag gac gcc ttc cct gag gtg tac gac aag atc 1536
Lys Gln Leu Gly Cys Gln Asp Ala Phe Pro Glu Val Tyr Asp Lys Ile
      500                505                510

tgc aag gcg gca agg cac tga 1557
Cys Lys Ala Ala Arg His
      515

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&lt;210&gt; SEQ ID NO 14

&lt;211&gt; LENGTH: 518

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Mus musculus

&lt;400&gt; SEQUENCE: 14

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Met Glu Pro Ala Val Ser Leu Ala Val Cys Ala Leu Leu Phe Leu Leu
1      5      10      15

Trp Val Arg Val Lys Gly Leu Glu Phe Val Leu Ile His Gln Arg Trp
20     25     30

Val Phe Val Cys Leu Phe Leu Leu Pro Leu Ser Leu Ile Phe Asp Ile
35     40     45

Tyr Tyr Tyr Val Arg Ala Trp Val Val Phe Lys Leu Ser Ser Ala Pro
50     55     60

Arg Leu His Glu Gln Arg Val Arg Asp Ile Gln Lys Gln Val Arg Glu
65     70     75     80

Trp Lys Glu Gln Gly Ser Lys Thr Phe Met Cys Thr Gly Arg Pro Gly
85     90     95

Trp Leu Thr Val Ser Leu Arg Val Gly Lys Tyr Lys Lys Thr His Lys
100    105    110

Asn Ile Met Ile Asn Leu Met Asp Ile Leu Glu Val Asp Thr Lys Lys
115    120    125

Gln Ile Val Arg Val Glu Pro Leu Val Ser Met Gly Gln Val Thr Ala
130    135    140

Leu Leu Asn Ser Ile Gly Trp Thr Leu Pro Val Leu Pro Glu Leu Asp
145    150    155    160

Asp Leu Thr Val Gly Gly Leu Ile Met Gly Thr Gly Ile Glu Ser Ser
165    170    175

Ser His Lys Tyr Gly Leu Phe Gln His Ile Cys Thr Ala Tyr Glu Leu
180    185    190

Ile Leu Ala Asp Gly Ser Phe Val Arg Cys Thr Pro Ser Glu Asn Ser
195    200    205

Asp Leu Phe Tyr Ala Val Pro Trp Ser Cys Gly Thr Leu Gly Phe Leu
210    215    220

Val Ala Ala Glu Ile Arg Ile Ile Pro Ala Lys Lys Tyr Val Lys Leu
225    230    235    240

Arg Phe Glu Pro Val Arg Gly Leu Glu Ala Ile Cys Glu Lys Phe Thr
245    250    255

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Arg Glu Ser Gln Arg Leu Glu Asn His Phe Val Glu Gly Leu Leu Tyr  
 260 265 270  
 Ser Leu Asp Glu Ala Val Ala Val Ile Met Thr Gly Val Met Thr Asp  
 275 280 285  
 Asp Val Glu Ser Ser Lys Leu Asn Ser Ile Gly Ser Tyr Tyr Lys Pro  
 290 295 300  
 Trp Phe Phe Lys His Val Glu Asn Tyr Leu Lys Thr Asn Arg Glu Gly  
 305 310 315 320  
 Leu Glu Tyr Ile Pro Leu Arg His Tyr Tyr His Arg His Thr Arg Ser  
 325 330 335  
 Ile Phe Trp Glu Leu Gln Asp Ile Ile Pro Phe Gly Asn Asn Pro Ile  
 340 345 350  
 Phe Arg Tyr Leu Phe Gly Trp Met Val Pro Pro Lys Ile Ser Leu Leu  
 355 360 365  
 Lys Leu Thr Gln Gly Glu Thr Leu Arg Lys Leu Tyr Glu Gln His His  
 370 375 380  
 Val Val Gln Asp Met Leu Val Pro Met Lys Cys Met Ser Gln Ala Leu  
 385 390 395 400  
 His Thr Phe Gln Asn Asp Ile His Val Tyr Pro Ile Trp Leu Cys Pro  
 405 410 415  
 Phe Ile Leu Pro Ser Gln Pro Gly Leu Val His Pro Lys Gly Asp Glu  
 420 425 430  
 Ala Glu Leu Tyr Val Asp Ile Gly Ala Tyr Gly Glu Pro Arg Val Lys  
 435 440 445  
 His Phe Glu Ala Arg Ser Cys Met Arg Gln Leu Glu Lys Phe Val Arg  
 450 455 460  
 Ser Val His Gly Phe Gln Met Leu Tyr Ala Asp Cys Tyr Met Asn Arg  
 465 470 475 480  
 Glu Glu Phe Trp Glu Met Phe Asp Gly Ser Leu Tyr His Lys Leu Arg  
 485 490 495  
 Lys Gln Leu Gly Cys Gln Asp Ala Phe Pro Glu Val Tyr Asp Lys Ile  
 500 505 510  
 Cys Lys Ala Ala Arg His  
 515

<210> SEQ ID NO 15  
 <211> LENGTH: 1551  
 <212> TYPE: DNA  
 <213> ORGANISM: Homo sapiens  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1551)

<400> SEQUENCE: 15

atg gag ccc gcc gtg tcg ctg gcc gtg tgc gcg ctg ctc ttc ctg ctg	48
Met Glu Pro Ala Val Ser Leu Ala Val Cys Ala Leu Leu Phe Leu Leu	
1 5 10 15	
tggtgtgcgcctgaagggctggagttcgtgctc atc cac cag cgc tgg	96
Trp Val Arg Leu Lys Gly Leu Glu Phe Val Leu Ile His Gln Arg Trp	
20 25 30	
gtgttcgtgtgctctctctctg ccgctctcgctt atc ttc gat atc	144
Val Phe Val Cys Leu Phe Leu Leu Pro Leu Ser Leu Ile Phe Asp Ile	
35 40 45	
tac tac tac gtg cgc gcc tgg gtg gtg ttc aag ctc agc agc gct ccg	192
Tyr Tyr Tyr Val Arg Ala Trp Val Val Phe Lys Leu Ser Ser Ala Pro	
50 55 60	

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cgc ctg cac gag cag cgc gtg cgg gac atc cag aag cag gtg cgg gaa	240
Arg Leu His Glu Gln Arg Val Arg Asp Ile Gln Lys Gln Val Arg Glu	
65 70 75 80	
tgg aag gag cag ggt agc aag acc ttc atg tgc acg ggg cgc cct ggc	288
Trp Lys Glu Gln Gly Ser Lys Thr Phe Met Cys Thr Gly Arg Pro Gly	
85 90 95	
tgg ctc act gtc tca cta cgt gtc ggg aag tac aag aag aca cac aaa	336
Trp Leu Thr Val Ser Leu Arg Val Gly Lys Tyr Lys Lys Thr His Lys	
100 105 110	
aac atc atg atc aac ctg atg gac att ctg gaa gtg gac acc aag aaa	384
Asn Ile Met Ile Asn Leu Met Asp Ile Leu Glu Val Asp Thr Lys Lys	
115 120 125	
cag att gtc cgt gtg gag ccc ttg gtg acc atg ggc cag gtg act gcc	432
Gln Ile Val Arg Val Glu Pro Leu Val Thr Met Gly Gln Val Thr Ala	
130 135 140	
ctg ctg acc tcc att ggc tgg act ctc ccc gtg ttg cct gag ctt gat	480
Leu Leu Thr Ser Ile Gly Trp Thr Leu Pro Val Leu Pro Glu Leu Asp	
145 150 155 160	
gac ctc aca gtg ggg ggc ttg atc atg ggc aca ggc atc gag tca tca	528
Asp Leu Thr Val Gly Gly Leu Ile Met Gly Thr Gly Ile Glu Ser Ser	
165 170 175	
tcc cac aag tac ggc ctg ttc caa cac atc tgc act gct tac gag ctg	576
Ser His Lys Tyr Gly Leu Phe Gln His Ile Cys Thr Ala Tyr Glu Leu	
180 185 190	
gtc ctg gct gat ggc agc ttt gtg cga tgc act ccg tcc gaa aac tca	624
Val Leu Ala Asp Gly Ser Phe Val Arg Cys Thr Pro Ser Glu Asn Ser	
195 200 205	
gac ctg ttc tat gcc gta ccc tgg tcc tgt ggg acg ctg ggt ttc ctg	672
Asp Leu Phe Tyr Ala Val Pro Trp Ser Cys Gly Thr Leu Gly Phe Leu	
210 215 220	
gtg gcc gct gag atc cgc atc atc cct gcc aag aag tac gtc aag ctg	720
Val Ala Ala Glu Ile Arg Ile Ile Pro Ala Lys Lys Tyr Val Lys Leu	
225 230 235 240	
cgt ttc gag cca gtg cgg ggc ctg gag gct atc tgt gcc aag ttc acc	768
Arg Phe Glu Pro Val Arg Gly Leu Glu Ala Ile Cys Ala Lys Phe Thr	
245 250 255	
cac gag tcc cag cgg cag gag aac cac ttc gtg gaa ggg ctg ctc tac	816
His Glu Ser Gln Arg Gln Glu Asn His Phe Val Glu Gly Leu Leu Tyr	
260 265 270	
tcc ctg gat gag gct gtc att atg aca ggg gtc atg aca gat gag gca	864
Ser Leu Asp Glu Ala Val Ile Met Thr Gly Val Met Thr Asp Glu Ala	
275 280 285	
gag ccc agc aag ctg aat agc att ggc aat tac tac aag ccg tgg ttc	912
Glu Pro Ser Lys Leu Asn Ser Ile Gly Asn Tyr Tyr Lys Pro Trp Phe	
290 295 300	
ttt aag cat gtg gag aac tat ctg aag aca aac cga gag ggc ctg gag	960
Phe Lys His Val Glu Asn Tyr Leu Lys Thr Asn Arg Glu Gly Leu Glu	
305 310 315 320	
tac att ccc ttg aga cac tac tac cac cgc cac acg cgc agc atc ttc	1008
Tyr Ile Pro Leu Arg His Tyr Tyr His Arg His Thr Arg Ser Ile Phe	
325 330 335	
tgg gag ctc cag gac atc atc ccc ttt ggc aac aac ccc atc ttc cgc	1056
Trp Glu Leu Gln Asp Ile Ile Pro Phe Gly Asn Asn Pro Ile Phe Arg	
340 345 350	
tac ctc ttt ggc tgg atg gtg cct ccc aag atc tcc ctc ctg aag ctg	1104
Tyr Leu Phe Gly Trp Met Val Pro Pro Lys Ile Ser Leu Leu Lys Leu	
355 360 365	
acc cag ggt gag acc ctg cgc aag ctg tac gag cag cac cac gtg gtg	1152
Thr Gln Gly Glu Thr Leu Arg Lys Leu Tyr Glu Gln His His Val Val	
370 375 380	

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cag gac atg ctg gtg ccc atg aag tgc ctg cag cag gcc ctg cac acc 1200  
 Gln Asp Met Leu Val Pro Met Lys Cys Leu Gln Gln Ala Leu His Thr  
 385 390 395 400

ttc caa aac gac atc cac gtc tac ccc atc tgg ctg tgt ccg ttc atc 1248  
 Phe Gln Asn Asp Ile His Val Tyr Pro Ile Trp Leu Cys Pro Phe Ile  
 405 410 415

ctg ccc agc cag cca gcc cta gtg cac ccc aaa gga aat gag gca gag 1296  
 Leu Pro Ser Gln Pro Gly Leu Val His Pro Lys Gly Asn Glu Ala Glu  
 420 425 430

ctc tac atc gac att gga gca tat ggg gag ccg cgt gtg aaa cac ttt 1344  
 Leu Tyr Ile Asp Ile Gly Ala Tyr Gly Glu Pro Arg Val Lys His Phe  
 435 440 445

gaa gcc agg tcc tgc atg agg cag ctg gag aag ttt gtc cgc agc gtg 1392  
 Glu Ala Arg Ser Cys Met Arg Gln Leu Glu Lys Phe Val Arg Ser Val  
 450 455 460

cat gcc ttc cag atg ctg tat gcc gac tgc tac atg aac cgg gag gag 1440  
 His Gly Phe Gln Met Leu Tyr Ala Asp Cys Tyr Met Asn Arg Glu Glu  
 465 470 475 480

ttc tgg gag atg ttt gat gcc tcc ttg tac cac aag ctg cga gag aag 1488  
 Phe Trp Glu Met Phe Asp Gly Ser Leu Tyr His Lys Leu Arg Glu Lys  
 485 490 495

ctg ggt tgc cag gac gcc ttc ccc gag gtg tac gac aag atc tgc aag 1536  
 Leu Gly Cys Gln Asp Ala Phe Pro Glu Val Tyr Asp Lys Ile Cys Lys  
 500 505 510

gcc gcc agg cac tga 1551  
 Ala Ala Arg His  
 515

<210> SEQ ID NO 16  
 <211> LENGTH: 516  
 <212> TYPE: PRT  
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Met Glu Pro Ala Val Ser Leu Ala Val Cys Ala Leu Leu Phe Leu Leu  
 1 5 10 15

Trp Val Arg Leu Lys Gly Leu Glu Phe Val Leu Ile His Gln Arg Trp  
 20 25 30

Val Phe Val Cys Leu Phe Leu Leu Pro Leu Ser Leu Ile Phe Asp Ile  
 35 40 45

Tyr Tyr Tyr Val Arg Ala Trp Val Val Phe Lys Leu Ser Ser Ala Pro  
 50 55 60

Arg Leu His Glu Gln Arg Val Arg Asp Ile Gln Lys Gln Val Arg Glu  
 65 70 75 80

Trp Lys Glu Gln Gly Ser Lys Thr Phe Met Cys Thr Gly Arg Pro Gly  
 85 90 95

Trp Leu Thr Val Ser Leu Arg Val Gly Lys Tyr Lys Lys Thr His Lys  
 100 105 110

Asn Ile Met Ile Asn Leu Met Asp Ile Leu Glu Val Asp Thr Lys Lys  
 115 120 125

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

Leu Leu Thr Ser Ile Gly Trp Thr Leu Pro Val Leu Pro Glu Leu Asp  
 145 150 155 160

