(19) DANMARK

(10) **DK/EP 3158076 T3**

Patent- og Varemærkestyrelsen

(12) Oversættelse af europæisk patentskrift

(51) Int.Cl.: ***C 12 P 19/14 (2006.01)***

(45) Oversættelsen bekendtgjort den: **2021-09-13**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2021-06-16**

(86) Europæisk ansøgning nr.: **15732869.1**

(86) Europæisk indleveringsdag: **2015-06-12**

(87) Den europæiske ansøgnings publiceringsdag: **2017-04-26**

(86) International ansøgning nr.: **FR2015051557**

(87) Internationalt publikationsnr.: **WO2015193588**

(30) Prioritet: **2014-06-20 FR 1455701**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **VARIANTER AF EXOGLUCANASER MED FORBEDRET AKTIVITET OG ANVENDELSER DERAF**

(56) Fremdragne publikationer:
WO-A1-2014/081884
WO-A2-2014/078546
FR-A1- 2 782 323
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VARIANTS OF EXOGLUCANASES HAVING IMPROVED ACTIVITY AND USES THEREOF

The possibility of producing ethanol from cellulose has received much attention because of the availability of large amounts of raw material as well as the interest of ethanol as a fuel. Cellulosic natural raw materials for such a process are referred to by the term "biomass". Many types of biomass, for example wood, agricultural residues, herbaceous cultures and municipal solid wastes, have been considered as potential raw materials for biofuel production. These matters primarily consist of cellulose, hemicellulose and lignin.

Cellulose is a polymer consisting of glucose molecules bonded by beta 1-4 bonds, which resist degradation or depolymerisation very well. Once the cellulose is converted into glucose, the latter is easily fermented into biofuel, for example ethanol, using a yeast.

The oldest methods studied to convert cellulose into glucose are based on acid hydrolysis. This process may be carried out in the presence of concentrated or diluted acids. However, several drawbacks such as the poor recovery of acid when using concentrated acids and the low production of glucose when using diluted acids affect the economics of the acid hydrolysis process.

To overcome the drawbacks of the acid hydrolysis process, the cellulose conversion processes have focused more recently on enzyme hydrolysis, using cellulose-type enzymes. However, this enzyme hydrolysis of the lignocellulose biomass (for example, cellulose) has the drawback of being an expensive industrial process. Therefore, it is necessary to use strains of increasingly effective cellulose-secreting microorganisms. As such, many microorganisms include enzymes that hydrolyse cellulose, such as the fungi *Trichoderma*, *Aspergillus*, *Humicola*, *Fusarium* as well as bacteria such as *Thermomonospora*, *Bacillus*, *Cellulomonas* and *Streptomyces*. The enzymes secreted by these microorganisms have three types of activities useful in the conversion of cellulose into glucose and are divided into three groups: endoglucanases, which randomly attack cellulose fibers internally, exoglucanases which will attack the ends of the fibers while releasing cellobiose, and beta-glucosidases which will hydrolyse this cellobiose into glucose. Other classes of enzymes such as hemicellulases or the recently-discovered enzyme class of monooxygenase polysaccharides may also participate to the effectiveness of the hydrolysis.

There is a strong industrial interest in the reduction of the cost of the enzyme hydrolysis, and this reduction is achieved through the use of a reduced dose of enzymes and therefore of more effective enzyme cocktails. Consequently, several patent applications describe natural enzymes with greater capabilities than those of *Trichoderma reesei*, or variants improved by genetic engineering. Mention may be made of the patent applications US2010304464, WO2010066411, WO2014078546, WO2012149403, and WO2013029176 regarding exoglucanases, the applications WO2007109441, WO2012149192 and WO2010076388 regarding endoglucanases, the applications WO2010029259, WO2010135836 or WO2010022518 regarding beta-glucosidases, or still the applications WO12135659, WO12149344 regarding monooxygenase polysaccharides. Mention may also be made of the following accession numbers regarding exoglucanases: BBG53179, BAG13668. In turn, the reference ASR93979 describes an enzyme having a lignocellulosic activity.

The accession number Uniprot I1RIJ1 refers to a protein having a putative glucanase activity.

The patent application FR2782323 describes a method for producing recombined polynucleotide sequences *in vitro*.

The application WO2014081884 describes modified secreted proteins.

Enzymes hydrolysing the lignocellulosic biomass are classified in the CAZy system (Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., & Henrissat, B. (2009). The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic acids research, 37, D233-8) on primarily structural criteria. Exoglucanases may belong to the families GH 6, 7, 9, 48, and 74.

For a hydrolysis of lignocellulosic biomass to be effective and economically-profitable, the enzyme mixture should include balanced proportions of enzymes having various enzyme activities, including, yet without exclusion, exoglucanases, endoglucanases, xylanases and beta-glucosidases. As example, in the native mixtures of *Trichoderma reesei*, it is generally noticed the presence of 60-70% of exoglucanases, 15-20% of endoglucanases, a few percents of hemicellulases and about 5-10% of beta-glucosidases. This mixture is suitable for hydrolysing most pretreated substrates (for example, wheat straw steam exploded in acid conditions) with acceptable yields. The already large proportion of exoglucanases in the mixture indicates that it will be difficult to increase the amount of these enzymes without adversely affecting the other activities.

The *Trichoderma reesei* genome includes two exoglucanases, one originating from the family 6 (CBH2, cel6a) and the other one originating from the family 7 (CBH1, Cel7a). They respectively hydrolyse the non-reducing (EC3.2.1.176) and reducing (EC3.2.1.91) ends of cellulose into cellobiose.

The hydrolysis and the fermentation could be carried out according to different schemes. The most common one consists of separate hydrolysis and fermentation (SHF – Separate Hydrolysis and Fermentation). This method allows optimising each step by maintaining optimum reaction conditions. This fermentation is performed extemporaneously, at a temperature comprised between about 28°C and about 30°C, whereas the hydrolysis generally takes place at a temperature of at least 45°C. However, in SHF, the sugars released at the end of reaction are present at a very high concentration and cause an inhibition of the enzymes, thereby slowing down the effectiveness of the process. To avoid these drawbacks, another type of processes may be considered. In SSF, the two steps (hydrolysis and fermentation of the hexoses) take place simultaneously, thereby preventing the accumulation of the sugars at concentrations that might be inhibiting for the enzymes. The investment costs are also reduced thanks to the use of one single reactor. The hydrolysis rate is higher because of the absence of inhibition as the other sugars are used immediately for fermentation into ethanol. In this method, the temperature of the reactor necessarily consists of a trade-off between the optimum hydrolysis and fermentation temperatures, typically between about 30°C and about 35°C. However, at such a temperature, the activity of the cellulolytic enzymes is reduced by about 30%.

SSF also enables the expression of enzymes degrading cellulose in the organism that ferments the sugars, which allows limiting, or in an extreme case suppressing, the need for enzymes produced at a separate step.

Consequently, the obtainment of enzymes maintaining an effective exoglucanase activity at optimum hydrolysis and fermentation temperatures (namely between 30°C and 50°C) while keeping the proportion of all enzymes of the mixture would be a significant gain for the process of conversion of lignocellulosic biomass into biofuel.

The inventors have developed a polypeptide having an improved exoglucanase activity, in particular in comparison with the exoglucanase activity of the reference protein CBH2 of sequence SEQ ID NO: 2, CBH2 corresponds to the exoglucanase 2 of *Trichoderma reesei*.

In this perspective, after many researches, the Applicants had the great merit of finding an isolated or purified polypeptide having an improved exoglucanase activity in comparison with the exoglucanase activity of the reference protein CBH2 (SEQ ID NO: 2).

Hence, the description relates to a polypeptide selected from the group consisting of:

i. an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26 and SEQ ID NO: 28; and

ii. an amino acid sequence featuring a percentage of identical residues, with respect to the sequence SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26 and SEQ ID NO: 28, (identity percentage) of at least 70%, preferably of at least 75%, 80%, 85%, 90%, 95%, 98% or 99%.

The invention is defined by the claims.

Preferably, the polypeptide as described before is characterised in that its expression in a fermentative organism is at least equal to the expression of the reference protein CBH2 (SEQ ID NO: 2).

According to the invention, the percent identity of a given sequence with respect to SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 28 corresponds to the number of identical residues between this given sequence and SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 28 divided by the number of residues in SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 28.

The polypeptide of the invention has an exoglucanase activity improved by at least 10%, preferably by at least 20%, preferably by at least 30%, still more preferably by at least 40%, at a temperature of about 35°C and/or about 50°C, in comparison with the exoglucanase activity of the polypeptide CBH2 of amino acid sequences SEQ ID No. 2.

For example, those skilled in the art could determine the increase, or in other words the improvement of the enzyme activity, either using a substrate like the cellulose Avicel®, the cellulose PASC or with a chromogenic substrate (p-Nitrophenyl glycoside), for example pNP lactose. The enzyme activity will be respectively revealed by colorimetric analysis of the reducing sugars or of the released nitrophenol.

An example of a protocol, that those skilled in the a use to determine whether a polypeptide according to the invention has an improved enzyme activity in comparison with that of the reference protein CBH2 (SEQ ID NO : 2), is as follows:

- preparation of a stock culture of *Y. lipolytica* expressing a recombinant enzyme according to the invention overnight at 28°C;
- seeding of an expression medium with a volume of stock culture allowing having an optical density at 600 nm equal to 0.2 at the beginning of the culture;
- culturing of said cells at 28°C for 96 hours;
- centrifugation at 8000 rpm for 5 minutes;
- incubation of 100 µl of supernatant with 100 µl of 0.1 M citrate phosphate buffer at pH 6 containing 1% of reduced cellodextrins (CD) for 4 hours at 35°C and 50°C;
- sampling of 100 µL of reaction;
- addition of 100 µL of DNS reactant;
- incubation for 5 minutes at 100°C;
- incubation for 3 minutes on ice;
- centrifugation for 10 minutes at 3000 rpm;
- reading of the OD at 540 nm on 150 µL.

Another object of the invention is purified or isolated nucleic acid encoding at least one polypeptide as described before, according to the invention. TABLE 1 hereinbelow comprises the identifications of the nucleic and peptide sequences for the reference gene CBH2 of *T. reesei*, the putative exoglucanases of *Nectria haematococca* (NH) and *Giberella Zeae* (GZ), as well as for the polypeptides and polynucleotides of the invention.

TABLE 1

Clones	Nucleic acid	Polypeptide
CBH2 (natural)	SEQ ID NO: 1	SEQ ID NO: 2
35B7	SEQ ID NO: 3	SEQ ID NO: 4
95B7	SEQ ID NO: 5	SEQ ID NO: 6
100F11	SEQ ID NO: 7	SEQ ID NO: 8
139F12	SEQ ID NO: 9	SEQ ID NO: 10
157B11	SEQ ID NO: 11	SEQ ID NO: 12
161A1	SEQ ID NO: 13	SEQ ID NO: 14
161C12	SEQ ID NO: 15	SEQ ID NO: 16

189H8	SEQ ID NO: 17	SEQ ID NO: 18
196D9	SEQ ID NO: 19	SEQ ID NO: 20
198E11	SEQ ID NO: 21	SEQ ID NO: 22
251B4	SEQ ID NO: 23	SEQ ID NO: 24
251C4	SEQ ID NO: 25	SEQ ID NO: 26
382A2	SEQ ID NO: 27	SEQ ID NO: 28
Gene GZ	SEQ ID NO: 29	SEQ ID NO: 30
Gene NH-7	SEQ ID NO: 31	SEQ ID NO: 32

The invention also relates to a purified or isolated nucleic acid encoding at least one polypeptide as described before, according to the invention.

Preferably, said purified or isolated nucleic acid may be selected from the following sequences: SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25 and SEQ ID NO: 27.

According to the invention, the nucleic acid as described before may be operationally linked to a promoter, a terminator or any other sequence necessary to the expression thereof in a host cell.

The invention also covers a vector comprising at least one nucleic acid as described before, according to the invention.

According to the invention, by "vector", it should be understood any DNA sequence wherein it is possible to insert foreign nucleic acid fragments, the vectors allowing introducing foreign DNA into a host cell. As examples of vectors, mention may be made in a non-exhaustive manner of: plasmids, cosmids, yeast artificial chromosomes (YAC), bacterial artificial chromosome (BAC), artificial chromosomes derived from the bacteriophage P1 (PAC) or vectors derived from viruses.

The vector according to the invention may also carry a selection marker. By "selection marker", it should be understood a gene whose expression confers on the cells containing it a characteristic allowing selecting them. For example, it consists of an antibiotic resistance gene.

