

TITLE OF THE INVENTION

HYALURONIC ACID-PRODUCING RECOMBINANT CELLS

FIELD OF THE INVENTION

5 The present invention relates to the field of bio-production of hyaluronic acid.

BACKGROUND OF THE INVENTION

10 Hyaluronic acid, also known as hyaluronan or HA, is a naturally occurring high molecular weight polysaccharide composed of D-glucuronic acid and N-acetyl-D-glucosamine, linked via alternating β -(1 $\rightarrow$ 4) and β -(1 $\rightarrow$ 3) glycosidic bonds and having the chemical formula (C₁₄H₂₁NO₁₁)_n. Hyaluronic acid can be 25,000 disaccharide repeats in length. Polymers of hyaluronic acid can range in size from 5,000 to 20,000,000 Da *in vivo*.

15 HA has many applications in the medical and cosmetic fields including scaffolding for tissue engineering, dermatological fillers, and viscosupplementation for osteoarthritis treatment. In particular, a reduction in HA mass or molecular weight via degradation or slowing of synthesis affects physical and chemical properties such as tissue volume, viscosity, and elasticity. There is therefore a constant need for HA production.

 Today's main known sources of HA are human umbilical cords, rooster combs and fermentation of certain microorganisms.

20 Fermentation methods for preparing HA from microorganisms are particularly of interest since they facilitate the production of a large quantity of HA through possible scale-up, at a reduced cost. Contrary to isolating HA from animal sources which provide hyaluronic acid of very high molecular weights, microbial fermentation allows to control, to a certain extent, the size of the starting molecular weight. This avoids the need for further fractionation steps by mechanical, physical or chemical means.

25 In this aim, bacterial cultures such as group A and C hemolytic streptococci have been shown to represent good sources of HA (US5316926, US4801539, JP2009011315). However, such bacteria, and in particular *Streptococcus zooepidemicus* which is mainly used in the art, is not generally recognized as safe. Recombinant *Bacillus* host cells have also shown the ability to produce HA in the range of 20 to 800 KDa (US2008038780). It has also been demonstrated in US2006168690 that transforming plant cells to include a DNA

encoding hyaluronic acid synthase successfully allowed for the production of HA. Finally, production of small molecules of HA has also been demonstrated in yeasts such as in a recombinant *Pichia pastoris* in CN104263666.

Contrary to other microorganisms that are commonly used for the production of biological molecules, yeasts are generally recognised as safe. They can grow rapidly and be cultivated at higher density, compared to bacteria, and do not require an aseptic environment. Furthermore, yeast cells can be more easily separated from the culture medium than bacteria, greatly simplifying the process for product extraction and purification. Finally, yeasts present the advantage of being more resistant to pH changes in the culture medium, and as such represent stronger fermentation systems.

However, among yeasts, distinguishing features between species may also present certain challenges. This is mainly due to the difference in metabolisms between the species. For example, genome integrations in *P. pastoris* are stable but often a high variability of clones from one transformation is encountered, displaying various productivity characteristics or changes in their physiology. This requires a time-consuming screening process so as to find the clone with the optimal features for the desired application. This clonal variability is an important drawback for the further development of *P. pastoris* as a platform for producing value-added chemicals. Another drawback of using *P. pastoris* is that it is a methanotrophic organism.

On the contrary, *Saccharomyces cerevisiae* is particularly useful as a tool for the production of molecules of interest since it has a long safe history when it comes to being used by humans (such as in wine, beer, or bread) and as such is a well-established model whose genetic information is well known in the art. *S. cerevisiae* further presents the advantage of being generally recognised as safe for humans and animals. What's more, the acidification of the medium which occurs when cultivating this yeast reduces the possibility of contamination of the bio fermenters, and therefore eliminates the need to add antibiotics to the medium. Finally, many genetic tools have been developed allowing for stable modifications of its genome (integration within the chromosome).

Accordingly, there is still a need in the art for further hyaluronic acid production methods allowing its highly efficient synthesis and secretion. In particular, there is still a need to provide production methods that are cost-efficient and supply hyaluronic acid which is safe for human application.

In a particular case, there is still a need in the art for hyaluronic acid methods allowing to obtain large amounts of hyaluronic acid of a particular and controlled size.