Asp Leu Thr Val Gly Gly Leu Ile Met Gly Thr Gly Ile Glu Ser Ser  
 165 170 175

Ser His Lys Tyr Gly Leu Phe Gln His Ile Cys Thr Ala Tyr Glu Leu

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180	185	190
Val Leu Ala Asp Gly Ser Phe	Val Arg Cys Thr Pro Ser Glu Asn Ser	
195	200	205
Asp Leu Phe Tyr Ala Val Pro Trp Ser Cys Gly Thr Leu Gly Phe Leu		
210	215	220
Val Ala Ala Glu Ile Arg Ile Ile Pro Ala Lys Lys Tyr Val Lys Leu		
225	230	235
Arg Phe Glu Pro Val Arg Gly Leu Glu Ala Ile Cys Ala Lys Phe Thr		
245	250	255
His Glu Ser Gln Arg Gln Glu Asn His Phe Val Glu Gly Leu Leu Tyr		
260	265	270
Ser Leu Asp Glu Ala Val Ile Met Thr Gly Val Met Thr Asp Glu Ala		
275	280	285
Glu Pro Ser Lys Leu Asn Ser Ile Gly Asn Tyr Tyr Lys Pro Trp Phe		
290	295	300
Phe Lys His Val Glu Asn Tyr Leu Lys Thr Asn Arg Glu Gly Leu Glu		
305	310	315
Tyr Ile Pro Leu Arg His Tyr Tyr His Arg His Thr Arg Ser Ile Phe		
325	330	335
Trp Glu Leu Gln Asp Ile Ile Pro Phe Gly Asn Asn Pro Ile Phe Arg		
340	345	350
Tyr Leu Phe Gly Trp Met Val Pro Pro Lys Ile Ser Leu Leu Lys Leu		
355	360	365
Thr Gln Gly Glu Thr Leu Arg Lys Leu Tyr Glu Gln His His Val Val		
370	375	380
Gln Asp Met Leu Val Pro Met Lys Cys Leu Gln Gln Ala Leu His Thr		
385	390	395
Phe Gln Asn Asp Ile His Val Tyr Pro Ile Trp Leu Cys Pro Phe Ile		
405	410	415
Leu Pro Ser Gln Pro Gly Leu Val His Pro Lys Gly Asn Glu Ala Glu		
420	425	430
Leu Tyr Ile Asp Ile Gly Ala Tyr Gly Glu Pro Arg Val Lys His Phe		
435	440	445
Glu Ala Arg Ser Cys Met Arg Gln Leu Glu Lys Phe Val Arg Ser Val		
450	455	460
His Gly Phe Gln Met Leu Tyr Ala Asp Cys Tyr Met Asn Arg Glu Glu		
465	470	475
Phe Trp Glu Met Phe Asp Gly Ser Leu Tyr His Lys Leu Arg Glu Lys		
485	490	495
Leu Gly Cys Gln Asp Ala Phe Pro Glu Val Tyr Asp Lys Ile Cys Lys		
500	505	510
Ala Ala Arg His		
515		

<210> SEQ ID NO 17  
 <211> LENGTH: 1422  
 <212> TYPE: DNA  
 <213> ORGANISM: Saccharomyces cerevisiae  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1422)

<400> SEQUENCE: 17

atg gca aag gat aat agt gag aag ctg cag gtc cag gga gag gag aaa  
 Met Ala Lys Asp Asn Ser Glu Lys Leu Gln Val Gln Gly Glu Glu Lys  
 1 5 10 15

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aag tcc aag caa ccg gtt aat ttc ctg cct cag ggt aaa tgg ctg aag Lys Ser Lys Gln Pro Val Asn Phe Leu Pro Gln Gly Lys Trp Leu Lys 20 25 30	96
cca aat gaa atc gaa tat gag ttt ggt ggg act act ggt gtt att ggt Pro Asn Glu Ile Glu Tyr Glu Phe Gly Gly Thr Thr Gly Val Ile Gly 35 40 45	144
atg ctg atc ggg ttt cca ctg cta atg tac tat atg tgg att tgt gcg Met Leu Ile Gly Phe Pro Leu Leu Met Tyr Tyr Met Trp Ile Cys Ala 50 55 60	192
gaa ttt tat cac ggt aag gtt gcc cta ccc aag gct ggt gaa tcg tgg Glu Phe Tyr His Gly Lys Val Ala Leu Pro Lys Ala Gly Glu Ser Trp 65 70 75 80	240
atg cac ttt atc aag cac cta tac cag tta gtc ttg gag aac ggt atc Met His Phe Ile Lys His Leu Tyr Gln Leu Val Leu Glu Asn Gly Ile 85 90 95	288
cca gaa aag tat gac tgg act att ttc tta aca ttt tgg gtg ttt cag Pro Glu Lys Tyr Asp Trp Thr Ile Phe Leu Thr Phe Trp Val Phe Gln 100 105 110	336
atc att ttc tac tat acg ttg ccc ggg att tgg aca aaa ggt caa cca Ile Ile Phe Tyr Tyr Thr Leu Pro Gly Ile Trp Thr Lys Gly Gln Pro 115 120 125	384
ttg tct cat ttg aag gga aaa caa ttg cct tac ttt tgt aat gcc atg Leu Ser His Leu Lys Gly Lys Gln Leu Pro Tyr Phe Cys Asn Ala Met 130 135 140	432
tgg acc ttg tat gta act acc act ttg gtc ttg gtt ttg cac ttt acc Trp Thr Leu Tyr Val Thr Thr Thr Leu Val Leu Val Leu His Phe Thr 145 150 155 160	480
aat ctt ttt aga ttg tat gtc att att gac cgt ttt ggg agg atc atg Asn Leu Phe Arg Leu Tyr Val Ile Ile Asp Arg Phe Gly Arg Ile Met 165 170 175	528
aca tgt gcc att att tca ggg ttt gcc ttc tcc atc ata ttg tac tta Thr Cys Ala Ile Ile Ser Gly Phe Ala Phe Ser Ile Ile Leu Tyr Leu 180 185 190	576
tgg act tta ttt atc tca cat gac tat cat aga atg aca gga aac cat Trp Thr Leu Phe Ile Ser His Asp Tyr His Arg Met Thr Gly Asn His 195 200 205	624
cta tat gat ttc ttc atg gga gct cca cta aac cct agg tgg ggg att Leu Tyr Asp Phe Phe Met Gly Ala Pro Leu Asn Pro Arg Trp Gly Ile 210 215 220	672
ttg gac ttg aag atg ttt ttc gag gtt aga tta cct tgg ttc acc ctt Leu Asp Leu Lys Met Phe Phe Glu Val Arg Leu Pro Trp Phe Thr Leu 225 230 235 240	720
tac ttt atc act ttg ggt gcc tgt ttg aag cag tgg gag act tac ggc Tyr Phe Ile Thr Leu Gly Ala Cys Leu Lys Gln Trp Glu Thr Tyr Gly 245 250 255	768
tat gtg aca cca caa ttg ggg gtt gtc atg tta gct cat tgg ttg tac Tyr Val Thr Pro Gln Leu Gly Val Val Met Leu Ala His Trp Leu Tyr 260 265 270	816
gcg aac gca tgt gct aaa ggt gaa gaa ttg att gtt cca acc tgg gac Ala Asn Ala Cys Ala Lys Gly Glu Glu Leu Ile Val Pro Thr Trp Asp 275 280 285	864
atg gct tac gaa aag ttt gga ttt atg ctg atc ttc tgg aat att gcc Met Ala Tyr Glu Lys Phe Gly Phe Met Leu Ile Phe Trp Asn Ile Ala 290 295 300	912
ggg gtc cca tac act tac tgt cat tgt acg ttg tat ttg tac tac cat Gly Val Pro Tyr Thr Tyr Cys His Cys Thr Leu Tyr Leu Tyr Tyr His 305 310 315 320	960
gac cca tct gaa tat cac tgg tct aca ctg tac aat gtt tcg ctg tac Asp Pro Ser Glu Tyr His Trp Ser Thr Leu Tyr Asn Val Ser Leu Tyr	1008

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	325	330	335	
gtt gtt cta tta tgc gcc tac tac ttc ttt gac acg gca aat gct cag				1056
Val Val Leu Leu Cys Ala Tyr Tyr Phe Phe Asp Thr Ala Asn Ala Gln	340	345	350	
aaa aat gcc ttc aga aag caa atg tct ggt gac aag aca ggt agg aag				1104
Lys Asn Ala Phe Arg Lys Gln Met Ser Gly Asp Lys Thr Gly Arg Lys	355	360	365	
act ttc cca ttt ttg cca tac caa att ttg aag aat cca aag tat atg				1152
Thr Phe Pro Phe Leu Pro Tyr Gln Ile Leu Lys Asn Pro Lys Tyr Met	370	375	380	
gtt acc tcc aat gga tcg tac cta ttg att gat ggt tgg tac act ttg				1200
Val Thr Ser Asn Gly Ser Tyr Leu Leu Ile Asp Gly Trp Tyr Thr Leu	385	390	395	400
gct aga aaa att cac tac act gcc gat tgg act caa tct ctc gtt tgg				1248
Ala Arg Lys Ile His Tyr Thr Ala Asp Trp Thr Gln Ser Leu Val Trp	405	410	415	
gcc ttg tct tgc ggg ttc aac tcg gtg ttc cca tgg ttt ttc cca gta				1296
Ala Leu Ser Cys Gly Phe Asn Ser Val Phe Pro Trp Phe Phe Pro Val	420	425	430	
ttc ttc ctt gtt gtc ctg att cac aga gcc ttc aga gac caa gca aaa				1344
Phe Phe Leu Val Val Leu Ile His Arg Ala Phe Arg Asp Gln Ala Lys	435	440	445	
tgt aag aga aag tac gga aaa gat tgg gat gag tat tgt aaa cat tgc				1392
Cys Lys Arg Lys Tyr Gly Lys Asp Trp Asp Glu Tyr Cys Lys His Cys	450	455	460	
cct tac gtc ttt att cct tat gtt ttc tag				1422
Pro Tyr Val Phe Ile Pro Tyr Val Phe	465	470		
 <210> SEQ ID NO 18				
<211> LENGTH: 473				
<212> TYPE: PRT				
<213> ORGANISM: Saccharomyces cerevisiae				
 <400> SEQUENCE: 18				
Met Ala Lys Asp Asn Ser Glu Lys Leu Gln Val Gln Gly Glu Glu Lys	1	5	10	15
Lys Ser Lys Gln Pro Val Asn Phe Leu Pro Gln Gly Lys Trp Leu Lys	20	25	30	
Pro Asn Glu Ile Glu Tyr Glu Phe Gly Gly Thr Thr Gly Val Ile Gly	35	40	45	
Met Leu Ile Gly Phe Pro Leu Leu Met Tyr Tyr Met Trp Ile Cys Ala	50	55	60	
Glu Phe Tyr His Gly Lys Val Ala Leu Pro Lys Ala Gly Glu Ser Trp	65	70	75	80
Met His Phe Ile Lys His Leu Tyr Gln Leu Val Leu Glu Asn Gly Ile	85	90	95	
Pro Glu Lys Tyr Asp Trp Thr Ile Phe Leu Thr Phe Trp Val Phe Gln	100	105	110	
Ile Ile Phe Tyr Tyr Thr Leu Pro Gly Ile Trp Thr Lys Gly Gln Pro	115	120	125	
Leu Ser His Leu Lys Gly Lys Gln Leu Pro Tyr Phe Cys Asn Ala Met	130	135	140	
Trp Thr Leu Tyr Val Thr Thr Thr Leu Val Leu Val Leu His Phe Thr	145	150	155	160
Asn Leu Phe Arg Leu Tyr Val Ile Ile Asp Arg Phe Gly Arg Ile Met	165	170	175	

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Thr Cys Ala Ile Ile Ser Gly Phe Ala Phe Ser Ile Ile Leu Tyr Leu  
 180 185 190

Trp Thr Leu Phe Ile Ser His Asp Tyr His Arg Met Thr Gly Asn His  
 195 200 205

Leu Tyr Asp Phe Phe Met Gly Ala Pro Leu Asn Pro Arg Trp Gly Ile  
 210 215 220

Leu Asp Leu Lys Met Phe Phe Glu Val Arg Leu Pro Trp Phe Thr Leu  
 225 230 235 240

Tyr Phe Ile Thr Leu Gly Ala Cys Leu Lys Gln Trp Glu Thr Tyr Gly  
 245 250 255

Tyr Val Thr Pro Gln Leu Gly Val Val Met Leu Ala His Trp Leu Tyr  
 260 265 270

Ala Asn Ala Cys Ala Lys Gly Glu Glu Leu Ile Val Pro Thr Trp Asp  
 275 280 285

Met Ala Tyr Glu Lys Phe Gly Phe Met Leu Ile Phe Trp Asn Ile Ala  
 290 295 300

Gly Val Pro Tyr Thr Tyr Cys His Cys Thr Leu Tyr Leu Tyr Tyr His  
 305 310 315 320

Asp Pro Ser Glu Tyr His Trp Ser Thr Leu Tyr Asn Val Ser Leu Tyr  
 325 330 335

Val Val Leu Leu Cys Ala Tyr Tyr Phe Phe Asp Thr Ala Asn Ala Gln  
 340 345 350

Lys Asn Ala Phe Arg Lys Gln Met Ser Gly Asp Lys Thr Gly Arg Lys  
 355 360 365

Thr Phe Pro Phe Leu Pro Tyr Gln Ile Leu Lys Asn Pro Lys Tyr Met  
 370 375 380

Val Thr Ser Asn Gly Ser Tyr Leu Leu Ile Asp Gly Trp Tyr Thr Leu  
 385 390 395 400

Ala Arg Lys Ile His Tyr Thr Ala Asp Trp Thr Gln Ser Leu Val Trp  
 405 410 415

Ala Leu Ser Cys Gly Phe Asn Ser Val Phe Pro Trp Phe Phe Pro Val  
 420 425 430

Phe Phe Leu Val Val Leu Ile His Arg Ala Phe Arg Asp Gln Ala Lys  
 435 440 445

Cys Lys Arg Lys Tyr Gly Lys Asp Trp Asp Glu Tyr Cys Lys His Cys  
 450 455 460

Pro Tyr Val Phe Ile Pro Tyr Val Phe  
 465 470

<210> SEQ ID NO 19  
 <211> LENGTH: 1152  
 <212> TYPE: DNA  
 <213> ORGANISM: Saccharomyces cerevisiae  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1152)

<400> SEQUENCE: 19

atg agt gaa aca gaa ttg aga aaa aga cag gcc caa ttc act agg gag	48
Met Ser Glu Thr Glu Leu Arg Lys Arg Gln Ala Gln Phe Thr Arg Glu	
1 5 10 15	
tta cat ggt gat gat att ggt aaa aag aca ggt ttg agt gca ttg atg	96
Leu His Gly Asp Asp Ile Gly Lys Lys Thr Gly Leu Ser Ala Leu Met	
20 25 30	
tcg aag aac aac tct gcc caa aag gaa gcc gtt cag aag tac ttg aga	144
Ser Lys Asn Asn Ser Ala Gln Lys Glu Ala Val Gln Lys Tyr Leu Arg	
35 40 45	