Another object of the invention is an isolated host cell comprising either at least one of the polypeptides as described before, according to the invention, or at least one

of the nucleic acids as described before, according to the invention, or at least one of the vectors as described before, according to the invention.

Those skilled in the art could introduce one of the polypeptides, one of the nucleic acids or one of the vectors as described before in the host cell by well-known conventional methods. For example, mention may be made of the treatment with calcium chloride, electroporation, the use of a particle gun.

According to one embodiment, those skilled in the art could introduce into the host cell and by conventional methods several copies of a nucleic acid encoding a polypeptide having an improved exoglucanase activity according to the invention.

According to one embodiment, the isolated host cell as described before according to the invention is selected from *Trichoderma*, *Aspergillus*, *Neurospora*, *Humicola*, *Myceliophthora*, *Chrysosporium*, *Penicillium*, *Fusarium*, *Thermomonospora*, *Bacillus*, *Pseudomonas*, *Escherichia*, *Clostridium*, *Cellulomonas*, *Streptomyces*, *Yarrowia*, *Pichia* and *Saccharomyces*.

According to a preferred embodiment, the isolated host cell as described before according to the invention is selected from *Trichoderma reesei*, *Trichoderma viridae*, *Trichoderma koningii*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus wentii*, *Aspergillus oryzae*, *Aspergillus phoenicis*, *Myceliophthora thermopila*, *Chrysosporium lucknowense*, *Neurospora crassa*, *Humicola grisae*, *Penicillium pinophilum*, *Penicillium oxalicum*, *Escherichia coli*, *Clostridium acetobutylicum*, *Clostridium saccharolyticum*, *Clostridium benjerinckii*, *Clostridium butylicum*, *Pichia pastoris*, *Yarrowia lipolityca* and *Saccharomyces cerevisiae*.

According to a preferred embodiment, the isolated host cell as described before according to the invention is selected from *Trichoderma reesei* and *Saccharomyces cerevisiae*.

Another object of the invention is the use of any one of the polypeptides described before, according to the invention for hydrolysing cellulose.

Another object of the invention is the use of any one of the polypeptides described before, according to the invention for producing biofuel.

According to the invention, the term biofuel may be defined as referring to any product originating from the transformation of biomass and being able to be used for energy purposes. On the one hand and without limitation, as examples of biogases, mention may be made of the products that could be incorporated (possibly after a

subsequent transformation) into a fuel or be a fuel on its own, such as alcohols (ethanol, butanol and/or isopropanol depending on the type of used fermentative organism), solvents (acetone), acids (butyric), lipids and derivatives thereof (fatty acids with short or long chains, esters of fatty acids), as well as hydrogen.

Preferably, the biofuel according to the invention is an alcohol, for example ethanol, butanol and/or isopropanol. More preferably, the biofuel according to the invention is ethanol.

According to another embodiment, the biofuel is a biogas.

According to another embodiment, the product is a molecule interesting the chemical industry, like, another alcohol such as 1,2-propanediol, 1,3-propanediol, 1,4-butanediol, 2,3-butanediol, organic acids like acetic, propionic, acrylic, butyric, succinic, malic, fumaric, citric, itaconic acid, or hydroxyl acids like glycolic, hydroxypropionic or lactic acid.

An embodiment of production of an enzyme cocktail useful for hydrolysing lignocellulose is described hereinbelow.

The filamentous fungi strains, preferably *Trichoderma*, more preferably *T. reesei*, capable of expressing at least one polypeptide according to the invention are cultivated in fermenters, in the presence of a carbon substrate, such as lactose or glucose, selected for the growth of the microorganism. In one embodiment, depending on its nature, this carbon substrate is introduced into the fermenter before sterilisation or is sterilised separately and introduced into the fermenter after sterilisation of the latter to obtain an initial concentration of 20 to 35 g/L.

Afterwards, an aqueous solution containing the selected substrate for the production of enzymes is added. Finally, an enzyme composition acting on the lignocellulosic biomass produced by the fungi is recovered by filtering the culture medium. In this composition, beta-glucosidase, the endoglucanase and the exoglucanase according to the invention are found in particular.

According to one embodiment, the aqueous solution containing the selected substrate for the production of the enzymes is prepared at the concentration of 200-250 g/L. Preferably, this solution further contains an inductor substrate such as lactose. This aqueous solution is injected after exhaustion of the initial carbon substrate so as to bring in an optimised amount, comprised between 35 and 45 mg/g of cells ("fed batch"). During this "fed batch" phase, the sugar residual concentration in the culture medium is lower

than 1 g/L and the enzymes acting on the lignocellulosic biomass are secreted by the fungus. These may be recovered by filtering the culture medium.

Another object of the invention is an enzyme composition capable of acting upon lignocellulosic biomass, said enzyme composition being produced by filamentous fungi and comprising at least one polypeptide having an improved exoglucanase activity in comparison with the exoglucanase activity of the reference protein CBH2. By "filamentous fungi", it should be understood in particular *Trichoderma*, more preferably *T. reesei*.

Finally, an object of the invention is a method for producing biofuel from biomass, comprising the following successive steps:

- suspending the biomass to be hydrolysed in aqueous phase;
- hydrolysing the lignocellulosic biomass in the presence of an enzyme composition as described before the lignocellulosic biomass so as to produce a hydrolysate containing glucose;
- fermenting in the presence of a fermentative organism the glucose from the hydrolysate so as to produce a fermentation wort;
- separating the biofuel from the fermentation wort.

In one embodiment, the biomass to be hydrolysed is suspended in aqueous phase at 6 to 40% of dry matter, preferably 20 to 30%. The pH is adjusted between 4 and 5.5, preferably between 4.8 and 5.2 and the temperature between 40°C and 60°C, preferably between 45°C and 50°C. The hydrolysis reaction begins by the addition of the enzyme composition acting on the lignocellulosic biomass; the amount that is commonly used in from 10 to 30 mg of excreted protein per gram of pretreated substrate or less. In general, the reaction lasts from 15 to 48 hours. The reaction is followed by dosing the released sugars, in particular glucose. The sugar solution is separated from the non-hydrolysed solid fraction, consisting essentially of lignin, by filtering or centrifugation and treated afterwards in a fermentation unit.

After the fermentation step, the biofuel is separated from the fermentation wort, for example by distillation.

Another object of the invention is a method for producing biofuel from biomass, characterised in that it comprises the following successive steps:

- suspending the biomass to be hydrolysed in aqueous phase;

- simultaneously adding to the suspension an enzyme composition acting on the lignocellulosic biomass as defined before and a fermentative organism and fermenting the mixture so as to produce a fermentation wort;
- separating the biofuel from the fermentation wort.

Preferably, the enzyme composition and the fermentative organism are added simultaneously and then incubated at a temperature comprised between 30°C and 35°C to produce a fermentation wort.

According to this embodiment, the cellulose present in the biomass is converted into glucose, and at the same time, in the same reactor, the fermentative organism (for example a yeast) converts the glucose into the end product according to a SSF (simultaneous Saccharification and Fermentation) process known to those skilled in the art. Depending on the metabolic and hydrolytic capabilities of the fermentative organism, the proper progress of the operation may require the addition of a substantial amount of exogenous cellulolytic mixture.

In another embodiment, the fermentative organism produces the polypeptide object of the invention by secretion or at the surface of its cell, possibly together with other enzymes acting on the lignocellulose biomass, thereby limiting or suppressing the need for enzymes produced by the filamentous fungi. Preferably, the fermentative organism is a host cell as described before, according to the invention.

Preferably, the host cells with the enzyme composition and/or the fermentative organism are added and then incubated at a temperature comprised between 30°C and 35°C to produce a fermentation wort.

Thus, the use of the polypeptide having a better exoglucanase activity according to the present invention has the advantage of obtaining a better glucose production yield while using less enzyme than ever before, which has also an economic advantage.

Other aspects, objects, advantages and features of the invention, will come out upon reading the following non-restrictive description which describes preferred embodiments of the invention provided through examples and the figures.

Figure 1 is a MALDI-TOF mass spectrum representing the cellodextrins DP3 to DP11 used for screening.

EXAMPLES

EXAMPLE 1: Preparation of reduced cellodextrins (DP 3-11)

1-Hydrolysis of cellulose (Adapted from Y-H. Percival Zhang, L. R. Lynd Analytical Biochemistry 322 (2003), 225-232.)

$$\text{Avicel} + HCl + H_2SO_4 \xrightarrow{4h,\ 25°C} \text{Cellodextrins}$$

20 g of cellulose (Avicel CAS Number 9004-34-6, Sigma-Aldrich Saint-Quentin Fallavier) are added by portions and under strong stirring to 160 mL of a solution cooled down to 0°C of hydrochloric acid. Sulfuric acid, cooled beforehand, is added to the solution in several times (4 x 10 mL). The reaction is maintained under stirring for 4 hours at 25°C before being poured into 1.8 L of acetone cooled down to -20°C. After 2 hours of stirring, the precipitate is filtered, taken up in 400 mL of cooled acetone and then filtered again. The solid is then taken up in 600 mL of water, and then stirred overnight to solubilise the cellodextrins. After filtering of the solid, the soluble fraction containing the cellodextrins is neutralised with 300 g of Amberlite IRA 400 OH^- resin and then lyophilised. Afterwards, the lyophilisate is suspended again in 500 mL of methanol in the presence of ultrasounds for 30 minutes to solubilise the sugars with low molecular weight before being filtered and then lyophilised again to end up with 6.8 g of DP 3-11 cellodextrins.

For screening, it has been chosen to work with substrates with the greatest molecular weight as possible to mimic the closest the structure of cellulose. Yet, the cellodextrins with high molecular weight are not soluble, that preventing a good repeatability of the tests.

Hence, a range of DP 5-7 cellodextrins has been chosen, which represents a good trade-off between the necessary high molecular weight and the solubility of the cellodextrins.

Figure 1 shows a MALDI-TOF mass spectrum typically obtained according to the method described hereinabove.

Figure 1 shows that the isolated oligosaccharides primarily consist of DP 5-7.

2-Reduction of the cellodextrins

400 mg of sodium borohydride are added to 2 g of DP 3-11 cellodextrins diluted in 120 mL of water. After 3 hours under stirring at room temperature, the solution is neutralised by addition of Amberlite H^- IR 120 resin, and then lyophilised, to end up with 2 g of quantitatively reduced cellodextrins. (C. Schou, G. Rasmussen, M-B. Kaltoft, B. Henrissat, M. Schulein Eur. J. Biochem. 217, 947-953 (1993)).

A dosing of the isolated cellodextrins with BCA (bicinchoninic acid) allows checking up the total reduction of the reducing ends (Y.-H. Percival Zhang, L. R. Lynd Biomacromolecules 2005, 6, 1510-1515).

EXAMPLE 2: evolution by L-shuffling

The sequence of the gene of the cellobiohydrolase 2 of *Trichoderma reesei* (SEQ ID NO: 1) has been subjected to a L-shuffling round according to the patented method described in the patent EP1104457 with the genes of a putative endoglucanase of *Giberella zeae* PH-1 (SEQ ID NO: 29) and of a hypothetical protein NECHADRAFT_73991 of *Nectria haematococca* mpVI (SEQ ID NO: 31) having respectively 63% and 69% of homology with the parent gene CBH2 (SEQ ID NO: 1). The nucleic sequence encoding the signal peptide (SEQ ID NO: 33) has been deleted during cloning, and replaced with that of yeast, of sequence SEQ ID NO: 34 (sequence of the corresponding signal peptide: SEQ ID NO: 35).

1-High-throughput screening

A high-throughput screening test has been developed in order to select the best clones derived from the L-shuffling, that is to say those having at least 20% of improvement of the cellobiohydrolase activity in comparison with the reference enzyme CBH2 (SEQ IS NO: 2).

The high-throughput screening test has been carried out according to the following steps:

- agar isolation of *Y. lipolytica* clones expressing the L-shuffling variants of the enzyme according to the invention and setting into pre-cultures in a YNBcasa medium (yeast nitrogen base 1.7 g/L, NH_4Cl 10 g/L, casamino acids 2 g/L, pH 7) of said colonies for 36 hours at 28°C;
- inoculation of a YTD medium (yeast extract 10 g/L, tryptone 20g/L, glucose 2.5 g/L, pH 6.8) added to tetracycline at 12.5 µg/mL at 5% with the pre-culture and then incubation for 20 hours at 28°C;
- inoculation of the expression medium containing the inductor (oleic acid) at 20 g/L at 10% with the previous culture and then incubation for 96 hours at 28°C;
- centrifugation for 5 minutes at 1500 rpm;
- sampling of 100 µL of supernatant;
- addition of 100 µL of reduced CDs at 1 g/L in a 0.1 M citrate phosphate buffer pH 6;

- incubation for 24 hours at 35°C;
- centrifugation for 5 minutes at 2500 rpm;
- sampling of 80 µL of supernatant;
- addition of 80 µL of DNS reactant;
- incubation for 12 minutes at 105°C and then 5 minutes on ice;
- reading of the optical density (OD) at 540 nm on 120 µL.