SUMMARY OF THE INVENTION

5 The present invention accordingly relates to the following items:

Item 1: a recombinant yeast cell producing hyaluronic acid (HA) wherein the recombinant cell comprises:

 (a) one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity;

10 (b) one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity;

 (c) one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity wherein the polypeptide having hyaluronidase activity comprises a secretion signal so that hyaluronic acid, in particular of a desired molecular weight (HAMW)

15 is produced by the recombinant yeast cell, and

 (d) (i) one or more recombinant nucleic acids encoding a polypeptide having a glutamine synthetase (GLN1) activity; and/or

 (ii) one or more disrupted endogeneous nucleic acids encoding a glutamate synthase (GLT1);

20 wherein said recombinant yeast cell belongs to the *Saccharomyces* genus, or to the *Candida* genus, or to the *Kluyveromyces* genus, or to the *Ogataea* genus, or to the *Yarrowia* genus, or to the *Debaryomyces* genus, or to the *Ashbya* genus.

 As illustrated in the examples, the recombinant yeasts of the invention allow for the production of hyaluronic acid in a yeast cell which is not naturally able to produce
25 hyaluronic acid. It is further demonstrated in the examples that the size of the hyalaruonic acid produced by the recombinant yeast may be controlled.

 Said advantageous properties can be further increased by recombining the yeast with additional modifications described here-after.

Item 2: A recombinant host cell producing hyaluronic acid (HA) wherein the
30 recombinant host cell comprises:

 (a) one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity;

(b) one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity;

(c) one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity wherein the polypeptide having hyaluronidase activity comprises a secretion signal and an anchoring signal so that hyaluronic acid, in particular of a desired molecular weight (HAMW) is produced by the host cell; and

(d) (i) one or more recombinant nucleic acids encoding a polypeptide having a glutamine synthetase (GLN1) activity; and/or

(ii) one or more disrupted endogeneous nucleic acids encoding a glutamate synthase (GLT1).

Item 3: The recombinant cell according to item 1 or 2, wherein the molecular weight of the HA is in the range of less than 50kDa, preferably in the range of about 20kDa to about 50kDa.

Item 4: The recombinant cell according to item 1 or 2, wherein the molecular weight of the HA is in the range of greater than 50kDa, preferably in the range of about 50kDa to about 250kDa.

Item 5: The recombinant cell according to item 1 or 2, wherein the molecular weight of the HA is in the range of greater than 100kDa, preferably in the range of about 100kDa to about 1500kDa.

Item 6: The recombinant cell according to any one of items 1 to 5, wherein the nucleic acid encoding a polypeptide having a glutamine synthetase activity is obtained or derived from *Saccharomyces cerevisiae*.

Item 7: The recombinant cell according to any one of items 1 to 6, wherein the nucleic acid encoding a polypeptide having hyaluronidase activity is obtained or derived from at least one of *Cupiennius salei*, *Loxosceles intermedia*, *Hirudo nipponia*, *Bothrops atrox* or *Tityus serrulatus*.

Item 8: The recombinant cell according to any one of items 1 to 7 wherein the nucleic acid encoding a polypeptide having hyaluronan synthase activity is obtained or derived from at least one of *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Chlorella virus CviK1*, *Chlorella virus IL-5-2s1*, *Chlorella virus CZ-2*, *Chlorella virus CVG-1*, *Xenopus laevis* or *Pasteurella multocida*, and is in particular obtained or derived from at least one of *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Chlorella virus CviK1*, *Chlorella virus IL-5-2s1*, *Chlorella virus CZ-2*, *Chlorella virus CVG-1*, or *Xenopus laevis*.

Item 9: The recombinant cell according to any one of items 1 to 8, wherein the nucleic acid encoding a polypeptide having UDP-Glucose dehydrogenase activity is obtained or derived from at least one of *Arabidopsis thaliana*, *Chlorella virus PBCV1* or *Streptococcus zooepidemicus*, and in particular from *Arabidopsis thaliana* or *Chlorella virus PBCV1*.

5 **Item 10:** The recombinant cell according to any one of items 1 to 9, wherein the recombinant cell comprises a recombinant nucleic acid encoding one or more of:

(i) a polypeptide having glutamine-fructose-6-phosphate amidotransferase (GFA1) activity; and/or

(ii) a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase (QRI1) activity.

10 **Item 11:** The recombinant cell according to any one of items 1 to 10, wherein the recombinant cell comprises at least one recombinant nucleic acid encoding one or more of:

(i) a polypeptide having Phosphoglucomutase-1 (PGM1) activity; and/or

15 (ii) a polypeptide having UTP-glucose-1-phosphate uridylyltransferase (UGP1) activity; and/or

(iii) a polypeptide having Glucosamine-6-phosphate N-acetyltransferase (GNA1) activity; and/or

(iv) a polypeptide having phosphoacetylglucosamine mutase (PCM1) activity.