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aat tgg gat ggt aga acc gat aaa gat gcc gaa gaa cgt cgt ctt gag Asn Trp Asp Gly Arg Thr Asp Lys Asp Ala Glu Glu Arg Arg Leu Glu 50 55 60	192
gat tat aat gaa gcc aca cat tcc tac tat aac gtc gtt aca gat ttc Asp Tyr Asn Glu Ala Thr His Ser Tyr Tyr Asn Val Val Thr Asp Phe 65 70 75 80	240
tat gaa tat ggt tgg ggt tcc tct ttc cat ttc agc aga ttt tat aaa Tyr Glu Tyr Gly Trp Gly Ser Ser Phe His Phe Ser Arg Phe Tyr Lys 85 90 95	288
ggt gag agt ttc gct gcc tcg ata gca aga cat gaa cat tat tta gct Gly Glu Ser Phe Ala Ala Ser Ile Ala Arg His Glu His Tyr Leu Ala 100 105 110	336
tac aag gct ggt att caa aga ggc gat tta gtt ctc gac gtt ggt tgt Tyr Lys Ala Gly Ile Gln Arg Gly Asp Leu Val Leu Asp Val Gly Cys 115 120 125	384
ggt gtt ggg ggc cca gca aga gag att gca aga ttt acc ggt tgt aac Gly Val Gly Gly Pro Ala Arg Glu Ile Ala Arg Phe Thr Gly Cys Asn 130 135 140	432
gtc atc ggt cta aac aat aac gat tac caa att gcc aag gca aaa tat Val Ile Gly Leu Asn Asn Asn Asp Tyr Gln Ile Ala Lys Ala Lys Tyr 145 150 155 160	480
tac gct aaa aaa tac aat ttg agt gac caa atg gac ttt gta aag ggt Tyr Ala Lys Lys Tyr Asn Leu Ser Asp Gln Met Asp Phe Val Lys Gly 165 170 175	528
gat ttc atg aaa atg gat ttc gaa gaa aac act ttc gac aaa gtt tat Asp Phe Met Lys Met Asp Phe Glu Glu Asn Thr Phe Asp Lys Val Tyr 180 185 190	576
gca att gag gcc aca tgt cac gct cca aaa tta gaa ggt gta tac agc Ala Ile Glu Ala Thr Cys His Ala Pro Lys Leu Glu Gly Val Tyr Ser 195 200 205	624
gaa atc tac aag gtt ttg aaa ccg ggt ggt acc ttt gct gtt tac gaa Glu Ile Tyr Lys Val Leu Lys Pro Gly Gly Thr Phe Ala Val Tyr Glu 210 215 220	672
tgg gta atg act gat aaa tat gac gaa aac aat cct gaa cat aga aag Trp Val Met Thr Asp Lys Tyr Asp Glu Asn Asn Pro Glu His Arg Lys 225 230 235 240	720
atc gct tat gaa att gaa cta ggt gat ggt atc cca aag atg ttc cat Ile Ala Tyr Glu Ile Glu Leu Gly Asp Gly Ile Pro Lys Met Phe His 245 250 255	768
gtc gac gtg gct agg aaa gca ttg aag aac tgt ggt ttc gaa gtc ctc Val Asp Val Ala Arg Lys Ala Leu Lys Asn Cys Gly Phe Glu Val Leu 260 265 270	816
gtt agc gaa gac ctg gcg gac aat gat gat gaa atc cct tgg tat tac Val Ser Glu Asp Leu Ala Asp Asn Asp Asp Glu Ile Pro Trp Tyr Tyr 275 280 285	864
cca tta act ggt gag tgg aag tac gtt caa aac tta gct aat ttg gcc Pro Leu Thr Gly Glu Trp Lys Tyr Val Gln Asn Leu Ala Asn Leu Ala 290 295 300	912
aca ttt ttc aga act tct tac ttg ggt aga caa ttt act aca gca atg Thr Phe Phe Arg Thr Ser Tyr Leu Gly Arg Gln Phe Thr Thr Ala Met 305 310 315 320	960
gtt act gta atg gaa aaa tta ggt cta gcc cca gaa ggt tcc aag gaa Val Thr Val Met Glu Lys Leu Gly Leu Ala Pro Glu Gly Ser Lys Glu 325 330 335	1008
gtt act gct gct cta gaa aat gct gcg gtt ggt tta gtt gcc ggt ggt Val Thr Ala Ala Leu Glu Asn Ala Ala Val Gly Leu Val Ala Gly Gly 340 345 350	1056
aag tcc aag tta ttc act cca atg atg ctt ttc gtc gct agg aag cca Lys Ser Lys Leu Phe Thr Pro Met Met Leu Phe Val Ala Arg Lys Pro	1104

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355          360          365
gaa aac gcc gaa acc ccc tcc caa act tcc caa gaa gca act caa taa    1152
Glu Asn Ala Glu Thr Pro Ser Gln Thr Ser Gln Glu Ala Thr Gln
370          375          380

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<210> SEQ ID NO 20
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

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<400> SEQUENCE: 20

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

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Lys Ser Lys Leu Phe Thr Pro Met Met Leu Phe Val Ala Arg Lys Pro  
 355 360 365

Glu Asn Ala Glu Thr Pro Ser Gln Thr Ser Gln Glu Ala Thr Gln  
 370 375 380

<210> SEQ ID NO 21  
 <211> LENGTH: 1617  
 <212> TYPE: DNA  
 <213> ORGANISM: *Saccharomyces cerevisiae*  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1617)

<400> SEQUENCE: 21

atg agt tct gtc gca gaa aat ata ata caa cat gcc act cat aat tct 48  
 Met Ser Ser Val Ala Glu Asn Ile Ile Gln His Ala Thr His Asn Ser  
 1 5 10 15

acg cta cac caa ttg gct aaa gac cag ccc tct gta ggc gtc act act 96  
 Thr Leu His Gln Leu Ala Lys Asp Gln Pro Ser Val Gly Val Thr Thr  
 20 25 30

gcc ttc agt atc ctg gat aca ctt aag tct atg tca tat ttg aaa ata 144  
 Ala Phe Ser Ile Leu Asp Thr Leu Lys Ser Met Ser Tyr Leu Lys Ile  
 35 40 45

ttt gct act tta atc tgt att ctt ttg gtt tgg gac caa gtt gca tat 192  
 Phe Ala Thr Leu Ile Cys Ile Leu Leu Val Trp Asp Gln Val Ala Tyr  
 50 55 60

caa atc aag aaa ggt tcc atc gca ggt cca aag ttt aag ttc tgg ccc 240  
 Gln Ile Lys Lys Gly Ser Ile Ala Gly Pro Lys Phe Lys Phe Trp Pro  
 65 70 75 80

atc atc ggt cca ttt ttg gaa tcc tta gat cca aag ttt gaa gaa tat 288  
 Ile Ile Gly Pro Phe Leu Glu Ser Leu Asp Pro Lys Phe Glu Glu Tyr  
 85 90 95

aag gct aag tgg gca tcc ggt cca ctt tca tgt gtt tct att ttc cat 336  
 Lys Ala Lys Trp Ala Ser Gly Pro Leu Ser Cys Val Ser Ile Phe His  
 100 105 110

aaa ttt gtt gtt atc gca tct act aga gac ttg gca aga aag atc ttg 384  
 Lys Phe Val Val Ile Ala Ser Thr Arg Asp Leu Ala Arg Lys Ile Leu  
 115 120 125

caa tct tcc aaa ttc gtc aaa cct tgc gtt gtc gat gtt gct gtg aag 432  
 Gln Ser Ser Lys Phe Val Lys Pro Cys Val Val Asp Val Ala Val Lys  
 130 135 140

atc tta aga cct tgc aat tgg gtt ttt ttg gac ggt aaa gct cat act 480  
 Ile Leu Arg Pro Cys Asn Trp Val Phe Leu Asp Gly Lys Ala His Thr  
 145 150 155 160

gat tac aga aaa tca tta aac ggt ctt ttc act aaa caa gct ttg gct 528  
 Asp Tyr Arg Lys Ser Leu Asn Gly Leu Phe Thr Lys Gln Ala Leu Ala  
 165 170 175

caa tac tta cct tca ttg gaa caa atc atg gat aag tac atg gat aag 576  
 Gln Tyr Leu Pro Ser Leu Glu Gln Ile Met Asp Lys Tyr Met Asp Lys  
 180 185 190

ttt gtt cgt tta tct aag gag aat aac tac gag ccc cag gtc ttt ttc 624  
 Phe Val Arg Leu Ser Lys Glu Asn Asn Tyr Glu Pro Gln Val Phe Phe  
 195 200 205

cat gaa atg aga gaa att ctt tgc gcc tta tca ttg aac tct ttc tgt 672  
 His Glu Met Arg Glu Ile Leu Cys Ala Leu Ser Leu Asn Ser Phe Cys  
 210 215 220

ggg aac tat att acc gaa gat caa gtc aga aag att gct gat gat tac 720  
 Gly Asn Tyr Ile Thr Glu Asp Gln Val Arg Lys Ile Ala Asp Asp Tyr  
 225 230 235 240

tat ttg gtt aca gca gca ttg gaa tta gtc aac ttc cca att att atc 768

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Tyr	Leu	Val	Thr	Ala	Ala	Leu	Glu	Leu	Val	Asn	Phe	Pro	Ile	Ile	Ile		
				245					250					255			
cct	tac	act	aaa	aca	tgg	tat	ggt	aag	aaa	act	gca	gac	atg	gcc	atg	816	
Pro	Tyr	Thr	Lys	Thr	Trp	Tyr	Gly	Lys	Lys	Thr	Ala	Asp	Met	Ala	Met		
			260					265					270				
aag	att	ttc	gaa	aac	tgt	gct	caa	atg	gct	aag	gat	cat	att	gct	gca	864	
Lys	Ile	Phe	Glu	Asn	Cys	Ala	Gln	Met	Ala	Lys	Asp	His	Ile	Ala	Ala		
		275					280					285					
ggt	ggt	aag	cca	gtt	tgt	gtt	atg	gat	gct	tgg	tgt	aag	ttg	atg	cac	912	
Gly	Gly	Lys	Pro	Val	Cys	Val	Met	Asp	Ala	Trp	Cys	Lys	Leu	Met	His		
	290					295					300						
gat	gca	aag	aat	agt	aac	gat	gat	gat	tct	aga	atc	tac	cac	aga	gag	960	
Asp	Ala	Lys	Asn	Ser	Asn	Asp	Asp	Asp	Ser	Arg	Ile	Tyr	His	Arg	Glu		
	305				310					315					320		
ttt	act	aac	aag	gaa	atc	tcc	gaa	gct	ggt	ttc	act	ttc	tta	ttt	gct	1008	
Phe	Thr	Asn	Lys	Glu	Ile	Ser	Glu	Ala	Val	Phe	Thr	Phe	Leu	Phe	Ala		
			325						330					335			
tct	caa	gat	gcc	tct	tct	tct	tta	gct	tgt	tgg	ttg	ttc	caa	att	gtt	1056	
Ser	Gln	Asp	Ala	Ser	Ser	Ser	Leu	Ala	Cys	Trp	Leu	Phe	Gln	Ile	Val		
			340					345					350				
gct	gac	cgt	cca	gat	gtc	tta	gct	aag	atc	aga	gaa	gaa	caa	ttg	gct	1104	
Ala	Asp	Arg	Pro	Asp	Val	Leu	Ala	Lys	Ile	Arg	Glu	Glu	Gln	Leu	Ala		
	355						360					365					
ggt	cgt	aac	aat	gac	atg	tct	acc	gaa	ttg	aac	ttg	gat	ttg	att	gag	1152	
Val	Arg	Asn	Asn	Asp	Met	Ser	Thr	Glu	Leu	Asn	Leu	Asp	Leu	Ile	Glu		
	370					375					380						
aaa	atg	aag	tac	acc	aat	atg	gtc	ata	aaa	gaa	act	ttg	cgt	tac	aga	1200	
Lys	Met	Lys	Tyr	Thr	Asn	Met	Val	Ile	Lys	Glu	Thr	Leu	Arg	Tyr	Arg		
	385				390					395				400			
cct	cct	gtc	ttg	atg	ggt	cca	tat	ggt	ggt	aag	aag	aat	ttc	cca	ggt	1248	
Pro	Pro	Val	Leu	Met	Val	Pro	Tyr	Val	Val	Lys	Lys	Asn	Phe	Pro	Val		
				405					410					415			
tcc	cct	aac	tat	acc	gca	cca	aag	ggc	gct	atg	tta	att	cca	acc	tta	1296	
Ser	Pro	Asn	Tyr	Thr	Ala	Pro	Lys	Gly	Ala	Met	Leu	Ile	Pro	Thr	Leu		
			420					425					430				
tac	cca	gct	tta	cat	gat	cct	gaa	ggt	tac	gaa	aat	cct	gat	gag	ttc	1344	
Tyr	Pro	Ala	Leu	His	Asp	Pro	Glu	Val	Tyr	Glu	Asn	Pro	Asp	Glu	Phe		
			435				440					445					
atc	cct	gaa	aga	tgg	gta	gaa	ggc	tct	aag	gct	agt	gaa	gca	aag	aag	1392	
Ile	Pro	Glu	Arg	Trp	Val	Glu	Gly	Ser	Lys	Ala	Ser	Glu	Ala	Lys	Lys		
	450					455					460						
aat	tgg	ttg	ggt	ttt	ggt	tgt	ggt	cca	cac	ggt	tgc	tta	ggt	caa	aca	1440	
Asn	Trp	Leu	Val	Phe	Gly	Cys	Gly	Pro	His	Val	Cys	Leu	Gly	Gln	Thr		
	465				470					475				480			
tat	gtc	atg	att	acc	ttc	gcc	gct	ttg	ttg	ggt	aaa	ttt	gca	cta	tat	1488	
Tyr	Val	Met	Ile	Thr	Phe	Ala	Ala	Leu	Leu	Gly	Lys	Phe	Ala	Leu	Tyr		
				485					490					495			
act	gat	ttc	cat	cat	aca	gtg	act	cca	tta	agt	gaa	aaa	atc	aag	ggt	1536	
Thr	Asp	Phe	His	His	Thr	Val	Thr	Pro	Leu	Ser	Glu	Lys	Ile	Lys	Val		
				500				505					510				
ttc	gct	aca	att	ttc	cca	aaa	gat	gat	ttg	tta	ctg	act	ttc	aaa	aag	1584	
Phe	Ala	Thr	Ile	Phe	Pro	Lys	Asp	Asp	Leu	Leu	Leu	Thr	Phe	Lys	Lys		
		515					520					525					
aga	gac	cca	att	act	gga	gaa	gtc	ttc	gaa	taa						1617	
Arg	Asp	Pro	Ile	Thr	Gly	Glu	Val	Phe	Glu								
	530					535											

&lt;210&gt; SEQ ID NO 22

&lt;211&gt; LENGTH: 538

&lt;212&gt; TYPE: PRT

-continued

<213> ORGANISM: *Saccharomyces cerevisiae*

&lt;400&gt; SEQUENCE: 22

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

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Pro Pro Val Leu Met Val Pro Tyr Val Val Lys Lys Asn Phe Pro Val  
 405 410 415

Ser Pro Asn Tyr Thr Ala Pro Lys Gly Ala Met Leu Ile Pro Thr Leu  
 420 425 430

Tyr Pro Ala Leu His Asp Pro Glu Val Tyr Glu Asn Pro Asp Glu Phe  
 435 440 445

Ile Pro Glu Arg Trp Val Glu Gly Ser Lys Ala Ser Glu Ala Lys Lys  
 450 455 460

Asn Trp Leu Val Phe Gly Cys Gly Pro His Val Cys Leu Gly Gln Thr  
 465 470 475 480

Tyr Val Met Ile Thr Phe Ala Ala Leu Leu Gly Lys Phe Ala Leu Tyr  
 485 490 495

Thr Asp Phe His His Thr Val Thr Pro Leu Ser Glu Lys Ile Lys Val  
 500 505 510

Phe Ala Thr Ile Phe Pro Lys Asp Asp Leu Leu Leu Thr Phe Lys Lys  
 515 520 525

Arg Asp Pro Ile Thr Gly Glu Val Phe Glu  
 530 535

<210> SEQ ID NO 23  
 <211> LENGTH: 1578  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Truncated  
 HMG construct  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1578)