In these screening conditions, an improvement of the cellobiohydrolase activity (increase of the OD at 540 nm) in comparison with the reference enzyme CBH2 (SEQ ID NO: 2) has been found in several clones. Among these clones, mention may be made of the clones 35B7, 95B7, 100F11, 139F12, 157B11, 161A1, 161C12, 189H8, 196D9, 198E11, 251B4, 251C4 and 382A2 encoding respectively the enzymes SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26 and SEQ ID NO: 28.

2-Determination of the improvement of the cellobiohydrolase activity

2-1/ On the reduced cellodextrin substrate

In order to estimate the relative kcat of the variants selected at the L-shuffling first-round in comparison with the reference enzyme CBH2 (SEQ ID NO: 2), it is proceeded in the following manner:

- preparation of a stock culture of *Y. lipolytica* expressing a recombinant enzyme according to the invention overnight at 28°C;
- seeding of an expression medium with a volume of stock culture allowing having an optical density at 600 nm equal to 0.2 at the beginning of the culture;
- culturing of said cells at 28°C for 96 hours;
- centrifugation at 8000 rpm for 5 minutes;
- incubation of 100 µl of supernatant with 100 µl of 0.1 M citrate phosphate buffer at pH 6 containing 1% of reduced CDs for 4 hours at 35°C and 50°C;
- sampling of 100 µL of reaction;
- addition of 100 µL of DNS reactant;
- incubation for 5 minutes at 100°C;
- incubation for 3 minutes on ice;
- centrifugation for 10 minutes at 3000 rpm;
- reading of the optical density at 540 nm on 150 µL.

According to the invention, the calculation of the kcats is done in the following manner:

- plotting of the curve of the ODs at 540 nm as a function of the culture supernatant volume in the test;
- subtraction of the value of the negative control;
- division by the coefficient of the glucose sample (different amounts of glucose are revealed with the DNS);
- division by the reaction time (240 minutes).

Table 2 shows the values of the kcats as well as the improvement factors obtained for the clones 35B7, 95B7, 100F11, 139F12, 157B11, 161A1, 161C12, 189H8, 196D9, 198E11, 251B4, 251C4 and 382A2 encoding respectively the enzymes SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26 and SEQ ID NO: 28 in comparison with the reference protein CBH2 (SEQ ID NO: 2) in these experimental conditions.

TABLE 2: improvement of the cellobiohydrolase activity on reduced CDs

		35°C		50°C	
	Clone	**Kcat (min^{-1})**	**Improvement factor**	**Kcat (min^{-1})**	**Improvement factor**
First-round clones	**35B7**	0.166	3.8	0.2345	1.6
	95B7	0.287	6.6	0.715	4.8
	100F11	0.0508	1.2	0.1375	0.9
	139F12	0.1719	3.9	0.2328	1.6
	157B11	0.113	2.6	0.2061	1.4
	161A1	0.0577	1.3	0.1175	0.8
	161C12	0.1086	2.5	0.2162	1.4
	189H8	0.0872	2.0	0.1792	1.2
	196D9	0.1055	2.4	0.1969	1.3
	198E11	0.1218	2.8	0.1757	1.2
	251B4	0.0495	1.1	0.0865	0.6
	251C4	0.0623	1.4	0.1315	0.9

	382A2	0.315	7.2	0.552	3.7
Reference protein	**cbh2**	0.0436	1	0.1501	1

The results show an improvement of the enzyme activity in comparison with the reference enzyme CBH2 (SEQ ID No: 2) for the clones 35B7, 95B7, 100F11, 139F12, 157B11, 161A1, 161C12, 189H8, 196D9, 198E11, 251B4, 251C4 and 382A2, encoding respectively the enzymes SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26 and SEQ ID NO: 28 whether at 35°C or at 50°C.

2-2/ <u>On the Avicel substrate</u>

Afterwards, the improvement of the activity of the clones 35B7, 95B7, 100F11, 139F12, 157B11, 161A1, 161C12, 189H8, 196D9, 198E11, 251B4, 251C4 and 382A2, encoding respectively the enzymes SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26 and SEQ ID NO: 28 has been confirmed on a second substrate: Avicel.

The determination of the improvement of the activity on this substrate is performed by endpoint measurement according to the following protocol:

- preparation of a stock culture of *Y. lipolytica* expressing a recombinant enzyme according to the invention overnight at 28°C;
- seeding of an expression medium with a volume of stock culture allowing having an optical density at 600 nm equal to 0.2 at the beginning of the culture;
- culturing of said cells at 28°C for 96 hours;
- centrifugation at 8000 rpm for 5 minutes;
- incubation of 100 µl of supernatant with 100 µl of 0.1 M citrate phosphate buffer at pH 6 containing 1% of Avicel for 18 hours at 35 and 50°C;
- sampling of 100 µL of reaction;
- addition of 100 µL of DNS reactant;
- incubation for 5 minutes at 100°C;
- incubation for 3 minutes on ice;
- centrifugation for 10 minutes at 3000 rpm;
- reading of the optical density at 540 nm on 150 µL.

Table 3 shows the value of the ODs at 540 nm (after subtraction of the value of the negative control) as well as the improvement factor obtained for the clones 35B7, 95B7, 100F11, 139F12, 157B11, 161A1, 161C12, 189H8, 196D9, 198E11, 251B4, 251C4 and 382A2 in these experimental conditions.

TABLE 3: improvement of the cellobiohydrolase activity on Avicel

		35°C		50°C	
	Clone	**Delta OD 540 nm**	**Improvement factor**	**Delta OD 540 nm**	**Improvement factor**
First-round clones	**35B7**	0.0617	1.0	0.0948	0.8
	95B7	0.0396	0.6	0.0555	0.4
	100F11	0.038	0.6	0.0159	0.1
	139F12	0.06	0.9	0.0365	0.3
	157B11	0.0456	0.7	0.0319	0.3
	161A1	0.0508	0.8	0.0237	0.2
	161C12	0.0564	0.9	0.0595	0.5
	189H8	0.0676	1.0	0.0573	0.5
	196D9	0.0565	0.9	0.0874	0.7
	198E11	0.0867	1.3	0.0546	0.4
	251B4	0.0765	1.2	0.0622	0.5
	251C4	0.063	1.0	0.0889	0.7
	382A2	0.2476	3.8	0.2256	1.8
Reference protein	**cbh2**	0.0644	1	0.1252	1

The results of Table 3 show an improvement of the enzyme activity in comparison with the reference enzyme CBH2 (SEQ ID No: 2) at 35°C for the clones 198E11 and 251B4 (respectively SEQ ID No: 22 and 24) as well as an improvement of the enzyme activity in comparison with the reference enzyme CBH2 (SEQ ID No: 2) at 35°C and at 50°C for the clone 382A2 (SEQ ID No: 28).

SEQUENCE LISTING

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<220>
<223> Clone 100F11

<400> 8

Ala Pro Val Glu Glu Arg Gln Ser Cys Ser Asn Gly Val Trp Ser Gln
1 5 10 15

Cys Gly Gly Gln Asn Trp Ser Gly Thr Pro Cys Cys Thr Ser Gly Asn
20 25 30

Lys Cys Val Lys Val Asn Asp Phe Tyr Ser Gln Cys Gln Pro Gly Ser
35 40 45

Ala Asp Pro Ser Pro Thr Ser Thr Ile Val Ser Ala Thr Thr Thr Lys
50 55 60

Ala Thr Thr Thr Gly Ser Gly Gly Ser Val Thr Ser Pro Pro Pro Val
65 70 75 80

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

Tyr Arg Ser Glu Val Ser Thr Leu Ala Ile Pro Lys Leu Ser Gly Ala
100 105 110

Met Ala Thr Ala Ala Ala Lys Val Ala Asp Val Pro Ser Phe Gln Trp
115 120 125

Met Asp Thr Tyr Asp His Ile Ser Phe Met Glu Glu Ser Leu Ala Asp
130 135 140

Ile Arg Lys Ala Asn Lys Ala Gly Gly Asn Tyr Ala Gly Gln Phe Val
145 150 155 160

Val Tyr Asp Leu Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly
165 170 175

Glu Tyr Ser Leu Asp Lys Asp Gly Lys Asn Lys Tyr Lys Ala Tyr Ile
180 185 190

Ala Lys Ile Lys Gly Ile Leu Gln Asp Tyr Ser Asp Thr Arg Ile Ile
195 200 205

Leu Val Ile Glu Pro Asp Ser Leu Ala Asn Met Val Thr Asn Met Asn
210 215 220

Val Pro Lys Cys Ala Asn Ala Ala Ser Ala Tyr Lys Glu Leu Thr Ile
225 230 235 240

His Ala Leu Lys Glu Leu Asn Leu Pro Asn Val Ser Met Tyr Ile Asp
245 250 255

Ala Gly His Gly Gly Trp Leu Gly Trp Pro Ala Asn Leu Pro Pro Ala
260 265 270

Ala Gln Leu Tyr Gly Gln Leu Tyr Lys Asp Ala Gly Lys Pro Ser Arg
275 280 285

Leu Arg Gly Leu Val Thr Asn Val Ser Asn Tyr Asn Ala Trp Lys Leu
290 295 300

Ser Ser Lys Pro Asp Tyr Thr Glu Ser Asn Pro Asn Tyr Asp Glu Gln
305 310 315 320

Lys Tyr Ile His Ala Leu Ser Pro Leu Leu Glu Gln Glu Gly Trp Pro
325 330 335

Gly Ala Lys Phe Ile Val Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr
340 345 350

Gly Gln Lys Ala Trp Gly Asp Trp Cys Asn Ala Pro Gly Thr Gly Phe
355 360 365

Gly Ile Arg Pro Ser Ala Asn Thr Gly Asp Ser Leu Leu Asp Ala Phe
370 375 380

Val Trp Val Lys Pro Gly Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser
385 390 395 400

Ala Thr Arg Tyr Asp Tyr His Cys Gly Ala Ser Ala Ala Leu Gln Pro

405 410 415

Ala Pro Glu Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu
420 425 430

Thr Asn Ala Asn Pro Ser Phe Leu
435 440

<210> 9
<211> 1323
<212> DNA
<213> Artificial Sequence

<220>
<223> Clone 139F12

<400> 9
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aactggtccg gtactccttg ctgcaccagt ggaaacaagt gtgtcaaggt caacgacttc 120

tactcccagt gccagcctgg atctgcagac ccttctccca catccaccat tgtctccgcg 180

acgaccacca aggctactac tactggtagt ggaggctctg tcacctcgcc tcctcctgtt 240

gccaccaaca acccttttttc tggggtcgat ctgtgggcca ataactatta ccgctctgaa 300

gttagcactc tcgctattcc taagttgagc ggagccatgg ccactgctgc agcaaaggtc 360

gcagatgttc cctcttttca gtggatggat acttatgacc acatctcctt catggaggag 420

tctttggccg acatccgcaa ggccaacaag gctggcggta actatgccgg acagtttgtg 480

gtgtatgact tgccggatcg cgattgcgct gccgctgcct cgaatggcga atactctctt 540

gacaaggatg gcaagaacaa atataaggcc tatatcgcca agattaaggg tattctccag 600

gactattccg atacccggat cattctggtt attgagcctg actctcttgc caacatggtg 660

accaacatga acgtcccaaa gtgtgccaat gctgcttcag cctacaagga gctcaccatt 720

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gcctggaagc tatccagcaa gccagactac acggagagca accccaacta cgacgagcag 960

aagtacatcc acgctctgtc tcctcttctt gagcaggagg gctggcccgg tgccaagttc 1020

atcgtcgatc aaggtcgatc gggaaagcag cctaccggac agaaggcttg gggagactgg 1080

tgcaatgctc ccggcaccgg atttggtctc cgcccatccg caaacactgg ggacgccttg 1140

gtcgatgctt ttgtctgggt caagccaggc ggcgagtctg acggcaccag cgacaccagt 1200

gcggctcgat acgactacca ctgtggtgct tctgccgcct tgcaaccggc gcctgaggct 1260

ggtacctggt tccaagccta ctttgagcag cttctcaaga acgcaaaccc atcgttcctg 1320

taa 1323

<210> 10

<211> 440

<212> PRT

<213> Artificial Sequence

<220>

<223> Clone 139F12

<400> 10

Ala Pro Val Glu Glu Arg Gln Ser Cys Ser Asn Gly Val Trp Ser Gln
1 5 10 15

Cys Gly Gly Gln Asn Trp Ser Gly Thr Pro Cys Cys Thr Ser Gly Asn
20 25 30

Lys Cys Val Lys Val Asn Asp Phe Tyr Ser Gln Cys Gln Pro Gly Ser
35 40 45

Ala Asp Pro Ser Pro Thr Ser Thr Ile Val Ser Ala Thr Thr Thr Lys
50 55 60

Ala Thr Thr Thr Gly Ser Gly Gly Ser Val Thr Ser Pro Pro Pro Val
65 70 75 80

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

Tyr Arg Ser Glu Val Ser Thr Leu Ala Ile Pro Lys Leu Ser Gly Ala
100 105 110

Met Ala Thr Ala Ala Ala Lys Val Ala Asp Val Pro Ser Phe Gln Trp
115 120 125

Met Asp Thr Tyr Asp His Ile Ser Phe Met Glu Glu Ser Leu Ala Asp
130 135 140

Ile Arg Lys Ala Asn Lys Ala Gly Gly Asn Tyr Ala Gly Gln Phe Val
145 150 155 160

Val Tyr Asp Leu Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly
165 170 175

Glu Tyr Ser Leu Asp Lys Asp Gly Lys Asn Lys Tyr Lys Ala Tyr Ile
180 185 190

Ala Lys Ile Lys Gly Ile Leu Gln Asp Tyr Ser Asp Thr Arg Ile Ile
195 200 205

Leu Val Ile Glu Pro Asp Ser Leu Ala Asn Met Val Thr Asn Met Asn
210 215 220

Val Pro Lys Cys Ala Asn Ala Ala Ser Ala Tyr Lys Glu Leu Thr Ile
225 230 235 240

His Ala Leu Lys Glu Leu Asn Leu Pro Asn Val Ser Met Tyr Ile Asp
245 250 255

Ala Gly His Gly Gly Trp Leu Gly Trp Pro Ala Asn Leu Pro Pro Ala
260 265 270

Ala Gln Leu Tyr Gly Gln Leu Tyr Lys Asp Ala Gly Lys Pro Ser Arg
275 280 285

Leu Arg Gly Leu Val Thr Asn Val Ser Asn Tyr Asn Ala Trp Lys Leu
290 295 300

Ser Ser Lys Pro Asp Tyr Thr Glu Ser Asn Pro Asn Tyr Asp Glu Gln
305 310 315 320

Lys Tyr Ile His Ala Leu Ser Pro Leu Leu Glu Gln Glu Gly Trp Pro
325 330 335

Gly Ala Lys Phe Ile Val Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr
340 345 350

Gly Gln Lys Ala Trp Gly Asp Trp Cys Asn Ala Pro Gly Thr Gly Phe
355 360 365

Gly Leu Arg Pro Ser Ala Asn Thr Gly Asp Ala Leu Val Asp Ala Phe
370 375 380

Val Trp Val Lys Pro Gly Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser
385 390 395 400

Ala Ala Arg Tyr Asp Tyr His Cys Gly Ala Ser Ala Ala Leu Gln Pro
405 410 415

Ala Pro Glu Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu
420 425 430

Lys Asn Ala Asn Pro Ser Phe Leu
435 440

<210> 11
<211> 1323
<212> DNA
<213> Artificial Sequence

<220>
<223> Clone 157B11

<400> 11

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aactggtccg gtactccttg ctgcaccagt ggaaacaagt gtgtcaaggt caacgacttc 120

tactcccagt gccagcctgg atctgcagac ccttctccca catccaccat tgtctccgcg 180

acgaccacca aggctactac tactggtagt ggaggctctg tcacctcgcc tcctcctgtt 240

gccaccaaca acccttttttc tggggtcgat ctgtgggcca ataactatta ccgctctgaa 300

gttagcactc tcgctattcc taagttgagc ggagccatgg ccactgctgc agcaaaggtc 360

gcagatgttc cctcttttca gtggatggat acttatgacc acatctcctt catggaggag 420

tctttggccg acatccgcaa ggccaacaag gctggcggta actatgccgg acagtttgtg 480

gtgtatgact tgccggatcg cgattgcgct gccgccgcct cgaatggcga atactctctc 540

gacaatgatg gcgccaacaa atataaggcc tatatcgcca agattaaggg tattctccag 600

gactattccg atacccggat cattctggtt attgagcctg actctcttgc caacatggtg 660

accaacatga acgtcccaaa gtgtgccaat gctgcttcag cctacaagga gctcaccatt 720

cacgccctca aggagctgaa ccttccaaat gtttccatgt atatcgacgc tggccatggt 780

ggatggcttg gctggccggc aaaccttcct ccggccgctc agctatacgg tcagctctac 840

aaggatgcag gcaagccgtc tcgccttcgc ggattggtca ccaatgtctc caactacaac 900

gcctggaagc tatccagcaa gccagactac acggagagca accccaacta cgacgagcag 960

aagtacatcc acgctctgtc tcctcttctt gagcaggagg gctggcccgg tgccaagttc 1020

atcgtcgatc aaggtcgatc gggaaagcag cctaccggac agaaggcttg gggagactgg 1080

tgcaatgctc ccggcaccgg atttggtctc cgcccatccg caaacactgg ggactcgttg 1140

ctggatgctt ttgtctgggt caagccaggc ggcgagtctg acggcaccag cgacaccagt 1200

gcgacccgat acgactacca ctgtggtgct tctgccgcct tgcaaccggc gcctgaggct 1260

ggtacctggt tccaagccta ctttgagcag cttctcacaa acgcaaaccc atcgttcctg 1320

taa 1323

<210> 12
<211> 440
<212> PRT
<213> Artificial Sequence

<220>
<223> Clone 157B11

<400> 12

Ala Pro Val Glu Glu Arg Gln Ser Cys Ser Asn Gly Val Trp Ser Gln
1 5 10 15

Cys Gly Gly Gln Asn Trp Ser Gly Thr Pro Cys Cys Thr Ser Gly Asn
20 25 30

Lys Cys Val Lys Val Asn Asp Phe Tyr Ser Gln Cys Gln Pro Gly Ser

35 40 45

Ala Asp Pro Ser Pro Thr Ser Thr Ile Val Ser Ala Thr Thr Thr Lys
50 55 60

Ala Thr Thr Thr Gly Ser Gly Gly Ser Val Thr Ser Pro Pro Pro Val
65 70 75 80

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

Tyr Arg Ser Glu Val Ser Thr Leu Ala Ile Pro Lys Leu Ser Gly Ala
100 105 110

Met Ala Thr Ala Ala Ala Lys Val Ala Asp Val Pro Ser Phe Gln Trp
115 120 125

Met Asp Thr Tyr Asp His Ile Ser Phe Met Glu Glu Ser Leu Ala Asp
130 135 140

Ile Arg Lys Ala Asn Lys Ala Gly Gly Asn Tyr Ala Gly Gln Phe Val
145 150 155 160

Val Tyr Asp Leu Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly
165 170 175

Glu Tyr Ser Leu Asp Asn Asp Gly Ala Asn Lys Tyr Lys Ala Tyr Ile
180 185 190

Ala Lys Ile Lys Gly Ile Leu Gln Asp Tyr Ser Asp Thr Arg Ile Ile
195 200 205

Leu Val Ile Glu Pro Asp Ser Leu Ala Asn Met Val Thr Asn Met Asn
210 215 220

Val Pro Lys Cys Ala Asn Ala Ala Ser Ala Tyr Lys Glu Leu Thr Ile
225 230 235 240

His Ala Leu Lys Glu Leu Asn Leu Pro Asn Val Ser Met Tyr Ile Asp
245 250 255

Ala Gly His Gly Gly Trp Leu Gly Trp Pro Ala Asn Leu Pro Pro Ala
260 265 270

Ala Gln Leu Tyr Gly Gln Leu Tyr Lys Asp Ala Gly Lys Pro Ser Arg
275 280 285

Leu Arg Gly Leu Val Thr Asn Val Ser Asn Tyr Asn Ala Trp Lys Leu
290 295 300

Ser Ser Lys Pro Asp Tyr Thr Glu Ser Asn Pro Asn Tyr Asp Glu Gln
305 310 315 320

Lys Tyr Ile His Ala Leu Ser Pro Leu Leu Glu Gln Glu Gly Trp Pro
325 330 335

Gly Ala Lys Phe Ile Val Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr
340 345 350

Gly Gln Lys Ala Trp Gly Asp Trp Cys Asn Ala Pro Gly Thr Gly Phe
355 360 365

Gly Leu Arg Pro Ser Ala Asn Thr Gly Asp Ser Leu Leu Asp Ala Phe
370 375 380

Val Trp Val Lys Pro Gly Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser
385 390 395 400

Ala Thr Arg Tyr Asp Tyr His Cys Gly Ala Ser Ala Ala Leu Gln Pro
405 410 415

Ala Pro Glu Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu
420 425 430

Thr Asn Ala Asn Pro Ser Phe Leu
435 440

<210> 13
<211> 1323
<212> DNA
<213> Artificial Sequence

<220>
<223> Clone 161A1

<400> 13
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aactggtccg gtactccttg ctgcaccagt ggaaacaagt gtgtcaaggt caacgacttc 120

tactcccagt gccagcctgg atctgcagac ccttctccca catccaccat tgtctccgcg 180

acgaccacca aggctactac tactggtagt ggaggctctg tcacctcgcc tcctcctgtt 240

gccaccaaca acccttttc tggggtcgat ctgtgggcca ataactatta ccgctctgaa 300

gttagcactc tcgctattcc taagttgagc ggagccatgg ccactgctgc agcaaaggtc 360

gcagatgttc cctcttttca gtggatggat acttatgacc acatctcctt catggaggag 420

tctttggccg acatccgcaa ggccaacaag gctggcggta actatgccgg acagtttgtg 480

gtgtatgact tgccggatcg cgattgcgct gccgctgcct cgaatggcga atactctctt 540

gacaaggatg gcaagaacaa atataaggcc tatatcgcca agattaaggg tattctccag 600

gactattccg atacccggat cattctggtt attgagcctg actctcttgc caacatggtg 660

accaacatga acgtcccaaa gtgtgccaat gctgcttcag cctacaagga gctcaccatt 720

cacgccctca aggagctgaa ccttccaaat gtttccatgt atatcgacgc tggccatggt 780

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gcctggaagc tatccagcaa gccagactac acggagagca accccaacta cgacgagcag 960

aagtacatcc acgctctgtc tcctcttctt gagcaggagg gctggcccgg tgccaagttc 1020

atcgtcgatc aaggtcgatc gggaaagcag cctaccggac agaaggcttg gggagactgg 1080

tgcaatgctc ccggcaccgg atttggtatc cgcccatccg caaacactgg ggacgccttg 1140

gtcgatgctt ttgtctgggt caagccaggc ggcgagtctg acggcaccag cgacaccagt 1200

gcgacccgat acgactacca ctgtggtgct tctgccgcct tgcaaccggc gcctgaggct 1260

ggtacctggt tccaagccta ctttgagcag cttctcaaga acgcaaaccc atcgttcctg 1320

taa 1323

<210> 14

<211> 440

<212> PRT

<213> Artificial Sequence

<220>

<223> Clone 161A1

<400> 14

Ala Pro Val Glu Glu Arg Gln Ser Cys Ser Asn Gly Val Trp Ser Gln
1 5 10 15

Cys Gly Gly Gln Asn Trp Ser Gly Thr Pro Cys Cys Thr Ser Gly Asn
20 25 30

Lys Cys Val Lys Val Asn Asp Phe Tyr Ser Gln Cys Gln Pro Gly Ser
35 40 45

Ala Asp Pro Ser Pro Thr Ser Thr Ile Val Ser Ala Thr Thr Thr Lys
50 55 60

Ala Thr Thr Thr Gly Ser Gly Gly Ser Val Thr Ser Pro Pro Pro Val
65 70 75 80

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

Tyr Arg Ser Glu Val Ser Thr Leu Ala Ile Pro Lys Leu Ser Gly Ala
100 105 110

Met Ala Thr Ala Ala Ala Lys Val Ala Asp Val Pro Ser Phe Gln Trp

115 120 125

Met Asp Thr Tyr Asp His Ile Ser Phe Met Glu Glu Ser Leu Ala Asp
130 135 140

Ile Arg Lys Ala Asn Lys Ala Gly Gly Asn Tyr Ala Gly Gln Phe Val
145 150 155 160

Val Tyr Asp Leu Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly
165 170 175