<400> SEQUENCE: 23

atg gac caa ttg gtg aaa act gaa gtc acc aag aag tct ttt act gct	48
Met Asp Gln Leu Val Lys Thr Glu Val Thr Lys Lys Ser Phe Thr Ala	
1 5 10 15	
cct gta caa aag gct tct aca cca gtt tta acc aat aaa aca gtc att	96
Pro Val Gln Lys Ala Ser Thr Pro Val Leu Thr Asn Lys Thr Val Ile	
20 25 30	
tct gga tgc aaa gtc aaa agt tta tca tct gcg caa tgc agc tca tca	144
Ser Gly Ser Lys Val Lys Ser Leu Ser Ser Ala Gln Ser Ser Ser	
35 40 45	
gga cct tca tca tct agt gag gaa gat gat tcc cgc gat att gaa agc	192
Gly Pro Ser Ser Ser Ser Glu Glu Asp Asp Ser Arg Asp Ile Glu Ser	
50 55 60	
ttg gat aag aaa ata cgt cct tta gaa gaa tta gaa gca tta tta agt	240
Leu Asp Lys Lys Ile Arg Pro Leu Glu Glu Leu Glu Ala Leu Leu Ser	
65 70 75 80	
agt gga aat aca aaa caa ttg aag aac aaa gag gtc gct gcc ttg gtt	288
Ser Gly Asn Thr Lys Gln Leu Lys Asn Lys Glu Val Ala Ala Leu Val	
85 90 95	
att cac ggt aag tta cct ttg tac gct ttg gag aaa aaa tta ggt gat	336
Ile His Gly Lys Leu Pro Leu Tyr Ala Leu Glu Lys Lys Leu Gly Asp	
100 105 110	
act acg aga gcg gtt gcg gta cgt agg aag gct ctt tca att ttg gca	384
Thr Thr Arg Ala Val Ala Val Arg Arg Lys Ala Leu Ser Ile Leu Ala	
115 120 125	
gaa gct cct gta tta gca tct gat cgt tta cca tat aaa aat tat gac	432
Glu Ala Pro Val Leu Ala Ser Asp Arg Leu Pro Tyr Lys Asn Tyr Asp	
130 135 140	
tac gac cgc gta ttt ggc gct tgt tgt gaa aat gtt ata ggt tac atg	480
Tyr Asp Arg Val Phe Gly Ala Cys Cys Glu Asn Val Ile Gly Tyr Met	

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145	150	155	160	
cct ttg ccc gtt ggt gtt ata ggc ccc ttg gtt atc gat ggt aca tct Pro Leu Pro Val Gly Val Ile Gly Pro Leu Val Ile Asp Gly Thr Ser 165 170 175				528
tat cat ata cca atg gca act aca gag ggt tgt ttg gta gct tct gcc Tyr His Ile Pro Met Ala Thr Thr Glu Gly Cys Leu Val Ala Ser Ala 180 185 190				576
atg cgt ggc tgt aag gca atc aat gct ggc ggt ggt gca aca act gtt Met Arg Gly Cys Lys Ala Ile Asn Ala Gly Gly Gly Ala Thr Thr Val 195 200 205				624
tta act aag gat ggt atg aca aga ggc cca gta gtc cgt ttc cca act Leu Thr Lys Asp Gly Met Thr Arg Gly Pro Val Val Arg Phe Pro Thr 210 215 220				672
ttg aaa aga tct ggt gcc tgt aag ata tgg tta gac tca gaa gag gga Leu Lys Arg Ser Gly Ala Cys Lys Ile Trp Leu Asp Ser Glu Glu Gly 225 230 235 240				720
caa aac gca att aaa aaa gct ttt aac tct aca tca aga ttt gca cgt Gln Asn Ala Ile Lys Lys Ala Phe Asn Ser Thr Ser Arg Phe Ala Arg 245 250 255				768
ctg caa cat att caa act tgt cta gca gga gat tta ctc ttc atg aga Leu Gln His Ile Gln Thr Cys Leu Ala Gly Asp Leu Leu Phe Met Arg 260 265 270				816
ttt aga aca act act ggt gac gca atg ggt atg aat atg att tct aaa Phe Arg Thr Thr Thr Gly Asp Ala Met Gly Met Asn Met Ile Ser Lys 275 280 285				864
ggt gtc gaa tac tca tta aag caa atg gta gaa gag tat ggc tgg gaa Gly Val Glu Tyr Ser Leu Lys Gln Met Val Glu Glu Tyr Gly Trp Glu 290 295 300				912
gat atg gag gtt gtc tcc gtt tct ggt aac tac tgt acc gac aaa aaa Asp Met Glu Val Val Ser Val Ser Gly Asn Tyr Cys Thr Asp Lys Lys 305 310 315 320				960
cca gct gcc atc aac tgg atc gaa ggt cgt ggt aag agt gtc gtc gca Pro Ala Ala Ile Asn Trp Ile Glu Gly Arg Gly Lys Ser Val Val Ala 325 330 335				1008
gaa gct act att cct ggt gat gtt gtc aga aaa gtg tta aaa agt gat Glu Ala Thr Ile Pro Gly Asp Val Val Arg Lys Val Leu Lys Ser Asp 340 345 350				1056
gtt tcc gca ttg gtt gag ttg aac att gct aag aat ttg gtt gga tct Val Ser Ala Leu Val Glu Leu Asn Ile Ala Lys Asn Leu Val Gly Ser 355 360 365				1104
gca atg gct ggg tct gtt ggt gga ttt aac gca cat gca gct aat tta Ala Met Ala Gly Ser Val Gly Gly Phe Asn Ala His Ala Ala Asn Leu 370 375 380				1152
gtg aca gct gtt ttc ttg gca tta gga caa gat cct gca caa aat gtt Val Thr Ala Val Phe Leu Ala Leu Gly Gln Asp Pro Ala Gln Asn Val 385 390 395 400				1200
gaa agt tcc aac tgt ata aca ttg atg aaa gaa gtg gac ggt gat ttg Glu Ser Ser Asn Cys Ile Thr Leu Met Lys Glu Val Asp Gly Asp Leu 405 410 415				1248
aga att tcc gta tcc atg cca tcc atc gaa gta ggt acc atc ggt ggt Arg Ile Ser Val Ser Met Pro Ser Ile Glu Val Gly Thr Ile Gly Gly 420 425 430				1296
ggt act gtt cta gaa cca caa ggt gcc atg ttg gac tta tta ggt gta Gly Thr Val Leu Glu Pro Gln Gly Ala Met Leu Asp Leu Leu Gly Val 435 440 445				1344
aga ggc ccg cat gct acc gct cct ggt acc aac gca cgt caa tta gca Arg Gly Pro His Ala Thr Ala Pro Gly Thr Asn Ala Arg Gln Leu Ala 450 455 460				1392
aga ata gtt gcc tgt gcc gtc ttg gca ggt gaa tta tcc tta tgt gct				1440



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Gly Val Glu Tyr Ser Leu Lys Gln Met Val Glu Glu Tyr Gly Trp Glu  
 290 295 300

Asp Met Glu Val Val Ser Val Ser Gly Asn Tyr Cys Thr Asp Lys Lys  
 305 310 315 320

Pro Ala Ala Ile Asn Trp Ile Glu Gly Arg Gly Lys Ser Val Val Ala  
 325 330 335

Glu Ala Thr Ile Pro Gly Asp Val Val Arg Lys Val Leu Lys Ser Asp  
 340 345 350

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

Ala Met Ala Gly Ser Val Gly Gly Phe Asn Ala His Ala Ala Asn Leu  
 370 375 380

Val Thr Ala Val Phe Leu Ala Leu Gly Gln Asp Pro Ala Gln Asn Val  
 385 390 395 400

Glu Ser Ser Asn Cys Ile Thr Leu Met Lys Glu Val Asp Gly Asp Leu  
 405 410 415

Arg Ile Ser Val Ser Met Pro Ser Ile Glu Val Gly Thr Ile Gly Gly  
 420 425 430

Gly Thr Val Leu Glu Pro Gln Gly Ala Met Leu Asp Leu Leu Gly Val  
 435 440 445

Arg Gly Pro His Ala Thr Ala Pro Gly Thr Asn Ala Arg Gln Leu Ala  
 450 455 460

Arg Ile Val Ala Cys Ala Val Leu Ala Gly Glu Leu Ser Leu Cys Ala  
 465 470 475 480

Ala Leu Ala Ala Gly His Leu Val Gln Ser His Met Thr His Asn Arg  
 485 490 495

Lys Pro Ala Glu Pro Thr Lys Pro Asn Asn Leu Asp Ala Thr Asp Ile  
 500 505 510

Asn Arg Leu Lys Asp Gly Ser Val Thr Cys Ile Lys Ser  
 515 520 525

<210> SEQ ID NO 25  
 <211> LENGTH: 1593  
 <212> TYPE: DNA  
 <213> ORGANISM: Saccharomyces cerevisiae  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1593)

<400> SEQUENCE: 25

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att ggt tta agt cat ttc ttg gct tta cca ttg gcc caa aga atc tct	96
Ile Gly Leu Ser His Phe Leu Ala Leu Pro Leu Ala Gln Arg Ile Ser	
20 25 30	
ttg atc ata ata att cct ttc att tac aat att gta tgg caa tta cta	144
Leu Ile Ile Ile Ile Pro Phe Ile Tyr Asn Ile Val Trp Gln Leu Leu	
35 40 45	
tat tct ttg aga aag gac cgt cca cct cta gtg ttt tac tgg att cca	192
Tyr Ser Leu Arg Lys Asp Arg Pro Pro Leu Val Phe Tyr Trp Ile Pro	
50 55 60	
tggt gtc ggt agt gct gtt gtg tac ggt atg aag cca tac gag ttt ttc	240
Trp Val Gly Ser Ala Val Val Tyr Gly Met Lys Pro Tyr Glu Phe Phe	
65 70 75 80	
gaa gaa tgt caa aag aaa tac ggt gat att ttt tca ttc gtt ttg tta	288
Glu Glu Cys Gln Lys Lys Tyr Gly Asp Ile Phe Ser Phe Val Leu Leu	

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			85				90				95					
gga	aga	gtc	atg	act	gtg	tat	tta	gga	cca	aag	ggt	cac	gaa	ttt	gtc	336
Gly	Arg	Val	Met	Thr	Val	Tyr	Leu	Gly	Pro	Lys	Gly	His	Glu	Phe	Val	
			100				105				110					
ttc	aac	gct	aag	ttg	gca	gat	gtt	tca	gca	gaa	gct	gct	tac	gct	cat	384
Phe	Asn	Ala	Lys	Leu	Ala	Asp	Val	Ser	Ala	Glu	Ala	Ala	Tyr	Ala	His	
			115				120				125					
ttg	act	act	cca	ggt	ttc	ggg	aaa	ggg	ggt	att	tac	gat	tgt	cca	aat	432
Leu	Thr	Thr	Pro	Val	Phe	Gly	Lys	Gly	Val	Ile	Tyr	Asp	Cys	Pro	Asn	
			130				135				140					
tct	aga	ttg	atg	gag	caa	aag	aag	ttt	ggt	aag	ggt	gct	cta	acc	aaa	480
Ser	Arg	Leu	Met	Glu	Gln	Lys	Lys	Phe	Val	Lys	Gly	Ala	Leu	Thr	Lys	
			145				150				155				160	
gaa	gcc	ttc	aag	agc	tac	ggt	cca	ttg	att	gct	gaa	gaa	gtg	tac	aag	528
Glu	Ala	Phe	Lys	Ser	Tyr	Val	Pro	Leu	Ile	Ala	Glu	Glu	Val	Tyr	Lys	
			165				170				175					
tac	ttc	aga	gac	tcc	aaa	aac	ttc	cgt	ttg	aat	gaa	aga	act	act	ggt	576
Tyr	Phe	Arg	Asp	Ser	Lys	Asn	Phe	Arg	Leu	Asn	Glu	Arg	Thr	Thr	Gly	
			180				185				190					
act	att	gac	gtg	atg	ggt	act	caa	cct	gaa	atg	act	att	ttc	acc	gct	624
Thr	Ile	Asp	Val	Met	Val	Thr	Gln	Pro	Glu	Met	Thr	Ile	Phe	Thr	Ala	
			195				200				205					
tca	aga	tca	tta	ttg	ggg	aag	gaa	atg	aga	gca	aaa	ttg	gat	acc	gat	672
Ser	Arg	Ser	Leu	Leu	Gly	Lys	Glu	Met	Arg	Ala	Lys	Leu	Asp	Thr	Asp	
			210				215				220					
ttt	gct	tac	ttg	tac	agt	gat	ttg	gat	aag	ggg	ttc	act	cca	atc	aac	720
Phe	Ala	Tyr	Leu	Tyr	Ser	Asp	Leu	Asp	Lys	Gly	Phe	Thr	Pro	Ile	Asn	
			225				230				235				240	
ttc	gtc	ttc	cct	aac	tta	cca	ttg	gaa	cac	tat	aga	aag	aga	gat	cac	768
Phe	Val	Phe	Pro	Asn	Leu	Pro	Leu	Glu	His	Tyr	Arg	Lys	Arg	Asp	His	
			245				250				255					
gct	caa	aag	gct	atc	tcc	ggg	act	tac	atg	tct	ttg	att	aag	gaa	aga	816
Ala	Gln	Lys	Ala	Ile	Ser	Gly	Thr	Tyr	Met	Ser	Leu	Ile	Lys	Glu	Arg	
			260				265				270					
aga	aag	aac	aac	gac	att	caa	gac	aga	gat	ttg	atc	gat	tcc	ttg	atg	864
Arg	Lys	Asn	Asn	Asp	Ile	Gln	Asp	Arg	Asp	Leu	Ile	Asp	Ser	Leu	Met	
			275				280				285					
aag	aac	tct	acc	tac	aag	gat	ggg	gtg	aag	atg	act	gat	caa	gaa	atc	912
Lys	Asn	Ser	Thr	Tyr	Lys	Asp	Gly	Val	Lys	Met	Thr	Asp	Gln	Glu	Ile	
			290				295				300					
gct	aac	ttg	tta	att	ggg	gtc	tta	atg	ggg	ggg	caa	cat	act	tct	gct	960
Ala	Asn	Leu	Leu	Ile	Gly	Val	Leu	Met	Gly	Gly	Gln	His	Thr	Ser	Ala	
			305				310				315				320	
gcc	act	tct	gct	tgg	att	ttg	ttg	cac	ttg	gct	gaa	aga	cca	gat	gtc	1008
Ala	Thr	Ser	Ala	Trp	Ile	Leu	Leu	His	Leu	Ala	Glu	Arg	Pro	Asp	Val	
			325				330				335					
caa	caa	gaa	ttg	tac	gaa	gaa	caa	atg	cgt	ggt	ttg	gat	ggg	ggg	aag	1056
Gln	Gln	Glu	Leu	Tyr	Glu	Glu	Gln	Met	Arg	Val	Leu	Asp	Gly	Gly	Lys	
			340				345				350					
aag	gaa	ttg	acc	tac	gat	tta	tta	caa	gaa	atg	cca	ttg	ttg	aac	caa	1104
Lys	Glu	Leu	Thr	Tyr	Asp	Leu	Leu	Gln	Glu	Met	Pro	Leu	Leu	Asn	Gln	
			355				360				365					
act	att	aag	gaa	act	cta	aga	atg	cac	cat	cca	ttg	cac	tct	ttg	ttc	1152
Thr	Ile	Lys	Glu	Thr	Leu	Arg	Met	His	His	Pro	Leu	His	Ser	Leu	Phe	
			370				375				380					
cgt	aag	ggt	atg	aaa	gat	atg	cac	ggt	cca	aac	act	tct	tat	gtc	atc	1200
Arg	Lys	Val	Met	Lys	Asp	Met	His	Val	Pro	Asn	Thr	Ser	Tyr	Val	Ile	
			385				390				395				400	
cca	gca	ggg	tat	cac	ggt	ttg	ggt	tct	cca	ggg	tac	act	cat	tta	aga	1248