Glu Tyr Ser Leu Asp Lys Asp Gly Lys Asn Lys Tyr Lys Ala Tyr Ile
180 185 190

Ala Lys Ile Lys Gly Ile Leu Gln Asp Tyr Ser Asp Thr Arg Ile Ile
195 200 205

Leu Val Ile Glu Pro Asp Ser Leu Ala Asn Met Val Thr Asn Met Asn
210 215 220

Val Pro Lys Cys Ala Asn Ala Ala Ser Ala Tyr Lys Glu Leu Thr Ile
225 230 235 240

His Ala Leu Lys Glu Leu Asn Leu Pro Asn Val Ser Met Tyr Ile Asp
245 250 255

Ala Gly His Gly Gly Trp Leu Gly Trp Pro Ala Asn Leu Pro Pro Ala
260 265 270

Ala Gln Leu Tyr Gly Gln Leu Tyr Lys Asp Ala Gly Lys Pro Ser Arg
275 280 285

Leu Arg Gly Leu Val Thr Asn Val Ser Asn Tyr Asn Ala Trp Lys Leu
290 295 300

Ser Ser Lys Pro Asp Tyr Thr Glu Ser Asn Pro Asn Tyr Asp Glu Gln
305 310 315 320

Lys Tyr Ile His Ala Leu Ser Pro Leu Leu Glu Gln Glu Gly Trp Pro
325 330 335

Gly Ala Lys Phe Ile Val Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr
340 345 350

Gly Gln Lys Ala Trp Gly Asp Trp Cys Asn Ala Pro Gly Thr Gly Phe
355 360 365

Gly Ile Arg Pro Ser Ala Asn Thr Gly Asp Ala Leu Val Asp Ala Phe
370 375 380

Val Trp Val Lys Pro Gly Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser
385 390 395 400

Ala Thr Arg Tyr Asp Tyr His Cys Gly Ala Ser Ala Ala Leu Gln Pro
405 410 415

Ala Pro Glu Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu
420 425 430

Lys Asn Ala Asn Pro Ser Phe Leu
435 440

<210> 15
<211> 1362
<212> DNA
<213> Artificial Sequence

<220>
<223> Clone 161C12

<400> 15
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tggtcgggtc cgacttgctg tgcttccgga agcacatgcg tctactccaa cgactattac 120

tcccagtgtc ttcccggcgc tgcaagctca agctcgtcca cgcgcgccgc gtcgacgact 180

tctcgagtat cccccacaac atcccggtcg agctccgcga cgcctccacc tggttctact 240

actaccagag tacctccagt cggatcggga accgctacgt attcaggcaa ccctttgtt 300

ggggtcactc cttgggccaa tgcatattac gcctctgaag ttagcagcct cgctattcct 360

agcttgactg gagccatggc cactgctgca gcagctgtcg caaaggttcc ctcttttatg 420

tggctagata ctcttgacaa gacccctctc atggagcaaa ccttggccga catccgcacc 480

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gattgcgctg cccttgcctc gaatggcgaa tactctctcg acaatgatgg cgccaacaaa 600

tataagaact atatccaaac cattaagaag attatccaga gctattccga tatccggata 660

ctcctggtta ttgagcctga ctctcttgcc aacctggtga ccaacatgga tgtcgccaag 720

tgtgccaagg ctcatgatgc ctacatcagc ctgacgaact acgccgtcac agaactgaac 780

cttccaaatg ttgcgatgta tttggacgct ggccatgcag gatggcttgg ctggccggca 840

aaccttcctc cggccgctca gctatacggt cagctctaca aggatgcagg caagccgtct 900

cgccttcgcg gattggtcac caatgtctcc aactacaacg cctggaagct atccagcaag 960

ccagactaca cggagagcaa ccccaactac gacgagcaga agtacatcca cgctctgtct 1020

cctcttcttg agcaggaggg ctggcccggt gccaagttca tcgtcgatca aggtcgatcg 1080

ggaaagcagc ctaccggaca gaaggcttgg ggagactggt gcaatgctcc cggcaccgga 1140

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tgtggtattg acggcgccgt caagccggcg cctgaggctg gtacctggtt ccaagcctac 1320

tttgagcagc ttctcaagaa cgcaaaccca tcgttcctgt aa 1362

<210> 16
<211> 453
<212> PRT
<213> Artificial Sequence

<220>
<223> Clone 161C12

<400> 16

Val Pro Leu Glu Glu Arg Gln Ala Cys Ser Ser Val Trp Gly Gln Cys
1 5 10 15

Gly Gly Gln Asn Trp Ser Gly Pro Thr Cys Cys Ala Ser Gly Ser Thr
20 25 30

Cys Val Tyr Ser Asn Asp Tyr Tyr Ser Gln Cys Leu Pro Gly Ala Ala
35 40 45

Ser Ser Ser Ser Ser Thr Arg Ala Ala Ser Thr Thr Ser Arg Val Ser
50 55 60

Pro Thr Thr Ser Arg Ser Ser Ser Ala Thr Pro Pro Pro Gly Ser Thr
65 70 75 80

Thr Thr Arg Val Pro Pro Val Gly Ser Gly Thr Ala Thr Tyr Ser Gly
85 90 95

Asn Pro Phe Val Gly Val Thr Pro Trp Ala Asn Ala Tyr Tyr Ala Ser
100 105 110

Glu Val Ser Ser Leu Ala Ile Pro Ser Leu Thr Gly Ala Met Ala Thr
115 120 125

Ala Ala Ala Ala Val Ala Lys Val Pro Ser Phe Met Trp Leu Asp Thr
130 135 140

Leu Asp Lys Thr Pro Leu Met Glu Gln Thr Leu Ala Asp Ile Arg Thr
145 150 155 160

Ala Asn Lys Asn Gly Gly Asn Tyr Ala Gly Gln Phe Val Val Tyr Asp
165 170 175

Leu Pro Asp Arg Asp Cys Ala Ala Leu Ala Ser Asn Gly Glu Tyr Ser
180 185 190

Leu Asp Asn Asp Gly Ala Asn Lys Tyr Lys Asn Tyr Ile Gln Thr Ile

195 200 205

Lys Lys Ile Ile Gln Ser Tyr Ser Asp Ile Arg Ile Leu Leu Val Ile
210 215 220

Glu Pro Asp Ser Leu Ala Asn Leu Val Thr Asn Met Asp Val Ala Lys
225 230 235 240

Cys Ala Lys Ala His Asp Ala Tyr Ile Ser Leu Thr Asn Tyr Ala Val
245 250 255

Thr Glu Leu Asn Leu Pro Asn Val Ala Met Tyr Leu Asp Ala Gly His
260 265 270

Ala Gly Trp Leu Gly Trp Pro Ala Asn Leu Pro Pro Ala Ala Gln Leu
275 280 285

Tyr Gly Gln Leu Tyr Lys Asp Ala Gly Lys Pro Ser Arg Leu Arg Gly
290 295 300

Leu Val Thr Asn Val Ser Asn Tyr Asn Ala Trp Lys Leu Ser Ser Lys
305 310 315 320

Pro Asp Tyr Thr Glu Ser Asn Pro Asn Tyr Asp Glu Gln Lys Tyr Ile
325 330 335

His Ala Leu Ser Pro Leu Leu Glu Gln Glu Gly Trp Pro Gly Ala Lys
340 345 350

Phe Ile Val Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr Gly Gln Lys
355 360 365

Ala Trp Gly Asp Trp Cys Asn Ala Pro Gly Thr Gly Phe Gly Leu Arg
370 375 380

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

Lys Pro Gly Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser Ala Ala Arg
405 410 415

Tyr Asp Tyr His Cys Gly Ile Asp Gly Ala Val Lys Pro Ala Pro Glu
420 425 430

Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu Lys Asn Ala
435 440 445

Asn Pro Ser Phe Leu
450

<210> 17

<211> 1323

<212> DNA

<213> Artificial Sequence

<220>

<223> Clone 189H8

<400> 17

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aactggtccg gtactccttg ctgcaccagt ggaaacaagt gtgtcaaggt caacgacttc 120

tactcccagt gccagcctgg atctgcagac ccttctccca catccaccat tgtctccgcg 180

acgaccacca aggctactac tactggtagt ggaggctctg tcacctcgcc tcctcctgtt 240

gccaccaaca acccttttttc tggggtcgat ctgtgggcca ataactatta ccgctctgaa 300

gttagcactc tcgctattcc taagttgagc ggagccatgg ccactgctgc agcaaaggtc 360

gcagatgttc cctcttttca gtggatggat acttatgacc acatctccct catggaggac 420

accttggtcg acatccgcaa ggccaacctg gctggcggta actatgccgg acagtttgtg 480

gtgtatgact tgccggatcg cgattgcgct gccgctgcct cgaatggcga atactctctt 540

gacaaggatg gcaagaacaa atataaggcc tatatcgcca agattaaggg tattctccag 600

gactattccg atacccggat cattctggtt attgagcctg actctcttgc caacatggtg 660

accaacatga acgtcccaaa gtgtgccaat gctgcttcag cctacaagga gctcaccatt 720

cacgccctca aggagctgaa ccttccaaat gtttccatgt atatcgacgc tggccatggt 780

ggatggcttg gctggccggc aaaccaaggc ccggccgcta aactatttgc aagcatctac 840

aaggatgcag gcaagccggc cgctcttcgc ggattggcaa ccaatgtcgc caactacaac 900

gcctggagcc tcagcagcgc tccaccttac acgcaaggcg cctctatcta cgacgagaag 960

agcttcatcc acgctatggg acctcttctt gagcagaatg gctggcctgg tgccaagttc 1020

atcgtcgatc aaggtcgatc gggaaagcag cctaccggac agaaggcttg gggagactgg 1080

tgcaatgctc ccggcaccgg atttggtctc cgcccatccg caaacactgg ggactcgttg 1140

ctggatgctt ttgtctgggt caagccaggc ggcgagtctg acggcaccag cgacaccagt 1200

gcgacccgat acgactacca ctgtggtgct tctgccgcct tgcaaccggc gcctgaggct 1260

ggtacctggt tccaagccta ctttgagcag cttctcacaa acgcaaaccc atcgttcctg 1320

taa 1323

<210> 18
<211> 440
<212> PRT
<213> Artificial Sequence

<220>
<223> Clone 189H8

<400> 18

Ala Pro Val Glu Glu Arg Gln Ser Cys Ser Asn Gly Val Trp Ser Gln
1 5 10 15

Cys Gly Gly Gln Asn Trp Ser Gly Thr Pro Cys Cys Thr Ser Gly Asn
20 25 30

Lys Cys Val Lys Val Asn Asp Phe Tyr Ser Gln Cys Gln Pro Gly Ser
35 40 45

Ala Asp Pro Ser Pro Thr Ser Thr Ile Val Ser Ala Thr Thr Thr Lys
50 55 60

Ala Thr Thr Thr Gly Ser Gly Gly Ser Val Thr Ser Pro Pro Pro Val
65 70 75 80

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

Tyr Arg Ser Glu Val Ser Thr Leu Ala Ile Pro Lys Leu Ser Gly Ala
100 105 110

Met Ala Thr Ala Ala Ala Lys Val Ala Asp Val Pro Ser Phe Gln Trp
115 120 125

Met Asp Thr Tyr Asp His Ile Ser Leu Met Glu Asp Thr Leu Val Asp
130 135 140

Ile Arg Lys Ala Asn Leu Ala Gly Gly Asn Tyr Ala Gly Gln Phe Val
145 150 155 160

Val Tyr Asp Leu Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly
165 170 175

Glu Tyr Ser Leu Asp Lys Asp Gly Lys Asn Lys Tyr Lys Ala Tyr Ile
180 185 190

Ala Lys Ile Lys Gly Ile Leu Gln Asp Tyr Ser Asp Thr Arg Ile Ile
195 200 205

Leu Val Ile Glu Pro Asp Ser Leu Ala Asn Met Val Thr Asn Met Asn
210 215 220

Val Pro Lys Cys Ala Asn Ala Ala Ser Ala Tyr Lys Glu Leu Thr Ile
225 230 235 240

His Ala Leu Lys Glu Leu Asn Leu Pro Asn Val Ser Met Tyr Ile Asp
245 250 255

Ala Gly His Gly Gly Trp Leu Gly Trp Pro Ala Asn Gln Gly Pro Ala

260 265 270

Ala Lys Leu Phe Ala Ser Ile Tyr Lys Asp Ala Gly Lys Pro Ala Ala
275 280 285

Leu Arg Gly Leu Ala Thr Asn Val Ala Asn Tyr Asn Ala Trp Ser Leu
290 295 300

Ser Ser Ala Pro Pro Tyr Thr Gln Gly Ala Ser Ile Tyr Asp Glu Lys
305 310 315 320

Ser Phe Ile His Ala Met Gly Pro Leu Leu Glu Gln Asn Gly Trp Pro
325 330 335

Gly Ala Lys Phe Ile Val Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr
340 345 350

Gly Gln Lys Ala Trp Gly Asp Trp Cys Asn Ala Pro Gly Thr Gly Phe
355 360 365

Gly Leu Arg Pro Ser Ala Asn Thr Gly Asp Ser Leu Leu Asp Ala Phe
370 375 380

Val Trp Val Lys Pro Gly Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser
385 390 395 400

Ala Thr Arg Tyr Asp Tyr His Cys Gly Ala Ser Ala Ala Leu Gln Pro
405 410 415

Ala Pro Glu Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu
420 425 430

Thr Asn Ala Asn Pro Ser Phe Leu
435 440

<210> 19
<211> 1362
<212> DNA
<213> Artificial Sequence

<220>
<223> Clone 196D9

<400> 19
gtgcctctag aggagcggca agcttgctca agcgtctggg gccaatgtgg tggccagaat 60

tggtcgggtc cgacttgctg tgcttccgga agcacatgcg tctactccaa cgactattac 120

tcccagtgtc ttcccggcgc tgcaagctca agctcgtcca cgcgcgccgc gtcgacgact 180

tctcgagtat cccccacaac atcccggtcg agctccgcga cgcctccacc tggttctact 240

actaccagag tacctccagt cggatcggga accgctacgt attcaggcaa ccctttgtt 300

ggggtcactc cttgggccaa tgcatattac gcctctgaag ttagcagcct cgctattcct 360

agcttgactg gagccatggc cactgctgca gcagctgtcg caaaggttcc ctcttttatg 420

tggctagata ctcttgacaa gacccctctc atggagcaaa ccttggccga catccgcacc 480

gccaacaaga atggcggtaa ctatgccgga cagtttgtgg tgtatgactt gccggatcgc 540

gattgcgctg cccttgcctc gaatggcgaa tactctattg ccgatggtgg cgtcgccaaa 600

tataagaact atatcgacac cattcgtcaa attgtcgtgg aatattccga tatccggacc 660

ctcctggtta ttgagcctga ctctcttacc aacctggtga ccaacatgaa cgtcccaaag 720

tgtgccaatg ctcagtcagc ctaccttgag tgcatcaact acgccgtcac acagctgaac 780

cttccaaatg ttgcgatgta tttggacgct ggccatgcag gatggcttgg ctggccggca 840

aaccaagacc cggccgctca gctatttgca aatgtttaca agaatgcatc gtctccgaga 900

gctcttcgcg gattggcaac caatgtcgcc aactacaacg ggtggaacat taccagcccc 960

ccatcgtaca cgcaaggcaa cgctgtctac aacgagaagc tgtacatcca cgctattgga 1020

cctcttcttg ccaatcacgg ctggtccaac gccttcttca tcactgatca aggtcgatcg 1080

ggaaagcagc ctaccggaca gcaacagtgg ggagactggt gcaatgctcc cggcaccgga 1140

tttggtctcc gcccatccgc aaacactggg gacgccttgg tcgatgcttt tgtctgggtc 1200

aagccaggcg gcgagtctga cggcaccagc gacaccagtg cggctcgata cgactaccac 1260

tgtggtattg acggcgccgt caagccggcg cctgaggctg gtacctggtt ccaagcctac 1320

tttgagcagc ttctcaagaa cgcaaaccca tcgttcctgt aa 1362

<210> 20

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<223> Clone 196D9

<400> 20

Val Pro Leu Glu Glu Arg Gln Ala Cys Ser Ser Val Trp Gly Gln Cys
1 5 10 15

Gly Gly Gln Asn Trp Ser Gly Pro Thr Cys Cys Ala Ser Gly Ser Thr
20 25 30

Cys Val Tyr Ser Asn Asp Tyr Tyr Ser Gln Cys Leu Pro Gly Ala Ala
35 40 45

Ser Ser Ser Ser Ser Thr Arg Ala Ala Ser Thr Thr Ser Arg Val Ser
50 55 60

Pro Thr Thr Ser Arg Ser Ser Ser Ala Thr Pro Pro Pro Gly Ser Thr
65 70 75 80

Thr Thr Arg Val Pro Pro Val Gly Ser Gly Thr Ala Thr Tyr Ser Gly
85 90 95

Asn Pro Phe Val Gly Val Thr Pro Trp Ala Asn Ala Tyr Tyr Ala Ser
100 105 110

Glu Val Ser Ser Leu Ala Ile Pro Ser Leu Thr Gly Ala Met Ala Thr
115 120 125

Ala Ala Ala Ala Val Ala Lys Val Pro Ser Phe Met Trp Leu Asp Thr
130 135 140

Leu Asp Lys Thr Pro Leu Met Glu Gln Thr Leu Ala Asp Ile Arg Thr
145 150 155 160

Ala Asn Lys Asn Gly Gly Asn Tyr Ala Gly Gln Phe Val Val Tyr Asp
165 170 175

Leu Pro Asp Arg Asp Cys Ala Ala Leu Ala Ser Asn Gly Glu Tyr Ser
180 185 190

Ile Ala Asp Gly Gly Val Ala Lys Tyr Lys Asn Tyr Ile Asp Thr Ile
195 200 205

Arg Gln Ile Val Val Glu Tyr Ser Asp Ile Arg Thr Leu Leu Val Ile
210 215 220

Glu Pro Asp Ser Leu Thr Asn Leu Val Thr Asn Met Asn Val Pro Lys
225 230 235 240

Cys Ala Asn Ala Gln Ser Ala Tyr Leu Glu Cys Ile Asn Tyr Ala Val
245 250 255

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

Ala Gly Trp Leu Gly Trp Pro Ala Asn Gln Asp Pro Ala Ala Gln Leu
275 280 285

Phe Ala Asn Val Tyr Lys Asn Ala Ser Ser Pro Arg Ala Leu Arg Gly
290 295 300

Leu Ala Thr Asn Val Ala Asn Tyr Asn Gly Trp Asn Ile Thr Ser Pro
305 310 315 320

Pro Ser Tyr Thr Gln Gly Asn Ala Val Tyr Asn Glu Lys Leu Tyr Ile
325 330 335

His Ala Ile Gly Pro Leu Leu Ala Asn His Gly Trp Ser Asn Ala Phe

340 345 350

Phe Ile Thr Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr Gly Gln Gln
355 360 365

Gln Trp Gly Asp Trp Cys Asn Ala Pro Gly Thr Gly Phe Gly Leu Arg
370 375 380

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

Lys Pro Gly Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser Ala Ala Arg
405 410 415

Tyr Asp Tyr His Cys Gly Ile Asp Gly Ala Val Lys Pro Ala Pro Glu
420 425 430

Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu Lys Asn Ala
435 440 445

Asn Pro Ser Phe Leu
450

<210> 21
<211> 1362

<212> DNA

<213> Artificial Sequence

<220>

<223> Clone 198E11

<400> 21

gtgcctctag aggagcggca agcttgctca agcgtctggg gccaatgtgg tggccagaat 60

tggtcgggtc cgacttgctg tgcttccgga agcacatgcg tctactccaa cgactattac 120

tcccagtgtc ttcccggcgc tgcaagctca agctcgtcca cgcgcgccgc gtcgacgact 180

tctcgagtat cccccacaac atcccggtcg agctccgcga cgcctccacc tggttctact 240

actaccagag tacctccagt cggatcggga accgctacgt attcaggcaa ccctttttgtt 300

ggggtcactc cttgggccaa tgcatattac gcctctgaag ttagcagcct cgctattcct 360

agcttgactg gagccatggc cactgctgca gcagctgtcg caaaggttcc ctcttttatg 420

tggctagata ctcttgacaa gacccctctc atggagcaaa ccttggccga catccgcacc 480

gccaacaaga atggcggtaa ctatgccgga cagtttgtgg tgtatgactt gccggatcgc 540

gattgcgctg ccgctgcctc gaatggcgaa tactctcttg acaaggatgg caagaacaaa 600

tataaggcct atatcgccaa gattaagggt attctccagg actattccga tacccggatc 660

attctggtta ttgagcctga ctctcttgcc aacatggtga ccaacatgaa cgtcccaaag 720

tgtgccaatg ctgcttcagc ctacaaggag ctcaccattc acgccctcaa ggagctgaac 780

cttccaaatg tttccatgta tatcgacgct ggccatggtg gatggcttgg ctggccggca 840

aaccttcctc cggccgctca gctatacggt cagctctaca aggatgcagg caagccgtct 900

cgccttcgcg gattggtcac caatgtctcc aactacaacg cctggaagct atccagcaag 960

ccagactaca cggagagcaa ccccaactac gacgagcaga agtacatcca cgctctgtct 1020

cctcttcttg agcaggaggg ctggcccggt gccaagttca tcgtcgatca aggtcgatcg 1080

ggaaagcagc ctaccggaca gaaggcttgg ggagactggt gcaatgtgat cggcaccgga 1140

tttggtattc gcccatccgc aaacactggg gactcgttgc tggattcgtt tgtctgggtc 1200

aagccaggcg gcgagtctga cggcaccagc gacaccagtg cgacccgata cgactaccac 1260

tgtggtgctt ctgccgcctt gcaaccggcg cctgaggctg gtacctggtt ccaagcctac 1320

tttgagcagc ttctcacaaa cgcaaaccca tcgttcctgt aa 1362

<210> 22
<211> 453
<212> PRT
<213> Artificial Sequence

<220>
<223> Clone 198E11

<400> 22

Val Pro Leu Glu Glu Arg Gln Ala Cys Ser Ser Val Trp Gly Gln Cys
1 5 10 15

Gly Gly Gln Asn Trp Ser Gly Pro Thr Cys Cys Ala Ser Gly Ser Thr
20 25 30

Cys Val Tyr Ser Asn Asp Tyr Tyr Ser Gln Cys Leu Pro Gly Ala Ala
35 40 45

Ser Ser Ser Ser Ser Thr Arg Ala Ala Ser Thr Thr Ser Arg Val Ser
50 55 60

Pro Thr Thr Ser Arg Ser Ser Ser Ala Thr Pro Pro Pro Gly Ser Thr
65 70 75 80

Thr Thr Arg Val Pro Pro Val Gly Ser Gly Thr Ala Thr Tyr Ser Gly
85 90 95

Asn Pro Phe Val Gly Val Thr Pro Trp Ala Asn Ala Tyr Tyr Ala Ser
100 105 110

Glu Val Ser Ser Leu Ala Ile Pro Ser Leu Thr Gly Ala Met Ala Thr
115 120 125

Ala Ala Ala Ala Val Ala Lys Val Pro Ser Phe Met Trp Leu Asp Thr
130 135 140

Leu Asp Lys Thr Pro Leu Met Glu Gln Thr Leu Ala Asp Ile Arg Thr
145 150 155 160

Ala Asn Lys Asn Gly Gly Asn Tyr Ala Gly Gln Phe Val Val Tyr Asp
165 170 175

Leu Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly Glu Tyr Ser
180 185 190

Leu Asp Lys Asp Gly Lys Asn Lys Tyr Lys Ala Tyr Ile Ala Lys Ile
195 200 205

Lys Gly Ile Leu Gln Asp Tyr Ser Asp Thr Arg Ile Ile Leu Val Ile
210 215 220

Glu Pro Asp Ser Leu Ala Asn Met Val Thr Asn Met Asn Val Pro Lys
225 230 235 240

Cys Ala Asn Ala Ala Ser Ala Tyr Lys Glu Leu Thr Ile His Ala Leu
245 250 255

Lys Glu Leu Asn Leu Pro Asn Val Ser Met Tyr Ile Asp Ala Gly His
260 265 270

Gly Gly Trp Leu Gly Trp Pro Ala Asn Leu Pro Pro Ala Ala Gln Leu
275 280 285

Tyr Gly Gln Leu Tyr Lys Asp Ala Gly Lys Pro Ser Arg Leu Arg Gly
290 295 300

Leu Val Thr Asn Val Ser Asn Tyr Asn Ala Trp Lys Leu Ser Ser Lys
305 310 315 320

Pro Asp Tyr Thr Glu Ser Asn Pro Asn Tyr Asp Glu Gln Lys Tyr Ile
325 330 335

His Ala Leu Ser Pro Leu Leu Glu Gln Glu Gly Trp Pro Gly Ala Lys
340 345 350

Phe Ile Val Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr Gly Gln Lys
355 360 365