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Pro	Ala	Gly	Tyr	His	Val	Leu	Val	Ser	Pro	Gly	Tyr	Thr	His	Leu	Arg	
				405					410					415		
gac	gaa	tac	ttc	cct	aat	gct	cac	caa	ttc	aac	att	cac	cgt	tgg	aac	1296
Asp	Glu	Tyr	Phe	Pro	Asn	Ala	His	Gln	Phe	Asn	Ile	His	Arg	Trp	Asn	
			420					425					430			
aaa	gat	tct	gcc	tcc	tct	tat	tcc	gtc	ggc	gaa	gaa	gtc	gat	tac	ggc	1344
Lys	Asp	Ser	Ala	Ser	Ser	Tyr	Ser	Val	Gly	Glu	Glu	Val	Asp	Tyr	Gly	
			435				440					445				
ttc	ggc	gcc	att	tct	aag	ggc	gtc	agc	tct	cca	tac	tta	cct	ttc	ggc	1392
Phe	Gly	Ala	Ile	Ser	Lys	Gly	Val	Ser	Ser	Pro	Tyr	Leu	Pro	Phe	Gly	
			450			455						460				
ggc	ggc	aga	cac	aga	tgt	atc	ggc	gaa	cac	ttt	gct	tac	tgt	cag	cta	1440
Gly	Gly	Arg	His	Arg	Cys	Ile	Gly	Glu	His	Phe	Ala	Tyr	Cys	Gln	Leu	
					470					475					480	
ggc	ggt	cta	atg	tcc	att	ttt	atc	aga	aca	tta	aaa	tgg	cat	tac	cca	1488
Gly	Val	Leu	Met	Ser	Ile	Phe	Ile	Arg	Thr	Leu	Lys	Trp	His	Tyr	Pro	
				485					490					495		
gag	ggc	aag	acc	ggt	cca	cct	cct	gac	ttt	aca	tct	atg	ggt	act	ctt	1536
Glu	Gly	Lys	Thr	Val	Pro	Pro	Pro	Asp	Phe	Thr	Ser	Met	Val	Thr	Leu	
			500					505					510			
cca	acc	ggc	cca	gcc	aag	atc	atc	tgg	gaa	aag	aga	aat	cca	gaa	caa	1584
Pro	Thr	Gly	Pro	Ala	Lys	Ile	Ile	Trp	Glu	Lys	Arg	Asn	Pro	Glu	Gln	
			515			520						525				
aag	atc	taa														1593
Lys	Ile															
		530														

<210> SEQ ID NO 26  
 <211> LENGTH: 530  
 <212> TYPE: PRT  
 <213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 26

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

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

<210> SEQ ID NO 27  
 <211> LENGTH: 1491  
 <212> TYPE: DNA  
 <213> ORGANISM: *Saccharomyces cerevisiae*  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1491)

<400> SEQUENCE: 27

atg tct gct gtt aac gtt gca cct gaa ttg att aat gcc gac aac aca  
 Met Ser Ala Val Asn Val Ala Pro Glu Leu Ile Asn Ala Asp Asn Thr

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1	5	10	15	
att acc tac gat gcg att gtc atc ggt gct ggt gtt atc ggt cca tgt				96
Ile Thr Tyr Asp Ala Ile Val Ile Gly Ala Gly Val Ile Gly Pro Cys	20	25	30	
ggt gct act ggt cta gca aga aag ggt aag aaa gtt ctt atc gta gaa				144
Val Ala Thr Gly Leu Ala Arg Lys Gly Lys Lys Val Leu Ile Val Glu	35	40	45	
cgt gac tgg gct atg cct gat aga att gtt ggt gaa ttg atg caa cca				192
Arg Asp Trp Ala Met Pro Asp Arg Ile Val Gly Glu Leu Met Gln Pro	50	55	60	
ggt ggt gtt aga gca ttg aga agt ctg ggt atg att caa tct atc aac				240
Gly Gly Val Arg Ala Leu Arg Ser Leu Gly Met Ile Gln Ser Ile Asn	65	70	75	80
aac atc gaa gca tat cct gtt acc ggt tat acc gtc ttt ttc aac ggc				288
Asn Ile Glu Ala Tyr Pro Val Thr Gly Tyr Thr Val Phe Phe Asn Gly	85	90	95	
gaa caa gtt gat att cca tac cct tac aag gcc gat atc cct aaa gtt				336
Glu Gln Val Asp Ile Pro Tyr Pro Tyr Lys Ala Asp Ile Pro Lys Val	100	105	110	
gaa aaa ttg aag gac ttg gtc aaa gat ggt aat gac aag gtc ttg gaa				384
Glu Lys Leu Lys Asp Leu Val Lys Asp Gly Asn Asp Lys Val Leu Glu	115	120	125	
gac agc act att cac atc aag gat tac gaa gat gat gaa aga gaa agg				432
Asp Ser Thr Ile His Ile Lys Asp Tyr Glu Asp Asp Glu Arg Glu Arg	130	135	140	
ggt gtt gct ttt gtt cat ggt aga ttc ttg aac aac ttg aga aac att				480
Gly Val Ala Phe Val His Gly Arg Phe Leu Asn Asn Leu Arg Asn Ile	145	150	155	160
act gct caa gag cca aat gtt act aga gtg caa ggt aac tgt att gag				528
Thr Ala Gln Glu Val Pro Asn Val Thr Arg Val Gln Gly Asn Cys Ile Glu	165	170	175	
ata ttg aag gat gaa aag aat gag gtt gtt ggt gcc aag gtt gac att				576
Ile Leu Lys Asp Glu Lys Asn Glu Val Val Gly Ala Lys Val Asp Ile	180	185	190	
gat ggc cgt ggc aag gtg gaa ttc aaa gcc cac ttg aca ttt atc tgt				624
Asp Gly Arg Gly Lys Val Glu Phe Lys Ala His Leu Thr Phe Ile Cys	195	200	205	
gac ggt atc ttt tca cgt ttc aga aag gaa ttg cac cca gac cat gtt				672
Asp Gly Ile Phe Ser Arg Phe Arg Lys Glu Leu His Pro Asp His Val	210	215	220	
cca act gtc ggt tct tcg ttt gtc ggt atg tct ttg ttc aat gct aag				720
Pro Thr Val Gly Ser Ser Phe Val Gly Met Ser Leu Phe Asn Ala Lys	225	230	235	240
aat cct gct cct atg cac ggt cac gtt att ctt ggt agt gat cat atg				768
Asn Pro Ala Pro Met His Gly His Val Ile Leu Gly Ser Asp His Met	245	250	255	
cca atc ttg gtt tac caa atc agt cca gaa gaa aca aga atc ctt tgt				816
Pro Ile Leu Val Tyr Gln Ile Ser Pro Glu Glu Thr Arg Ile Leu Cys	260	265	270	
gct tac aac tct cca aag gtc cca gct gat atc aag agt tgg atg att				864
Ala Tyr Asn Ser Pro Lys Val Pro Ala Asp Ile Lys Ser Trp Met Ile	275	280	285	
aag gat gtc caa cct ttc att cca aag agt cta cgt cct tca ttt gat				912
Lys Asp Val Gln Pro Phe Ile Pro Lys Ser Leu Arg Pro Ser Phe Asp	290	295	300	
gaa gcc gtc agc caa ggt aaa ttt aga gct atg cca aac tcc tac ttg				960
Glu Ala Val Ser Gln Gly Lys Phe Arg Ala Met Pro Asn Ser Tyr Leu	305	310	315	320
cca gct aga caa aac gac gtc act ggt atg tgt gtt atc ggt gac gct				1008

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Pro	Ala	Arg	Gln	Asn	Asp	Val	Thr	Gly	Met	Cys	Val	Ile	Gly	Asp	Ala	
				325					330					335		
cta	aat	atg	aga	cat	cca	ttg	act	ggt	ggt	ggt	atg	act	gtc	ggt	ttg	1056
Leu	Asn	Met	Arg	His	Pro	Leu	Thr	Gly	Gly	Gly	Met	Thr	Val	Gly	Leu	
			340					345					350			
cat	gat	ggt	gtc	ttg	ttg	att	aag	aaa	ata	ggt	gac	cta	gac	ttc	agc	1104
His	Asp	Val	Val	Leu	Leu	Ile	Lys	Lys	Ile	Gly	Asp	Leu	Asp	Phe	Ser	
		355				360						365				
gac	cgt	gaa	aag	ggt	ttg	gat	gaa	tta	cta	gac	tac	cat	ttc	gaa	aga	1152
Asp	Arg	Glu	Lys	Val	Leu	Asp	Glu	Leu	Leu	Asp	Tyr	His	Phe	Glu	Arg	
	370				375						380					
aag	agt	tac	gat	tcc	ggt	att	aac	ggt	ttg	tca	gtg	gct	ttg	tat	tct	1200
Lys	Ser	Tyr	Asp	Ser	Val	Ile	Asn	Val	Leu	Ser	Val	Ala	Leu	Tyr	Ser	
385				390						395				400		
ttg	ttc	gct	gct	gac	agc	gat	aac	ttg	aag	gca	tta	caa	aaa	ggt	tgt	1248
Leu	Phe	Ala	Ala	Asp	Ser	Asp	Asn	Leu	Lys	Ala	Leu	Gln	Lys	Gly	Cys	
				405					410					415		
ttc	aaa	tat	ttc	caa	aga	ggt	ggc	gat	tgt	gtc	aac	aaa	ccc	ggt	gaa	1296
Phe	Lys	Tyr	Phe	Gln	Arg	Gly	Gly	Asp	Cys	Val	Asn	Lys	Pro	Val	Glu	
			420					425					430			
ttt	ctg	tct	ggt	gtc	ttg	cca	aag	cct	ttg	caa	ttg	acc	agg	ggt	ttc	1344
Phe	Leu	Ser	Gly	Val	Leu	Pro	Lys	Pro	Leu	Gln	Leu	Thr	Arg	Val	Phe	
		435					440						445			
ttc	gct	gtc	gct	ttt	tac	acc	att	tac	ttg	aac	atg	gaa	gaa	cgt	ggt	1392
Phe	Ala	Val	Ala	Phe	Tyr	Thr	Ile	Tyr	Leu	Asn	Met	Glu	Glu	Arg	Gly	
		450				455					460					
ttc	ttg	gga	tta	cca	atg	gct	tta	ttg	gaa	ggt	att	atg	att	ttg	atc	1440
Phe	Leu	Gly	Leu	Pro	Met	Ala	Leu	Leu	Glu	Gly	Ile	Met	Ile	Leu	Ile	
	465				470					475				480		
aca	gct	att	aga	gta	ttc	acc	cca	ttt	ttg	ttt	ggt	gag	ttg	att	ggt	1488
Thr	Ala	Ile	Arg	Val	Phe	Thr	Pro	Phe	Leu	Phe	Gly	Glu	Leu	Ile	Gly	
				485					490					495		
taa																1491

<210> SEQ ID NO 28  
 <211> LENGTH: 496  
 <212> TYPE: PRT  
 <213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 28

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

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

<210> SEQ ID NO 29  
 <211> LENGTH: 1335  
 <212> TYPE: DNA  
 <213> ORGANISM: *Saccharomyces cerevisiae*  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1335)

<400> SEQUENCE: 29

atg gga aag cta tta caa ttg gca ttg cat ccg gtc gag atg aag gca

48

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Met	Gly	Lys	Leu	Leu	Gln	Leu	Ala	Leu	His	Pro	Val	Glu	Met	Lys	Ala	
1				5					10					15		
gct	ttg	aag	ctg	aag	ttt	tgc	aga	aca	ccg	cta	ttc	tcc	atc	tat	gat	96
Ala	Leu	Lys	Leu	Lys	Phe	Cys	Arg	Thr	Pro	Leu	Phe	Ser	Ile	Tyr	Asp	
			20					25					30			
cag	tcc	acg	tct	cca	tat	ctc	ttg	cac	tgt	ttc	gaa	ctg	ttg	aac	ttg	144
Gln	Ser	Thr	Ser	Pro	Tyr	Leu	Leu	His	Cys	Phe	Glu	Leu	Leu	Asn	Leu	
			35					40					45			
acc	tcc	aga	tcg	ttt	gct	gct	gtg	atc	aga	gag	ctg	cat	cca	gaa	ttg	192
Thr	Ser	Arg	Ser	Phe	Ala	Ala	Val	Ile	Arg	Glu	Leu	His	Pro	Glu	Leu	
		50					55					60				
aga	aac	tgt	ggt	act	ctc	ttt	tat	ttg	att	tta	agg	gct	ttg	gat	acc	240
Arg	Asn	Cys	Val	Thr	Leu	Phe	Tyr	Leu	Ile	Leu	Arg	Ala	Leu	Asp	Thr	
						70					75				80	
atc	gaa	gac	gat	atg	tcc	atc	gaa	cac	gat	ttg	aaa	att	gac	ttg	ttg	288
Ile	Glu	Asp	Asp	Met	Ser	Ile	Glu	His	Asp	Leu	Lys	Ile	Asp	Leu	Leu	
				85					90					95		
cgt	cac	ttc	cac	gag	aaa	ttg	ttg	tta	act	aaa	tgg	agt	ttc	gac	gga	336
Arg	His	Phe	His	Glu	Lys	Leu	Leu	Thr	Lys	Trp	Ser	Phe	Asp	Gly		
			100					105					110			
aat	gcc	ccc	gat	gtg	aag	gac	aga	gcc	ggt	ttg	aca	gat	ttc	gaa	tcg	384
Asn	Ala	Pro	Asp	Val	Lys	Asp	Arg	Ala	Val	Leu	Thr	Asp	Phe	Glu	Ser	
			115					120					125			
att	ctt	att	gaa	ttc	cac	aaa	ttg	aaa	cca	gaa	tat	caa	gaa	gtc	atc	432
Ile	Leu	Ile	Glu	Phe	His	Lys	Leu	Lys	Pro	Glu	Tyr	Gln	Glu	Val	Ile	
			130				135						140			
aag	gag	atc	acc	gag	aaa	atg	ggt	aat	ggt	atg	gcc	gac	tac	atc	tta	480
Lys	Glu	Ile	Thr	Glu	Lys	Met	Gly	Asn	Gly	Met	Ala	Asp	Tyr	Ile	Leu	
					150					155					160	
gat	gaa	aat	tac	aac	ttg	aat	ggg	ttg	caa	acc	gtc	cac	gac	tac	gac	528
Asp	Glu	Asn	Tyr	Asn	Leu	Asn	Gly	Leu	Gln	Thr	Val	His	Asp	Tyr	Asp	
				165					170					175		
gtg	tac	tgt	cac	tac	gta	gct	ggt	ttg	gtc	ggt	gat	ggt	ttg	acc	cgt	576
Val	Tyr	Cys	His	Tyr	Val	Ala	Gly	Leu	Val	Gly	Asp	Gly	Leu	Thr	Arg	
				180				185						190		
ttg	att	gtc	att	gcc	aag	ttt	gcc	aac	gaa	tct	ttg	tat	tct	aat	gag	624
Leu	Ile	Val	Ile	Ala	Lys	Phe	Ala	Asn	Glu	Ser	Leu	Tyr	Ser	Asn	Glu	
			195					200					205			
caa	ttg	tat	gaa	agc	atg	ggt	ctt	ttc	cta	caa	aaa	acc	aac	atc	atc	672
Gln	Leu	Tyr	Glu	Ser	Met	Gly	Leu	Phe	Leu	Gln	Lys	Thr	Asn	Ile	Ile	
			210					215					220			
aga	gat	tac	aat	gaa	gat	ttg	gtc	gat	ggt	aga	tcc	ttc	tgg	ccc	aag	720
Arg	Asp	Tyr	Asn	Glu	Asp	Leu	Val	Asp	Gly	Arg	Ser	Phe	Trp	Pro	Lys	
					230					235					240	
gaa	atc	tgg	tca	caa	tac	gct	cct	cag	ttg	aag	gac	ttc	atg	aaa	cct	768
Glu	Ile	Trp	Ser	Gln	Tyr	Ala	Pro	Gln	Leu	Lys	Asp	Phe	Met	Lys	Pro	
				245					250					255		
gaa	aac	gaa	caa	ctg	ggg	ttg	gac	tgt	ata	aac	cac	ctc	gtc	tta	aac	816
Glu	Asn	Glu	Gln	Leu	Gly	Leu	Asp	Cys	Ile	Asn	His	Leu	Val	Leu	Asn	
			260					265					270			
gca	ttg	agt	cat	ggt	atc	gat	gtg	ttg	act	tat	ttg	gcc	ggt	atc	cac	864
Ala	Leu	Ser	His	Val	Ile	Asp	Val	Leu	Thr	Tyr	Leu	Ala	Gly	Ile	His	
			275					280					285			
gag	caa	tcc	act	ttc	caa	ttt	tgt	gcc	att	ccc	caa	ggt	atg	gcc	att	912
Glu	Gln	Ser	Thr	Phe	Gln	Phe	Cys	Ala	Ile	Pro	Gln	Val	Met	Ala	Ile	
				290			295						300			
gca	acc	ttg	gct	ttg	gta	ttc	aac	aac	cgt	gaa	gtg	cta	cat	ggc	aat	960
Ala	Thr	Leu	Ala	Leu	Val	Phe	Asn	Asn	Arg	Glu	Val	Leu	His	Gly	Asn	
					305								310		315	320

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gta aag att cgt aag ggt act acc tgc tat tta att ttg aaa tca agg 1008
Val Lys Ile Arg Lys Gly Thr Thr Cys Tyr Leu Ile Leu Lys Ser Arg
      325                      330                      335

act ttg cgt ggc tgt gtc gag att ttt gac tat tac tta cgt gat atc 1056
Thr Leu Arg Gly Cys Val Glu Ile Phe Asp Tyr Tyr Leu Arg Asp Ile
      340                      345                      350

aaa tct aaa ttg gct gtg caa gat cca aat ttc tta aaa ttg aac att 1104
Lys Ser Lys Leu Ala Val Gln Asp Pro Asn Phe Leu Lys Leu Asn Ile
      355                      360                      365

caa atc tcc aag atc gaa cag ttt atg gaa gaa atg tac cag gat aaa 1152
Gln Ile Ser Lys Ile Glu Gln Phe Met Glu Glu Met Tyr Gln Asp Lys
      370                      375                      380

tta cct cct aac gtg aag cca aat gaa act cca att ttc ttg aaa gtt 1200
Leu Pro Pro Asn Val Lys Pro Asn Glu Thr Pro Ile Phe Leu Lys Val
      385                      390                      395                      400

aaa gaa aga tcc aga tac gat gat gaa ttg gtt cca acc caa caa gaa 1248
Lys Glu Arg Ser Arg Tyr Asp Asp Glu Leu Val Pro Thr Gln Gln Glu
      405                      410                      415

gaa gag tac aag ttc aat atg gtt tta tct atc atc ttg tcc gtt ctt 1296
Glu Glu Tyr Lys Phe Asn Met Val Leu Ser Ile Ile Leu Ser Val Leu
      420                      425                      430

ctt ggg ttt tat tat ata tac act tta cac aga gcg tga 1335
Leu Gly Phe Tyr Tyr Ile Tyr Thr Leu His Arg Ala
      435                      440

<210> SEQ ID NO 30
<211> LENGTH: 444
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 30
Met Gly Lys Leu Leu Gln Leu Ala Leu His Pro Val Glu Met Lys Ala
 1      5      10
Ala Leu Lys Leu Lys Phe Cys Arg Thr Pro Leu Phe Ser Ile Tyr Asp
 20     25     30
Gln Ser Thr Ser Pro Tyr Leu Leu His Cys Phe Glu Leu Leu Asn Leu
 35     40     45
Thr Ser Arg Ser Phe Ala Ala Val Ile Arg Glu Leu His Pro Glu Leu
 50     55     60
Arg Asn Cys Val Thr Leu Phe Tyr Leu Ile Leu Arg Ala Leu Asp Thr
 65     70     75     80
Ile Glu Asp Asp Met Ser Ile Glu His Asp Leu Lys Ile Asp Leu Leu
 85     90     95
Arg His Phe His Glu Lys Leu Leu Leu Thr Lys Trp Ser Phe Asp Gly
100    105    110
Asn Ala Pro Asp Val Lys Asp Arg Ala Val Leu Thr Asp Phe Glu Ser
115    120    125
Ile Leu Ile Glu Phe His Lys Leu Lys Pro Glu Tyr Gln Glu Val Ile
130    135    140
Lys Glu Ile Thr Glu Lys Met Gly Asn Gly Met Ala Asp Tyr Ile Leu
145    150    155    160
Asp Glu Asn Tyr Asn Leu Asn Gly Leu Gln Thr Val His Asp Tyr Asp
165    170    175
Val Tyr Cys His Tyr Val Ala Gly Leu Val Gly Asp Gly Leu Thr Arg
180    185    190
Leu Ile Val Ile Ala Lys Phe Ala Asn Glu Ser Leu Tyr Ser Asn Glu
195    200    205

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

<210> SEQ ID NO 31  
 <211> LENGTH: 1929  
 <212> TYPE: DNA  
 <213> ORGANISM: Saccharomyces cerevisiae  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1929)

<400> SEQUENCE: 31

atg gac aag aag aag gat cta ctg gag aac gaa caa ttt ctc cgc atc	48
Met Asp Lys Lys Lys Asp Leu Leu Glu Asn Glu Gln Phe Leu Arg Ile	
1 5 10 15	
caa aag ctc aac gct gcc gat gcg ggc aaa aga caa tct ata aca gtg	96
Gln Lys Leu Asn Ala Ala Asp Ala Gly Lys Arg Gln Ser Ile Thr Val	
20 25 30	
gac gac gag ggc gaa cta tat ggg tta gac acc tcc ggc aac tca cca	144
Asp Asp Glu Gly Glu Leu Tyr Gly Leu Asp Thr Ser Gly Asn Ser Pro	
35 40 45	
gcc aat gaa cac aca gct acc aca att aca cag aat cac agc gtg gtg	192
Ala Asn Glu His Thr Ala Thr Thr Ile Thr Gln Asn His Ser Val Val	
50 55 60	
gcc tca aac gga gac gtc gca ttc atc cca gga act gct acc gaa ggc	240
Ala Ser Asn Gly Asp Val Ala Phe Ile Pro Gly Thr Ala Thr Glu Gly	
65 70 75 80	
aat aca gag att gta act gaa gaa gtg att gag acc gat gat aac atg	288
Asn Thr Glu Ile Val Thr Glu Glu Val Ile Glu Thr Asp Asp Asn Met	
85 90 95	

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ttc aag acc cat gtg aag act tta agc tcc aaa gag aag gca cgg tat	336
Phe Lys Thr His Val Lys Thr Leu Ser Ser Lys Glu Lys Ala Arg Tyr	
100 105 110	
agg caa ggg tcc tct aac ttt ata tcg tat ttc gat gat atg tca ttt	384
Arg Gln Gly Ser Ser Asn Phe Ile Ser Tyr Phe Asp Asp Met Ser Phe	
115 120 125	
gaa cac agg ccc agt ata tta gat ggg tca gtt aac gag ccc ttc aag	432
Glu His Arg Pro Ser Ile Leu Asp Gly Ser Val Asn Glu Pro Phe Lys	
130 135 140	
acc aaa ttc gtg gga cct act tta gaa aag gag atc aga aga agg gag	480
Thr Lys Phe Val Gly Pro Thr Leu Glu Lys Glu Ile Arg Arg Arg Glu	
145 150 155 160	
aaa gag cta atg gcc atg cgc aaa aat tta cac cac cgc aag tcc tcc	528
Lys Glu Leu Met Ala Met Arg Lys Asn Leu His His Arg Lys Ser Ser	
165 170 175	
cca gat gct gtc gac tca gta ggg aaa aat gat ggc gcc gcc cca act	576
Pro Asp Ala Val Asp Ser Val Gly Lys Asn Asp Gly Ala Ala Pro Thr	
180 185 190	
act gtt cca act gcc gcc acc tca gaa acg gtg gtc acc gtt gaa acc	624
Thr Val Pro Thr Ala Ala Thr Ser Glu Thr Val Val Thr Val Glu Thr	
195 200 205	
acc ata att tca tcc aat ttc tcc ggg ttg tac gtg gcg ttt tgg atg	672
Thr Ile Ile Ser Ser Asn Phe Ser Gly Leu Tyr Val Ala Phe Trp Met	
210 215 220	
gct att gca ttt ggt gct gtc aag gct tta ata gac tat tat tac cag	720
Ala Ile Ala Phe Gly Ala Val Lys Ala Leu Ile Asp Tyr Tyr Tyr Gln	
225 230 235 240	
cat aat ggt agc ttc aag gat tcg gag atc ttg aaa ttt atg act acg	768
His Asn Gly Ser Phe Lys Asp Ser Glu Ile Leu Lys Phe Met Thr Thr	
245 250 255	
aat ttg ttc act gtg gca tcc gta gat ctt ttg atg tat ttg agc act	816
Asn Leu Phe Thr Val Ala Ser Val Asp Leu Leu Met Tyr Leu Ser Thr	
260 265 270	
tat ttt gtc gtt gga ata caa tac tta tgc aag tgg ggg gtc ttg aaa	864
Tyr Phe Val Val Gly Ile Gln Tyr Leu Cys Lys Trp Gly Val Leu Lys	
275 280 285	
tgg ggc act acc ggc tgg atc ttc acc tca att tac gag ttt ttg ttt	912
Trp Gly Thr Thr Gly Trp Ile Phe Thr Ser Ile Tyr Glu Phe Leu Phe	
290 295 300	
gtt atc ttc tac atg tat tta aca gaa aac atc cta aaa cta cac tgg	960
Val Ile Phe Tyr Met Tyr Leu Thr Glu Asn Ile Leu Lys Leu His Trp	
305 310 315 320	
ctg tcc aag atc ttc ctt ttt ttg cat tct tta gtt tta ttg atg aaa	1008
Leu Ser Lys Ile Phe Leu Phe Leu His Ser Leu Val Leu Leu Met Lys	
325 330 335	
atg cat tct ttc gcc ttc tac aat ggc tat cta tgg ggt ata aag gaa	1056
Met His Ser Phe Ala Phe Tyr Asn Gly Tyr Leu Trp Gly Ile Lys Glu	
340 345 350	
gaa cta caa ttt tcc aaa agc gct ctt gcc aaa tac aag gat tct ata	1104
Glu Leu Gln Phe Ser Lys Ser Ala Leu Ala Lys Tyr Lys Asp Ser Ile	
355 360 365	
aat gat cca aaa gtt att ggt gct ctt gag aaa agc tgt gag ttt tgt	1152
Asn Asp Pro Lys Val Ile Gly Ala Leu Glu Lys Ser Cys Glu Phe Cys	
370 375 380	
agt ttt gaa ttg agc tct cag tct tta agc gac caa act caa aaa ttc	1200
Ser Phe Glu Leu Ser Ser Gln Ser Leu Ser Asp Gln Thr Gln Lys Phe	
385 390 395 400	
ccc aac aat atc agt gca aaa agc ttt ttt tgg ttc acc atg ttt cca	1248
Pro Asn Asn Ile Ser Ala Lys Ser Phe Phe Trp Phe Thr Met Phe Pro	

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405	410	415	
acc cta att tac caa att gaa tat cca aga act aag gaa atc aga tgg			1296
Thr Leu Ile Tyr Gln Ile Glu Tyr Pro Arg Thr Lys Glu Ile Arg Trp			
420	425	430	
agc tac gta tta gaa aag atc tgc gcc atc ttc ggt acc att ttc tta			1344
Ser Tyr Val Leu Glu Lys Ile Cys Ala Ile Phe Gly Thr Ile Phe Leu			
435	440	445	
atg atg ata gat gct caa atc ttg atg tat cct gta gca atg aga gca			1392
Met Met Ile Asp Ala Gln Ile Leu Met Tyr Pro Val Ala Met Arg Ala			
450	455	460	
ttg gct gtg cgc aat tct gaa tgg act ggt ata ttg gat aga tta ttg			1440
Leu Ala Val Arg Asn Ser Glu Trp Thr Gly Ile Leu Asp Arg Leu Leu			
465	470	475	480
aaa tgg gtt gga ttg ctc gtt gat atc gtc cca ggg ttt atc gtg atg			1488
Lys Trp Val Gly Leu Leu Val Asp Ile Val Pro Gly Phe Ile Val Met			
485	490	495	
tac atc ttg gac ttc tat ttg att tgg gat gcc att ttg aac tgt gtg			1536
Tyr Ile Leu Asp Phe Tyr Leu Ile Trp Asp Ala Ile Leu Asn Cys Val			
500	505	510	
gct gaa ttg aca aga ttt ggc gac aga tat ttc tac ggt gac tgg tgg			1584
Ala Glu Leu Thr Arg Phe Gly Asp Arg Tyr Phe Tyr Gly Asp Trp Trp			
515	520	525	
aat tgt gtt agt tgg gca gac ttc agt aga att tgg aac atc cca gtg			1632
Asn Cys Val Ser Trp Ala Asp Phe Ser Arg Ile Trp Asn Ile Pro Val			
530	535	540	
cat aag ttt ttg tta aga cat gtt tac cat agt tca atg agt tca ttc			1680
His Lys Phe Leu Leu Arg His Val Tyr His Ser Ser Met Ser Ser Phe			
545	550	555	560
aaa ttg aac aag agt caa gca act ttg atg acc ttt ttc tta agt tcc			1728
Lys Leu Asn Lys Ser Gln Ala Thr Leu Met Thr Phe Phe Leu Ser Ser			
565	570	575	
gtc gtt cat gaa tta gca atg tac gtt atc ttc aag aaa ttg agg ttt			1776
Val Val His Glu Leu Ala Met Tyr Val Ile Phe Lys Lys Leu Arg Phe			
580	585	590	
tac ttg ttc ttc ttc caa atg ctg caa atg cca tta gta gct tta aca			1824
Tyr Leu Phe Phe Phe Gln Met Leu Gln Met Pro Leu Val Ala Leu Thr			
595	600	605	
aat act aaa ttc atg agg aac aga acc ata atc gga aat gtt att ttc			1872
Asn Thr Lys Phe Met Arg Asn Arg Thr Ile Ile Gly Asn Val Ile Phe			
610	615	620	
tgg ctc ggt atc tgc atg gga cca agt gtc atg tgt acg ttg tac ttg			1920
Trp Leu Gly Ile Cys Met Gly Pro Ser Val Met Cys Thr Leu Tyr Leu			
625	630	635	640
aca ttc taa			1929
Thr Phe			
<210> SEQ ID NO 32			
<211> LENGTH: 642			
<212> TYPE: PRT			
<213> ORGANISM: <i>Saccharomyces cerevisiae</i>			
<400> SEQUENCE: 32			
Met Asp Lys Lys Lys Asp Leu Leu Glu Asn Glu Gln Phe Leu Arg Ile			
1	5	10	15
Gln Lys Leu Asn Ala Ala Asp Ala Gly Lys Arg Gln Ser Ile Thr Val			
20	25	30	
Asp Asp Glu Gly Glu Leu Tyr Gly Leu Asp Thr Ser Gly Asn Ser Pro			
35	40	45	
Ala Asn Glu His Thr Ala Thr Thr Ile Thr Gln Asn His Ser Val Val			
50	55	60	