Ala Trp Gly Asp Trp Cys Asn Val Ile Gly Thr Gly Phe Gly Ile Arg
370 375 380

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

Lys Pro Gly Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser Ala Thr Arg

405 410 415

Tyr Asp Tyr His Cys Gly Ala Ser Ala Ala Leu Gln Pro Ala Pro Glu
420 425 430

Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu Thr Asn Ala
435 440 445

Asn Pro Ser Phe Leu
450

<210> 23
<211> 1323
<212> DNA
<213> Artificial Sequence

<220>
<223> Clone 251B4

<400> 23
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aactggtccg gtactccttg ctgcaccagt ggaaacaagt gtgtcaaggt caacgacttc 120

tactcccagt gccagcctgg atctgcagac ccttctccca catccaccat tgtctccgcg 180

acgaccacca aggctactac tactggtagt ggaggctctg tcacctcgcc tcctcctgtt 240

gccaccaaca acccttttc tggggtcgat ctgtgggcca ataactatta ccgctctgaa 300

gttagcactc tcgctattcc taagttgagc ggagccatgg ccactgctgc agcaaaggtc 360

gcagatgttc cctcttttca gtggatggat acttatgacc acatctcctt catggaggag 420

tctttggccg acatccgcac cgccaacaag aatggcggta actatgccgg acagtttgtg 480

gtgtatgact tgccggatcg cgattgcgct gccgccgcct cgaatggcga atactctctc 540

gacaatgatg gcgccaacaa atataagaac tatatccaaa ccattaagaa gattatccag 600

agctattccg atatccggat actcctggtt attgagcctg actctcttgc caacctggtg 660

accaacatgg atgtcgccaa gtgtgccaag gctcatgatg cctacatcag cctgacgaac 720

tacgccgtca cagaactgaa ccttccaaat gttgcgatgt atttggacgc tggccatgca 780

ggatggcttg gctggccggc aaaccaaggc ccggccgcta aactatttgc aagcatctac 840

aaggatgcag gcaagccggc cgctcttcgc ggattggcaa ccaatgtcgc caactacaac 900

gcctggaagc tatccagcaa gccagactac acggagagca accccaacta cgacgagcag 960

aagtacatcc acgctctgtc tcctcttctt gagcaggagg gctggcccgg tgccaagttc 1020

atcgtcgatc aaggtcgatc gggaaagcag cctaccggac agaaggcttg gggagactgg 1080

tgcaatgctc ccggcaccgg atttggtctc cgcccatccg caaacactgg ggacgccttg 1140

gtcgatgctt ttgtctgggt caagccaggc ggcgagtgtg acggcaccag cgacaccagt 1200

gcggctcgat acgactacca ctgtggtatt gacggcgccg tcaagccggc gcctgaggct 1260

ggtacctggt tccaagccta ctttgagcag cttctcacaa acgcaaaccc atcgttcctg 1320

taa 1323

<210> 24
<211> 440
<212> PRT
<213> Artificial Sequence

<220>
<223> Clone 251B4

<400> 24

Ala Pro Val Glu Glu Arg Gln Ser Cys Ser Asn Gly Val Trp Ser Gln
1 5 10 15

Cys Gly Gly Gln Asn Trp Ser Gly Thr Pro Cys Cys Thr Ser Gly Asn
20 25 30

Lys Cys Val Lys Val Asn Asp Phe Tyr Ser Gln Cys Gln Pro Gly Ser
35 40 45

Ala Asp Pro Ser Pro Thr Ser Thr Ile Val Ser Ala Thr Thr Thr Lys
50 55 60

Ala Thr Thr Thr Gly Ser Gly Gly Ser Val Thr Ser Pro Pro Pro Val
65 70 75 80

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

Tyr Arg Ser Glu Val Ser Thr Leu Ala Ile Pro Lys Leu Ser Gly Ala
100 105 110

Met Ala Thr Ala Ala Ala Lys Val Ala Asp Val Pro Ser Phe Gln Trp
115 120 125

Met Asp Thr Tyr Asp His Ile Ser Phe Met Glu Glu Ser Leu Ala Asp
130 135 140

Ile Arg Thr Ala Asn Lys Asn Gly Gly Asn Tyr Ala Gly Gln Phe Val
145 150 155 160

Val Tyr Asp Leu Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly
165 170 175

Glu Tyr Ser Leu Asp Asn Asp Gly Ala Asn Lys Tyr Lys Asn Tyr Ile
180 185 190

Gln Thr Ile Lys Lys Ile Ile Gln Ser Tyr Ser Asp Ile Arg Ile Leu
195 200 205

Leu Val Ile Glu Pro Asp Ser Leu Ala Asn Leu Val Thr Asn Met Asp
210 215 220

Val Ala Lys Cys Ala Lys Ala His Asp Ala Tyr Ile Ser Leu Thr Asn
225 230 235 240

Tyr Ala Val Thr Glu Leu Asn Leu Pro Asn Val Ala Met Tyr Leu Asp
245 250 255

Ala Gly His Ala Gly Trp Leu Gly Trp Pro Ala Asn Gln Gly Pro Ala
260 265 270

Ala Lys Leu Phe Ala Ser Ile Tyr Lys Asp Ala Gly Lys Pro Ala Ala
275 280 285

Leu Arg Gly Leu Ala Thr Asn Val Ala Asn Tyr Asn Ala Trp Lys Leu
290 295 300

Ser Ser Lys Pro Asp Tyr Thr Glu Ser Asn Pro Asn Tyr Asp Glu Gln
305 310 315 320

Lys Tyr Ile His Ala Leu Ser Pro Leu Leu Glu Gln Glu Gly Trp Pro
325 330 335

Gly Ala Lys Phe Ile Val Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr
340 345 350

Gly Gln Lys Ala Trp Gly Asp Trp Cys Asn Ala Pro Gly Thr Gly Phe
355 360 365

Gly Leu Arg Pro Ser Ala Asn Thr Gly Asp Ala Leu Val Asp Ala Phe
370 375 380

Val Trp Val Lys Pro Gly Gly Glu Cys Asp Gly Thr Ser Asp Thr Ser
385 390 395 400

Ala Ala Arg Tyr Asp Tyr His Cys Gly Ile Asp Gly Ala Val Lys Pro
405 410 415

Ala Pro Glu Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu
420 425 430

Thr Asn Ala Asn Pro Ser Phe Leu
435 440

<210> 25
<211> 1362
<212> DNA
<213> Artificial Sequence

<220>

<223> Clone 251C4

<400> 25

gtgcctctag aggagcggca agcttgctca agcgtctggg gccaatgtgg tggccagaat 60

tggtcgggtc cgacttgctg tgcttccgga agcacatgcg tctactccaa cgactattac 120

tcccagtgtc ttcccggcgc tgcaagctca agctcgtcca cgcgcgccgc gtcgacgact 180

tctcgagtat cccccacaac atcccggtcg agctccgcga cgcctccacc tggttctact 240

actaccagag tacctccagt cggatcggga accgctacgt attcaggcaa ccctttgtt 300

ggggtcactc cttgggccaa tgcatattac gcctctgaag ttagcagcct cgctattcct 360

agcttgactg gagccatggc cactgctgca gcagctgtcg caaaggttcc ctcttttatg 420

tggctagata ctcttgacaa gacccctctc atggagcaaa ccttggccga catccgcacc 480

gccaacaaga atggcggtaa ctatgccgga cagtttgtgg tgtatgactt gccggatcgc 540

gattgcgctg ccgctgcctc gaatggcgaa tactctattg ccgatggtgg cgtcgccaaa 600

tataagaact atatcgacac cattcgtcaa attgtcgtgg aatattccga tatccggacc 660

ctcctggtta ttgagcctga ctctcttgcc aacctggtga ccaacctcgg tactccaaag 720

tgtgccaatg ctcagtcagc ctaccttgag tgcatcaact acgccgtcac acagctgaac 780

cttccaaatg ttgcgatgta tttggacgct ggccatgcag gatggcttgg ctggccggca 840

aaccaagacc cggccgctca gctatttgca aatgtttaca agaatgcatc gtctccgaga 900

gctcttcgcg gattggcaac caatgtcgcc aactacaacg ggtggaacat taccagcccc 960

ccatcgtaca cgcaaggcaa cgctgtctac aacgagaagc tgtacatcca cgctattgga 1020

cctcttcttg ccaatcacgg ctggtccaac gccttcttca tcactgatca aggtcgatcg 1080

ggaaagcagc ctaccggaca gcaacagtgg ggagactggt gcaatgtgat cggcaccgga 1140

tttggtattc gcccatccgc aaacactggg gactcgttgc tggattcgtt tgtctgggtc 1200

aagccaggcg gcgagtgtga cggcaccagc gacaccagtg cgccacgatt tgactcccac 1260

tgtgcgctcc cagatgcctt gcaaccggcg cctgaggctg gtacctggtt ccaagcctac 1320

tttgagcagc ttctcacaaa cgcaaaccca tcgttcctgt aa 1362

<210> 26

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<223> Clone 251C4

<400> 26

Val Pro Leu Glu Glu Arg Gln Ala Cys Ser Ser Val Trp Gly Gln Cys
1 5 10 15

Gly Gly Gln Asn Trp Ser Gly Pro Thr Cys Cys Ala Ser Gly Ser Thr

20 25 30

Cys Val Tyr Ser Asn Asp Tyr Tyr Ser Gln Cys Leu Pro Gly Ala Ala
35 40 45

Ser Ser Ser Ser Ser Thr Arg Ala Ala Ser Thr Thr Ser Arg Val Ser
50 55 60

Pro Thr Thr Ser Arg Ser Ser Ser Ala Thr Pro Pro Pro Gly Ser Thr
65 70 75 80

Thr Thr Arg Val Pro Pro Val Gly Ser Gly Thr Ala Thr Tyr Ser Gly
85 90 95

Asn Pro Phe Val Gly Val Thr Pro Trp Ala Asn Ala Tyr Tyr Ala Ser
100 105 110

Glu Val Ser Ser Leu Ala Ile Pro Ser Leu Thr Gly Ala Met Ala Thr
115 120 125

Ala Ala Ala Ala Val Ala Lys Val Pro Ser Phe Met Trp Leu Asp Thr
130 135 140

Leu Asp Lys Thr Pro Leu Met Glu Gln Thr Leu Ala Asp Ile Arg Thr
145 150 155 160

Ala Asn Lys Asn Gly Gly Asn Tyr Ala Gly Gln Phe Val Val Tyr Asp
165 170 175

Leu Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly Glu Tyr Ser
180 185 190

Ile Ala Asp Gly Gly Val Ala Lys Tyr Lys Asn Tyr Ile Asp Thr Ile
195 200 205

Arg Gln Ile Val Val Glu Tyr Ser Asp Ile Arg Thr Leu Leu Val Ile
210 215 220

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

Cys Ala Asn Ala Gln Ser Ala Tyr Leu Glu Cys Ile Asn Tyr Ala Val
245 250 255

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

Ala Gly Trp Leu Gly Trp Pro Ala Asn Gln Asp Pro Ala Ala Gln Leu
275 280 285

Phe Ala Asn Val Tyr Lys Asn Ala Ser Ser Pro Arg Ala Leu Arg Gly
290 295 300

Leu Ala Thr Asn Val Ala Asn Tyr Asn Gly Trp Asn Ile Thr Ser Pro
305 310 315 320

Pro Ser Tyr Thr Gln Gly Asn Ala Val Tyr Asn Glu Lys Leu Tyr Ile
325 330 335

His Ala Ile Gly Pro Leu Leu Ala Asn His Gly Trp Ser Asn Ala Phe
340 345 350

Phe Ile Thr Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr Gly Gln Gln
355 360 365