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Ala Ser Asn Gly Asp Val Ala Phe Ile Pro Gly Thr Ala Thr Glu Gly  
65 70 75 80

Asn Thr Glu Ile Val Thr Glu Glu Val Ile Glu Thr Asp Asp Asn Met  
85 90 95

Phe Lys Thr His Val Lys Thr Leu Ser Ser Lys Glu Lys Ala Arg Tyr  
100 105 110

Arg Gln Gly Ser Ser Asn Phe Ile Ser Tyr Phe Asp Asp Met Ser Phe  
115 120 125

Glu His Arg Pro Ser Ile Leu Asp Gly Ser Val Asn Glu Pro Phe Lys  
130 135 140

Thr Lys Phe Val Gly Pro Thr Leu Glu Lys Glu Ile Arg Arg Arg Glu  
145 150 155 160

Lys Glu Leu Met Ala Met Arg Lys Asn Leu His His Arg Lys Ser Ser  
165 170 175

Pro Asp Ala Val Asp Ser Val Gly Lys Asn Asp Gly Ala Ala Pro Thr  
180 185 190

Thr Val Pro Thr Ala Ala Thr Ser Glu Thr Val Val Thr Val Glu Thr  
195 200 205

Thr Ile Ile Ser Ser Asn Phe Ser Gly Leu Tyr Val Ala Phe Trp Met  
210 215 220

Ala Ile Ala Phe Gly Ala Val Lys Ala Leu Ile Asp Tyr Tyr Tyr Gln  
225 230 235 240

His Asn Gly Ser Phe Lys Asp Ser Glu Ile Leu Lys Phe Met Thr Thr  
245 250 255

Asn Leu Phe Thr Val Ala Ser Val Asp Leu Leu Met Tyr Leu Ser Thr  
260 265 270

Tyr Phe Val Val Gly Ile Gln Tyr Leu Cys Lys Trp Gly Val Leu Lys  
275 280 285

Trp Gly Thr Thr Gly Trp Ile Phe Thr Ser Ile Tyr Glu Phe Leu Phe  
290 295 300

Val Ile Phe Tyr Met Tyr Leu Thr Glu Asn Ile Leu Lys Leu His Trp  
305 310 315 320

Leu Ser Lys Ile Phe Leu Phe Leu His Ser Leu Val Leu Leu Met Lys  
325 330 335

Met His Ser Phe Ala Phe Tyr Asn Gly Tyr Leu Trp Gly Ile Lys Glu  
340 345 350

Glu Leu Gln Phe Ser Lys Ser Ala Leu Ala Lys Tyr Lys Asp Ser Ile  
355 360 365

Asn Asp Pro Lys Val Ile Gly Ala Leu Glu Lys Ser Cys Glu Phe Cys  
370 375 380

Ser Phe Glu Leu Ser Ser Gln Ser Leu Ser Asp Gln Thr Gln Lys Phe  
385 390 395 400

Pro Asn Asn Ile Ser Ala Lys Ser Phe Phe Trp Phe Thr Met Phe Pro  
405 410 415

Thr Leu Ile Tyr Gln Ile Glu Tyr Pro Arg Thr Lys Glu Ile Arg Trp  
420 425 430

Ser Tyr Val Leu Glu Lys Ile Cys Ala Ile Phe Gly Thr Ile Phe Leu  
435 440 445

Met Met Ile Asp Ala Gln Ile Leu Met Tyr Pro Val Ala Met Arg Ala  
450 455 460

Leu Ala Val Arg Asn Ser Glu Trp Thr Gly Ile Leu Asp Arg Leu Leu  
465 470 475 480

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Lys Trp Val Gly Leu Leu Val Asp Ile Val Pro Gly Phe Ile Val Met  
485 490 495

Tyr Ile Leu Asp Phe Tyr Leu Ile Trp Asp Ala Ile Leu Asn Cys Val  
500 505 510

Ala Glu Leu Thr Arg Phe Gly Asp Arg Tyr Phe Tyr Gly Asp Trp Trp  
515 520 525

Asn Cys Val Ser Trp Ala Asp Phe Ser Arg Ile Trp Asn Ile Pro Val  
530 535 540

His Lys Phe Leu Leu Arg His Val Tyr His Ser Ser Met Ser Ser Phe  
545 550 555 560

Lys Leu Asn Lys Ser Gln Ala Thr Leu Met Thr Phe Phe Leu Ser Ser  
565 570 575

Val Val His Glu Leu Ala Met Tyr Val Ile Phe Lys Lys Leu Arg Phe  
580 585 590

Tyr Leu Phe Phe Phe Gln Met Leu Gln Met Pro Leu Val Ala Leu Thr  
595 600 605

Asn Thr Lys Phe Met Arg Asn Arg Thr Ile Ile Gly Asn Val Ile Phe  
610 615 620

Trp Leu Gly Ile Cys Met Gly Pro Ser Val Met Cys Thr Leu Tyr Leu  
625 630 635 640

Thr Phe

<210> SEQ ID NO 33  
<211> LENGTH: 60  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
primer

<400> SEQUENCE: 33

atgtcgaag ctacatataa ggaacgtgct gcattctcacc ccagctgaag cttcgtacgc 60

<210> SEQ ID NO 34  
<211> LENGTH: 62  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
primer

<400> SEQUENCE: 34

ttagttttgc tggccgcacc ttctcaaata tgcttcccag gcataggcca ctatgggatc 60

tg 62

<210> SEQ ID NO 35  
<211> LENGTH: 60  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
primer

<400> SEQUENCE: 35

gaatactcag gtagcgaag atgcaagagt tcgaatctct ccagctgaag cttcgtacgc 60

<210> SEQ ID NO 36  
<211> LENGTH: 62  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:

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

<400> SEQUENCE: 36

tctaccctat gaacatattc cattttgtaa ttctgtgtcg gcataggcca ctagtggatc 60

tg 62

<210> SEQ ID NO 37

<211> LENGTH: 60

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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

<400> SEQUENCE: 37

atgacagagc agaaagcct agtaaagcgt attacaaatg ccagctgaag cttcgtacgc 60

<210> SEQ ID NO 38

<211> LENGTH: 62

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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

<400> SEQUENCE: 38

ctacataaga acaccttggg tggagggaa acatcgttgga gcataggcca ctagtggatc 60

tg 62

<210> SEQ ID NO 39

<211> LENGTH: 60

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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

<400> SEQUENCE: 39

atgagtgaag cagaattgag aaaaagacag gcccaattca ccagctgaag cttcgtacgc 60

<210> SEQ ID NO 40

<211> LENGTH: 62

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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

<400> SEQUENCE: 40

ttattgagtt gcttcttggg aagtttggga gggggtttcg gcataggcca ctagtggatc 60

tg 62

<210> SEQ ID NO 41

<211> LENGTH: 60

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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

<400> SEQUENCE: 41

atgagttctg tcgcagaaaa tataatacaa catgccactc ccagctgaag cttcgtacgc 60



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<210> SEQ ID NO 48  
 <211> LENGTH: 28  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer  
  
 <400> SEQUENCE: 48  
  
 aactcgagtc agtgccttgc cgccttgc 28

<210> SEQ ID NO 49  
 <211> LENGTH: 1833  
 <212> TYPE: DNA  
 <213> ORGANISM: *Saccharomyces cerevisiae*  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1833)  
  
 <400> SEQUENCE: 49  
  
 atg acg gag act aag gat ttg ttg caa gac gaa gag ttt ctt aag atc 48  
 Met Thr Glu Thr Lys Asp Leu Leu Gln Asp Glu Glu Phe Leu Lys Ile  
 1 5 10 15  
  
 cgc aga ctc aat tcc gca gaa gcc aac aaa cgg cat tcg gtc acg tac 96  
 Arg Arg Leu Asn Ser Ala Glu Ala Asn Lys Arg His Ser Val Thr Tyr  
 20 25 30  
  
 gat aac gtg atc ctg cca cag gag tcc atg gag gtt tcg cca cgg tcg 144  
 Asp Asn Val Ile Leu Pro Gln Glu Ser Met Glu Val Ser Pro Arg Ser  
 35 40 45  
  
 tct acc acg tcg ctg gtg gag cca gtg gag tcg act gaa gga gtg gag 192  
 Ser Thr Thr Ser Leu Val Glu Pro Val Glu Ser Thr Glu Gly Val Glu  
 50 55 60  
  
 tcg act gag gcg gaa cgt gtg gca ggg aag cag gag cag gag gag gag 240  
 Ser Thr Glu Ala Glu Arg Val Ala Gly Lys Gln Glu Gln Glu Glu Glu  
 65 70 75 80  
  
 tac cct gtg gac gcc cac atg caa aag tac ctt tca cac ctg aag agc 288  
 Tyr Pro Val Asp Ala His Met Gln Lys Tyr Leu Ser His Leu Lys Ser  
 85 90 95  
  
 aag tct cgg tcg agg ttc cac cga aag gat gct agc aag tat gtg tcg 336  
 Lys Ser Arg Ser Arg Phe His Arg Lys Asp Ala Ser Lys Tyr Val Ser  
 100 105 110  
  
 ttt ttt ggg gac gtg agt ttt gat cct cgc ccc acg ctc ctg gac agc 384  
 Phe Phe Gly Asp Val Ser Phe Asp Pro Arg Pro Thr Leu Leu Asp Ser  
 115 120 125  
  
 gcc atc aac gtg ccc ttc cag acg act ttc aaa ggt ccg gtg ctg gag 432  
 Ala Ile Asn Val Pro Phe Gln Thr Thr Phe Lys Gly Pro Val Leu Glu  
 130 135 140  
  
 aaa cag ctc aaa aat tta cag ttg aca aag acc aag acc aag gcc acg 480  
 Lys Gln Leu Lys Asn Leu Gln Leu Thr Lys Thr Lys Thr Lys Ala Thr  
 145 150 155 160  
  
 gtg aag act acg gtg aag act acg gag aaa acg gac aag gca gat gcc 528  
 Val Lys Thr Thr Val Lys Thr Thr Glu Lys Thr Asp Lys Ala Asp Ala  
 165 170 175  
  
 ccc cca gga gaa aaa ctg gag tcg aac ttt tca ggg atc tac gtg ttc 576  
 Pro Pro Gly Glu Lys Leu Glu Ser Asn Phe Ser Gly Ile Tyr Val Phe  
 180 185 190  
  
 gca tgg atg ttc ttg ggc tgg ata gcc atc agg tgc tgc aca gat tac 624  
 Ala Trp Met Phe Leu Gly Trp Ile Ala Ile Arg Cys Cys Thr Asp Tyr  
 195 200 205  
  
 tat gcg tcg tac ggc agt gca tgg aat aag ctg gaa atc gtg cag tac 672  
 Tyr Ala Ser Tyr Gly Ser Ala Trp Asn Lys Leu Glu Ile Val Gln Tyr  
 210 215 220

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atg aca acg gac ttg ttc acg atc gca atg ttg gac ttg gca atg ttc Met Thr Thr Asp Leu Phe Thr Ile Ala Met Leu Asp Leu Ala Met Phe 225 230 235 240	720
ctg tgc act ttc ttc gtg gtt ttc gtg cac tgg ctg gtg aaa aag cgg Leu Cys Thr Phe Val Val Phe Val His Trp Leu Val Lys Lys Arg 245 250 255	768
atc atc aac tgg aag tgg act ggg ttc gtt gca gtg agc atc ttc gag Ile Ile Asn Trp Lys Trp Thr Gly Phe Val Ala Val Ser Ile Phe Glu 260 265 270	816
ttg gct ttc atc ccc gtg acg ttc ccc att tac gtc tac tac ttt gat Leu Ala Phe Ile Pro Val Thr Phe Pro Ile Tyr Val Tyr Tyr Phe Asp 275 280 285	864
ttc aac tgg gtc acg aga atc ttc ctg ttc ctg cac tcc gtg gtg ttt Phe Asn Trp Val Thr Arg Ile Phe Leu Phe Leu His Ser Val Val Phe 290 295 300	912
gtt atg aag agc cac tcg ttt gcc ttt tac aac ggg tat ctt tgg gac Val Met Lys Ser His Ser Phe Ala Phe Tyr Asn Gly Tyr Leu Trp Asp 305 310 315 320	960
ata aag cag gaa ctc gag tac tct tcc aaa cag ttg caa aaa tac aag Ile Lys Gln Glu Leu Glu Tyr Ser Ser Lys Gln Leu Gln Lys Tyr Lys 325 330 335	1008
gaa tct ttg tcc cca gag acc cgc gag att ctg caa aaa agt tgc gac Glu Ser Leu Ser Pro Glu Thr Arg Glu Ile Leu Gln Lys Ser Cys Asp 340 345 350	1056
ttt tgc ctt ttc gaa ttg aac tac cag acc aag gat aac gac ttc ccc Phe Cys Leu Phe Glu Leu Asn Tyr Gln Thr Lys Asp Asn Asp Phe Pro 355 360 365	1104
aac aac atc agt tgc agc aat ttc ttc atg ttc tgt ttg ttc ccc gtc Asn Asn Ile Ser Cys Ser Asn Phe Phe Met Phe Cys Leu Phe Pro Val 370 375 380	1152
ctc gtg tac cag atc aac tac cca aga acg tcg cgc atc aga tgg agg Leu Val Tyr Gln Ile Asn Tyr Pro Arg Thr Ser Arg Ile Arg Trp Arg 385 390 395 400	1200
tat gtg ttg gag aag gtg tgc gcc atc att ggc acc atc ttc ctc atg Tyr Val Leu Glu Lys Val Cys Ala Ile Ile Gly Thr Ile Phe Leu Met 405 410 415	1248
atg gtc acg gca cag ttc ttc atg cac ccg gtg gcc atg cgc tgt atc Met Val Thr Ala Gln Phe Phe Met His Pro Val Ala Met Arg Cys Ile 420 425 430	1296
cag ttc cac aac acg ccc acc ttc ggc ggc tgg atc ccc gcc acg caa Gln Phe His Asn Thr Pro Thr Phe Gly Gly Trp Ile Pro Ala Thr Gln 435 440 445	1344
gag tgg ttc cac ctg ctc ttc gac atg att ccg ggc ttc act gtt ctg Glu Trp Phe His Leu Leu Phe Asp Met Ile Pro Gly Phe Thr Val Leu 450 455 460	1392
tac atg ctc acg ttt tac atg ata tgg gac gct tta ttg aat tgc gtg Tyr Met Leu Thr Phe Tyr Met Ile Trp Asp Ala Leu Leu Asn Cys Val 465 470 475 480	1440
gcg gag ttg acc agg ttt gcg gac aga tat ttc tac ggc gac tgg tgg Ala Glu Leu Thr Arg Phe Ala Asp Arg Tyr Phe Tyr Gly Asp Trp Trp 485 490 495	1488
aat tgc gtt tcg ttt gaa gag ttt agc aga atc tgg aac gtc ccc gtt Asn Cys Val Ser Phe Glu Glu Phe Ser Arg Ile Trp Asn Val Pro Val 500 505 510	1536
cac aaa ttt tta cta aga cac gtg tac cac agc tcc atg ggc gca ttg His Lys Phe Leu Leu Arg His Val Tyr His Ser Ser Met Gly Ala Leu 515 520 525	1584
cat ttg agc aag agc caa gct aca tta ttt act ttt ttc ttg agt gcc His Leu Ser Lys Ser Gln Ala Thr Leu Phe Thr Phe Phe Leu Ser Ala 530 535 540 545	1632