Gln Trp Gly Asp Trp Cys Asn Val Ile Gly Thr Gly Phe Gly Ile Arg
370 375 380

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

Lys Pro Gly Gly Glu Cys Asp Gly Thr Ser Asp Thr Ser Ala Pro Arg
405 410 415

Phe Asp Ser His Cys Ala Leu Pro Asp Ala Leu Gln Pro Ala Pro Glu
420 425 430

Ala Gly Thr Trp Phe Gln Ala Tyr Phe Glu Gln Leu Leu Thr Asn Ala
435 440 445

Asn Pro Ser Phe Leu
450

<210> 27
<211> 1353
<212> DNA
<213> Artificial Sequence

<220>
<223> Clone 382A2

<400> 27
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agctggaatg gtgctacctg ctgccagtct ggtagctact gcagcaagat caacgactat 120

tactctcagt gcattcctgg agaaggcccc gccacttcca agtcgagcac tcttcctgct 180

tccaccacaa caactcagcc aacttccact tcgactgctg gaacctcttc cactaccaag 240

cctcctccag ctggatcggg aaccgctacg tattcaggca acccttactc tggggtcaac 300

ctttgggcca atagctatta ccgctctgaa gttaccaacc tcgctattcc taagttgagc 360

ggagccatgg ccactgctgc agcaaaggtc gcagatgttc cctcttttca gtggatggat 420

acttatgacc acatctcctt catggaggag tctttggccg acatccgcaa ggccaacaag 480

gctggcggta actatgccgg acagtttgtg gtgtatgact tgccggatcg cgattgcgct 540

gccgctgcct cgaatggcga atactctctt gacaaggatg gcaagaacaa atataaggcc 600

tatatcgcca agattaaggg tattctccag gactattccg atacccggat cattctggtt 660

attgagcctg actctcttgc caacatggtg accaacatga acgtcccaaa gtgtgccaat 720

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gtttccatgt atatcgacgc tggccatggt ggatggcttg gctggccggc aaaccttcct 840

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Thr Gln Pro Thr Ser Thr Ser Thr Ala Gly Thr Ser Ser Thr Thr Lys
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Pro Pro Pro Ala Gly Ser Gly Thr Ala Thr Tyr Ser Gly Asn Pro Tyr

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Ser Gly Val Asn Leu Trp Ala Asn Ser Tyr Tyr Arg Ser Glu Val Thr
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Lys Val Ala Asp Val Pro Ser Phe Gln Trp Met Asp Thr Tyr Asp His
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Ile Ser Phe Met Glu Glu Ser Leu Ala Asp Ile Arg Lys Ala Asn Lys
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Ala Gly Gly Asn Tyr Ala Gly Gln Phe Val Val Tyr Asp Leu Pro Asp
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Asp Gly Lys Asn Lys Tyr Lys Ala Tyr Ile Ala Lys Ile Lys Gly Ile
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Ser Leu Ala Asn Met Val Thr Asn Met Asn Val Pro Lys Cys Ala Asn
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275 280 285

Leu Tyr Lys Asp Ala Gly Lys Pro Ser Arg Leu Arg Gly Leu Val Thr
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Thr Glu Ser Asn Pro Asn Tyr Asp Glu Gln Lys Tyr Ile His Ala Leu
325 330 335

Ser Pro Leu Leu Glu Gln Glu Gly Trp Pro Gly Ala Lys Phe Ile Val
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Asp Gln Gly Arg Ser Gly Lys Gln Pro Thr Gly Gln Lys Ala Trp Gly
355 360 365

Asp Trp Cys Asn Ala Pro Gly Thr Gly Phe Gly Leu Arg Pro Ser Ala
370 375 380

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

Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser Ala Ala Arg Tyr Asp Tyr
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His Cys Gly Ile Asp Gly Ala Val Lys Pro Ala Pro Glu Ala Gly Thr
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Ala Thr Thr Thr Gly Ser Gly Gly Ser Val Thr Ser Pro Pro Pro Val
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Ala Thr Asn Asn Pro Phe Ser Gly Val Asp Leu Trp Ala Asn Asn Tyr
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Tyr Arg Ser Glu Val Ser Thr Leu Ala Ile Pro Lys Leu Ser Gly Ala
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Val Tyr Asp Leu Pro Asp Arg Asp Cys Ala Ala Ala Ala Ser Asn Gly
165 170 175

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

Pro Pro Pro Ala Gly Ser Gly Thr Ala Thr Tyr Ser Gly Asn Pro Tyr
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Ser Gly Val Asn Leu Trp Ala Asn Ser Tyr Tyr Arg Ser Glu Val Thr
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Asn Leu Ala Ile Pro Lys Leu Ser Gly Ala Met Ala Thr Ala Ala Ala
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Lys Val Ala Asp Val Pro Ser Tyr Gln Trp Met Asp Ser Phe Asp His
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Ile Ser Leu Met Glu Asp Thr Leu Val Asp Ile Arg Lys Ala Asn Leu
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Ser Leu Ala Asn Leu Val Thr Asn Met Asp Val Ala Lys Cys Ala Lys
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Asn Thr Gly Asp Ser Leu Leu Asp Ala Phe Val Trp Val Lys Pro Gly
385 390 395 400

Gly Glu Ser Asp Gly Thr Ser Asp Thr Ser Ala Thr Arg Tyr Asp Tyr
405 410 415

His Cys Gly Ala Ser Ala Ala Leu Gln Pro Ala Pro Glu Ala Gly Thr
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Phe Leu
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Ala Leu Pro Ser Pro Ile Thr Pro Ser Glu Ala Ala Val Leu Gln Lys
20 25 30

Arg

Patentkrav

1. Isoleret eller oprenset polypeptid **kendetegnet ved, at** det har en mindst 10% forbedret exoglucanaseaktivitet ved en temperatur på ca. 35°C og/eller ca. 50°C i forhold til exoglucanaseaktiviteten af referenceproteinet CBH2 af sekvens SEQ ID NO: 2, idet nævnte polypeptid er valgt fra gruppen bestående af:

i. en aminosyresekvens valgt fra SEQ ID NO : 28, SEQ ID NO : 6, SEQ ID NO : 4, SEQ ID NO : 8, SEQ ID NO : 10, SEQ ID NO : 12, SEQ ID NO : 14, SEQ ID NO : 16, SEQ ID NO : 18, SEQ ID NO : 20, SEQ ID NO : 22, SEQ ID NO : 24 og SEQ ID NO : 26; og

ii. en aminosyresekvens med, i forhold til sekvensen SEQ ID NO : 28, SEQ ID NO : 6, SEQ ID NO : 4, SEQ ID NO : 8, SEQ ID NO : 10, SEQ ID NO : 12, SEQ ID NO : 14, SEQ ID NO : 16, SEQ ID NO : 18, SEQ ID NO : 20, SEQ ID NO : 22, SEQ ID NO : 24 og SEQ ID NO : 26, en procentvis identitet på mindst 99%.

2. Oprenset eller isoleret nukleinsyre, **kendetegnet ved, at** den koder for mindst et polypeptid ifølge krav **1.**

3. Oprenset eller isoleret nukleinsyre ifølge krav **2** valgt fra følgende sekvenser: SEQ ID NO : 27, SEQ ID NO : 5, SEQ ID NO : 3,, SEQ ID NO : 7, SEQ ID NO : 9, SEQ ID NO : 11; SEQ ID NO : 13, SEQ ID NO : 15, SEQ ID NO : 17 og SEQ ID NO : 19, SEQ ID NO : 21, SEQ ID NO : 23 og SEQ ID NO : 25.

4. Vektor **kendetegnet ved, at** den omfatter mindst en nukleinsyre ifølge et af kravene **2 eller 3.**

5. Isoleret værtscelle **kendetegnet ved, at** den omfatter mindst et polypeptid ifølge krav **1**, eller mindst en nukleinsyre ifølge et af kravene **2 eller 3,** eller mindst en vektor ifølge krav **4**.

6. Isoleret værtscelle ifølge krav **5, kendetegnet ved, at** den er valgt fra *Trichoderma, Aspergillus, Neurospora, Humicola, Penicillium, Fusarium, Thermomonospora, Myceliophthora, Chrysosporium, Bacillus, Pseudomonas, Escherichia, Clostridium, Cellulomonas, Streptomyces, Yarrowia,*

Pichia og *Saccharomyces.*

7. Isoleret værtscelle ifølge et af kravene **5 eller 6, kendetegnet ved, at** den er valgt fra *Trichoderma reesei, Trichoderma viridae, Trichoderma koningii, Aspergillus niger, Aspergillus nidulans, Aspergillus wentii, Aspergillus oryzae, Aspergillus phoenicis, Neurospora crassa, Humicola grisae, Myceliophthora thermopila, Chrysosporium lucknowense, Penicillium pinophilum, Penicillium oxalicum, Escherichia coli, Clostridium acetobutylicum, Clostridium saccharolyticum, Clostridium benjerinckii, Clostridium butylicum, Pichia pastoris, Yarrowia lipolityca* og *Saccharomyces cerevisiae.*

8. Anvendelse af nævnte polypeptid ifølge krav **1** til hydrolyse af cellulosen.

9. Anvendelse af nævnte polypeptid ifølge krav **1** til fremstilling af biobrændstof.

10. Enzymsammensætning der er i stand til at påvirke den lignocelluloseholdige biomasse, idet nævnte enzymsammensætning er fremstillet af filamentøse svampe og omfatter mindst et polypeptid ifølge krav **1**.

11. Fremgangsmåde til fremstilling af biobrændstof fra lignocelluloseholdig biomasse, **kendetegnet ved, at** den omfatter følgende successive trin:

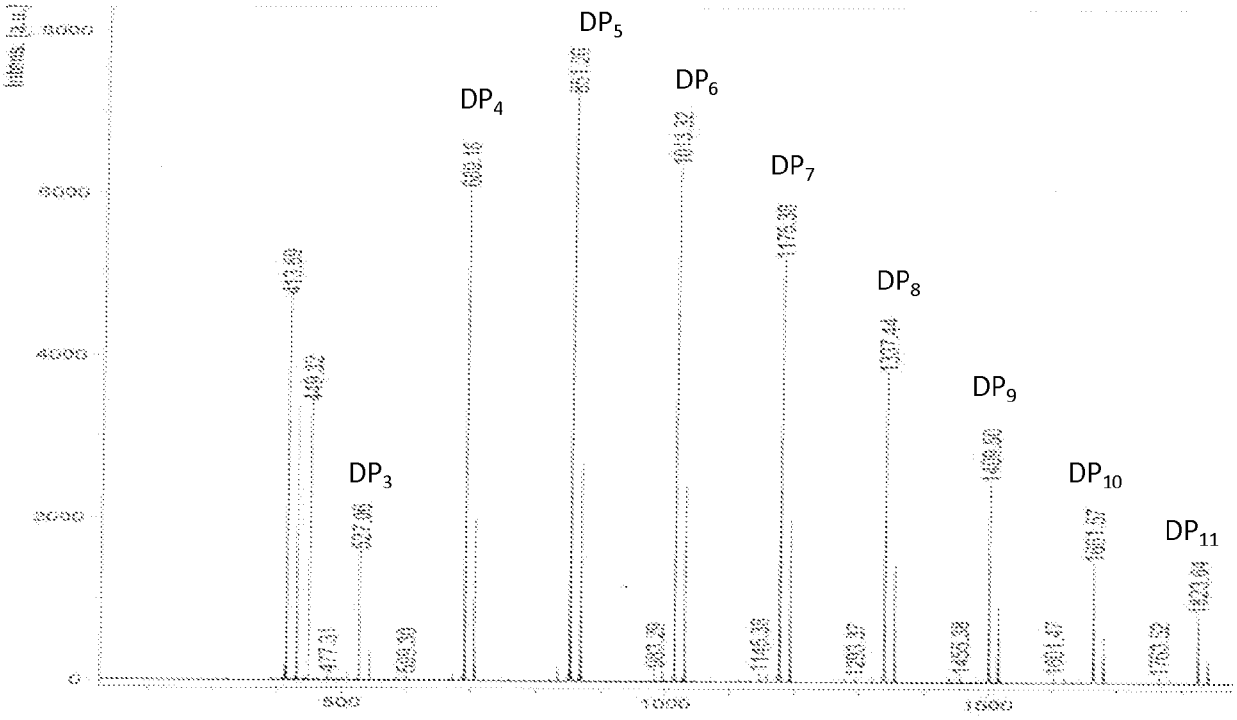
- opslæmning af biomassen, som skal hydrolyseres, i vandig fase;
- hydrolyse af biomassen under tilstedeværelsen af en enzymsammensætning ifølge krav **10** den lignocelluloseholdige biomasse for at fremstille et hydrolysat indeholdende glukose;
- fermentering under tilstedeværelsen af en fermenterende organisme glukosen fra hydrolysatet for at fremstille en fermenteret mask;
- adskillelse af biobrændstoffet fra den fermenterede mask.

12. Fremgangsmåde til fremstilling af biobrændstof fra biomasse, **kendetegnet ved, at** den omfatter følgende successive trin:

- opslæmning af biomassen, som skal hydrolyseres, i vandig fase;
- samtidig tilsætning til opslæmningen af en enzymsammensætning ifølge krav **10** og en gæringsorganisme og fermentering af blandingen for at fremstille en fermenteret mask;

- adskillelse af biobrændstoffet fra den fermenterede mask.

13. Fremgangsmåde ifølge et af kravene **11 eller 12**, hvor den fermenterende organisme er en værtscelle ifølge et af kravene **5 til 7**.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1823.64

FIGURE 1