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530	535	540	
gtg ttc cac gaa atg gcc atg ttc gcc att ttc aga agg gtt aga gga			1680
Val Phe His Glu Met Ala Met Phe Ala Ile Phe Arg Arg Val Arg Gly			
545	550	555	560
tat ctg ttc atg ttc caa ctg tcg cag ttt gtg tgg act gct ttg agc			1728
Tyr Leu Phe Met Phe Gln Leu Ser Gln Phe Val Trp Thr Ala Leu Ser			
	565	570	575
aac acc aag ttt cta cgg gca aga ccg cag ttg tcc aac gtt gtc ttt			1776
Asn Thr Lys Phe Leu Arg Ala Arg Pro Gln Leu Ser Asn Val Val Phe			
	580	585	590
tcg ttt ggt gtc tgt tca ggg ccc agt atc att atg acg ttg tac ctg			1824
Ser Phe Gly Val Cys Ser Gly Pro Ser Ile Ile Met Thr Leu Tyr Leu			
	595	600	605
acc tta tga			1833
Thr Leu			
610			
<210> SEQ ID NO 50			
<211> LENGTH: 610			
<212> TYPE: PRT			
<213> ORGANISM: <i>Saccharomyces cerevisiae</i>			
<400> SEQUENCE: 50			
Met Thr Glu Thr Lys Asp Leu Leu Gln Asp Glu Glu Phe Leu Lys Ile			
1	5	10	15
Arg Arg Leu Asn Ser Ala Glu Ala Asn Lys Arg His Ser Val Thr Tyr			
	20	25	30
Asp Asn Val Ile Leu Pro Gln Glu Ser Met Glu Val Ser Pro Arg Ser			
	35	40	45
Ser Thr Thr Ser Leu Val Glu Pro Val Glu Ser Thr Glu Gly Val Glu			
	50	55	60
Ser Thr Glu Ala Glu Arg Val Ala Gly Lys Gln Glu Gln Glu Glu Glu			
	65	70	75
Tyr Pro Val Asp Ala His Met Gln Lys Tyr Leu Ser His Leu Lys Ser			
	85	90	95
Lys Ser Arg Ser Arg Phe His Arg Lys Asp Ala Ser Lys Tyr Val Ser			
	100	105	110
Phe Phe Gly Asp Val Ser Phe Asp Pro Arg Pro Thr Leu Leu Asp Ser			
	115	120	125
Ala Ile Asn Val Pro Phe Gln Thr Thr Phe Lys Gly Pro Val Leu Glu			
	130	135	140
Lys Gln Leu Lys Asn Leu Gln Leu Thr Lys Thr Lys Thr Lys Ala Thr			
	145	150	155
Val Lys Thr Thr Val Lys Thr Thr Glu Lys Thr Asp Lys Ala Asp Ala			
	165	170	175
Pro Pro Gly Glu Lys Leu Glu Ser Asn Phe Ser Gly Ile Tyr Val Phe			
	180	185	190
Ala Trp Met Phe Leu Gly Trp Ile Ala Ile Arg Cys Cys Thr Asp Tyr			
	195	200	205
Tyr Ala Ser Tyr Gly Ser Ala Trp Asn Lys Leu Glu Ile Val Gln Tyr			
	210	215	220
Met Thr Thr Asp Leu Phe Thr Ile Ala Met Leu Asp Leu Ala Met Phe			
	225	230	235
Leu Cys Thr Phe Phe Val Val Phe Val His Trp Leu Val Lys Lys Arg			
	245	250	255
Ile Ile Asn Trp Lys Trp Thr Gly Phe Val Ala Val Ser Ile Phe Glu			

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260				265				270							
Leu	Ala	Phe	Ile	Pro	Val	Thr	Phe	Pro	Ile	Tyr	Val	Tyr	Tyr	Phe	Asp
	275						280					285			
Phe	Asn	Trp	Val	Thr	Arg	Ile	Phe	Leu	Phe	Leu	His	Ser	Val	Val	Phe
	290					295					300				
Val	Met	Lys	Ser	His	Ser	Phe	Ala	Phe	Tyr	Asn	Gly	Tyr	Leu	Trp	Asp
	305				310					315					320
Ile	Lys	Gln	Glu	Leu	Glu	Tyr	Ser	Ser	Lys	Gln	Leu	Gln	Lys	Tyr	Lys
			325						330					335	
Glu	Ser	Leu	Ser	Pro	Glu	Thr	Arg	Glu	Ile	Leu	Gln	Lys	Ser	Cys	Asp
			340						345					350	
Phe	Cys	Leu	Phe	Glu	Leu	Asn	Tyr	Gln	Thr	Lys	Asp	Asn	Asp	Phe	Pro
		355					360						365		
Asn	Asn	Ile	Ser	Cys	Ser	Asn	Phe	Phe	Met	Phe	Cys	Leu	Phe	Pro	Val
	370					375					380				
Leu	Val	Tyr	Gln	Ile	Asn	Tyr	Pro	Arg	Thr	Ser	Arg	Ile	Arg	Trp	Arg
	385				390					395					400
Tyr	Val	Leu	Glu	Lys	Val	Cys	Ala	Ile	Ile	Gly	Thr	Ile	Phe	Leu	Met
			405						410					415	
Met	Val	Thr	Ala	Gln	Phe	Phe	Met	His	Pro	Val	Ala	Met	Arg	Cys	Ile
			420						425					430	
Gln	Phe	His	Asn	Thr	Pro	Thr	Phe	Gly	Gly	Trp	Ile	Pro	Ala	Thr	Gln
		435					440						445		
Glu	Trp	Phe	His	Leu	Leu	Phe	Asp	Met	Ile	Pro	Gly	Phe	Thr	Val	Leu
	450					455					460				
Tyr	Met	Leu	Thr	Phe	Tyr	Met	Ile	Trp	Asp	Ala	Leu	Leu	Asn	Cys	Val
	465				470					475					480
Ala	Glu	Leu	Thr	Arg	Phe	Ala	Asp	Arg	Tyr	Phe	Tyr	Gly	Asp	Trp	Trp
			485						490					495	
Asn	Cys	Val	Ser	Phe	Glu	Glu	Phe	Ser	Arg	Ile	Trp	Asn	Val	Pro	Val
		500							505				510		
His	Lys	Phe	Leu	Leu	Arg	His	Val	Tyr	His	Ser	Ser	Met	Gly	Ala	Leu
		515					520						525		
His	Leu	Ser	Lys	Ser	Gln	Ala	Thr	Leu	Phe	Thr	Phe	Phe	Leu	Ser	Ala
	530					535					540				
Val	Phe	His	Glu	Met	Ala	Met	Phe	Ala	Ile	Phe	Arg	Arg	Val	Arg	Gly
	545				550					555					560
Tyr	Leu	Phe	Met	Phe	Gln	Leu	Ser	Gln	Phe	Val	Trp	Thr	Ala	Leu	Ser
			565						570					575	
Asn	Thr	Lys	Phe	Leu	Arg	Ala	Arg	Pro	Gln	Leu	Ser	Asn	Val	Val	Phe
		580							585				590		
Ser	Phe	Gly	Val	Cys	Ser	Gly	Pro	Ser	Ile	Ile	Met	Thr	Leu	Tyr	Leu
		595					600						605		
Thr	Leu														
	610														

&lt;210&gt; SEQ ID NO 51

&lt;211&gt; LENGTH: 33

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

&lt;400&gt; SEQUENCE: 51

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ctgcgccgc atcatgtctg ctgttaacgt tgc 33

<210> SEQ ID NO 52  
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<210> SEQ ID NO 56  
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 56

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<210> SEQ ID NO 57  
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<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 57

ctgcgccgc aatatggatt tggctttaga agtcg 35

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<210> SEQ ID NO 58
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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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<400> SEQUENCE: 58

aactcgagtc agttgttctt cttggtattt g                               31

<210> SEQ ID NO 59
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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ctgcggccgc actatggcaa aggataatag tgag                               34

<210> SEQ ID NO 60
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 60

ttctcgagct agaaaacata aggaataaag ac                               32

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The invention claimed is:

1. A method for preparing 7-dehydrocholesterol and/or cholesterol, the method comprising the steps of:

culturing a yeast organism which, compared to the wild type, has an increased activity of at least one of the activities selected from the group consisting of:

$\Delta 8$ - $\Delta 7$ -isomerase activity of a  $\Delta 8$ - $\Delta 7$ -isomerase having the amino acid sequence having at least 90% sequence identity to SEQ ID NO: 2,

$\Delta 5$ -desaturase activity of a  $\Delta 5$ -desaturase having the amino acid sequence having at least 90% sequence identity to SEQ ID NO: 8; and

$\Delta 24$ -reductase activity of a  $\Delta 24$ -reductase having the amino acid sequence having at least 90% sequence identity to SEQ ID NO: 14; and wherein the yeast organism additionally have increased HMG-CoA-reductase activity of HMG-CoA-reductase having the amino acid sequence having at least 90% sequence identity to SEQ ID NO: 24;

harvesting the yeast after culturing; and isolating the 7-dehydrocholesterol and/or cholesterol after harvesting.

2. The method of claim 1, wherein the yeast organism additionally has, compared to the wild type, an increased activity of at least one of the activities selected from the group consisting of lanosterol C14-demethylase activity, squalene-epoxidase activity, squalene-synthetase activity and sterol-acyltransferase activity.

3. The method of claim 1, wherein the yeast organism has an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity of a  $\Delta 8$ - $\Delta 7$ -isomerase

having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 2.

4. The method of claim 1, wherein the yeast organism has an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity of a  $\Delta 8$ - $\Delta 7$ -isomerase having the amino acid sequence of SEQ ID NO: 2.

5. The method of claim 1, wherein the yeast organism has an increased  $\Delta 5$ -desaturase activity of a  $\Delta 5$ -desaturase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8.

6. The method of claim 1, wherein the yeast organism has an increased  $\Delta 5$ -desaturase activity of a  $\Delta 5$ -desaturase having the amino acid sequence of SEQ ID NO: 8.

7. The method of claim 1, wherein the yeast organism has an increased  $\Delta 24$ -reductase activity of a  $\Delta 24$ -reductase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 14.

8. The method of claim 1, wherein the yeast organism has an increased  $\Delta 24$ -reductase activity of a  $\Delta 24$ -reductase having the amino acid sequence of SEQ ID NO: 14.

9. The method of claim 1, wherein the yeast organism has an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity of a  $\Delta 8$ - $\Delta 7$ -isomerase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 2, and an increased  $\Delta 5$ -desaturase activity of a  $\Delta 5$ -desaturase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8.

10. The method of claim 1, wherein the yeast organism has an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity of a  $\Delta 8$ - $\Delta 7$ -isomerase having the amino acid sequence of SEQ ID NO: 2, and an increased  $\Delta 5$ -desaturase activity of a  $\Delta 5$ -desaturase having the amino acid sequence of SEQ ID NO: 8.

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11. The method of claim 1, wherein the yeast organism has an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity of a  $\Delta 8$ - $\Delta 7$ -isomerase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 2, and an increased  $\Delta 24$ -reductase activity of a  $\Delta 24$ -reductase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 14.

12. The method of claim 1, wherein the yeast organism has an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity of a  $\Delta 8$ - $\Delta 7$ -isomerase having the amino acid sequence of SEQ ID NO: 2, and an increased  $\Delta 24$ -reductase activity of a  $\Delta 24$ -reductase having the amino acid sequence of SEQ ID NO: 14.

13. The method of claim 1, wherein the yeast organism has an increased  $\Delta 5$ -desaturase activity of a  $\Delta 5$ -desaturase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8, and an increased  $\Delta 24$ -reductase activity of a  $\Delta 24$ -reductase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 14.

14. The method of claim 1, wherein the yeast organism has an increased  $\Delta 5$ -desaturase activity of a  $\Delta 5$ -desaturase having the amino acid sequence of SEQ ID NO: 8, and an increased  $\Delta 24$ -reductase activity of a  $\Delta 24$ -reductase having the amino acid sequence of SEQ ID NO: 14.

15. The method of claim 1, wherein the yeast organism has an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity of a  $\Delta 8$ - $\Delta 7$ -isomerase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 2, an increased  $\Delta 5$ -desaturase activity of a  $\Delta 5$ -desaturase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8, and an increased  $\Delta 24$ -reductase activity of a

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$\Delta 24$ -reductase having the amino acid sequence having at least 95% sequence identity to SEQ ID NO: 14.

16. The method of claim 1, wherein the yeast organism has an increased  $\Delta 8$ - $\Delta 7$ -isomerase activity of a  $\Delta 8$ - $\Delta 7$ -isomerase having the amino acid sequence of SEQ ID NO: 2, an increased  $\Delta 5$ -desaturase activity of a  $\Delta 5$ -desaturase having the amino acid sequence of SEQ ID NO: 8, and an increased  $\Delta 24$ -reductase activity of a  $\Delta 24$ -reductase having the amino acid sequence of SEQ ID NO: 14.

17. The method of claim 1, wherein the yeast organism additionally has, compared to the wild type, increased HMG-CoA-reductase activity of an HMG-CoA-reductase having the amino acid sequence of SEQ ID NO: 24.

18. The method of claim 1, wherein the increased activity is from increased gene expression of a nucleic acid introduced into the yeast organism encoding the  $\Delta 8$ - $\Delta 7$ -isomerase,  $\Delta 5$ -desaturase, or  $\Delta 24$ -reductase.

19. The method of claim 3, wherein the increased  $\Delta 8$ - $\Delta 7$ -isomerase activity is from increased gene expression of a nucleic acid introduced into the yeast organism encoding the  $\Delta 8$ - $\Delta 7$ -isomerase.

20. The method of claim 5, wherein the increased  $\Delta 5$ -desaturase activity is from increased gene expression of a nucleic acid introduced into the yeast organism encoding the  $\Delta 5$ -desaturase.

21. The method of claim 7, wherein the increased  $\Delta 24$ -reductase activity is from increased gene expression of a nucleic acid introduced into the yeast organism encoding the  $\Delta 24$ -reductase.

\* \* \* \* \*