20 **Item 12:** The recombinant yeast cell according to any one of items 1 and 3 to 11, wherein the recombinant yeast cell is selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Saccharomyces bayanus*, *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces castelli*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces polysporus*, *Kluyveromyces thermotolerans*, *Ogataea polymorpha*, *Yarrowia lipolytica*, *Debaryomyces hansenii*, and *Ashbya gossypii*, and is preferably *Saccharomyces cerevisiae*.

25 **Item 13:** The recombinant host cell according to any one of items 2 to 11 wherein the recombinant host cell belongs to the *Saccharomycetales* order, in particular to the *Saccharomycetaceae* family, and is in particular selected from the group consisting of
30 *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Saccharomyces bayanus*, *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces castelli*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces polysporus*, *Kluyveromyces thermotolerans*, *Ogataea polymorpha*,

Yarrowia lypolytica, *Debaryomyces hansenii*, and *Ashbya gossypii*, and is preferably *Saccharomyces cerevisiae*.

Item 14: A method of producing hyaluronic acid (HA) of a desired molecular weight (HAMW) comprising:

5 (a) cultivating a recombinant cell as defined in any one of items 1 to 13 in a cultivation medium for a time sufficient to produce hyaluronic acid (HA) of the desired molecular weight; and

(b) optionally isolating or recovering the hyaluronic acid (HA) from the recombinant cell and/or from the cultivation medium.

10 **Item 15:** The method according to item 14, wherein the HA has a molecular weight of from about 20kDa to about 50kDa, and preferably from about 20 kDa to about 30 kDa.

Item 16: The method according to item 15, wherein the HA has a molecular weight of from about 30 kDa to about 50 kDa.

15 **Item 17:** The method according to item 14, wherein the molecular weight of the HA is of from about 50 kDa to about 150 kDa.

Item 18: The method according to item 14, wherein the molecular weight of the HA is of from about 150 kDa to about 1500 kDa.

Item 19: The method according to any one of items 14 to 18 wherein the recombinant cell comprises at least one recombinant nucleic acid encoding one or more of:

20 (i) a polypeptide having glutamine-fructose-6-phosphate amidotransferase (GFA1) activity; and/or

(ii) a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase (QRI1) activity.

25 **Item 20:** The method according to any one of items 14 to 19, wherein the recombinant cell comprises at least one recombinant nucleic acid encoding one or more of:

(i) a polypeptide having Phosphoglucomutase-1 (PGM1) activity;

(ii) a polypeptide having UTP-glucose-1-phosphate uridylyltransferase (UGP1) activity;

30 (iii) a polypeptide having Glucosamine-6-phosphate N-acetyltransferase (GNA1) activity; and/or

(iv) a polypeptide having phosphoacetylglucosamine mutase (PCM1) activity.

Item 21: The method according to any one of items 14 to 20, wherein the recombinant cell is a member of the genus *Saccharomyces*, and in particular is *Saccharomyces cerevisiae*.

Item 22: The method according according to any one of items 14 to 21, wherein
5 the time sufficient to produce hyaluronic acid (HA) of the desired molecular weight is a period of from about 35 hours to about 50 hours, preferably from about 40 hours to about 50 hours, preferably about 48 hours.

Item 23: The method according according to any one of items 14 to 22, wherein
10 the molecular weight of the hyaluronic acid is controlled by regulating the pH of the cultivation medium.

Item 24: The method according to any one of items 14 to 23, wherein the method is carried out on an industrial scale, preferably where the cultivation medium is at least about 100L, more preferably in the range of about 1000L to about 3000L, even more preferably about 10,000L, even more preferably about 100,000L, or even about 250,000L.

Item 25: Hyaluronic acid (HA) obtainable from a recombinant cell of any one of
15 items 1 to 10 or from the method according to any one of items 14 to 24.

Item 26: A cultivation medium comprising the HA according to item 25.

Item 27: A composition comprising the Hyaluronic acid (HA) according to item
20 24.

Item 28: An industrial product or a consumer product or a consumable
comprising (i) the HA according to item 25, (ii) the cultivation medium of item 26 or (iii) the composition of item 27.

Item 29: The industrial product or the consumer product or the consumable of
item 27, wherein the industrial product or the consumer product or the consumable is a
25 cosmetic product, a flavour product, a fragrance product, a food product, a food, a beverage, a texturant, a pharmaceutical composition, a dietary supplement, a nutraceutical, a cleaning product and/or a dental and/or an oral hygiene composition.

Item 30: Use of a recombinant cell as defined in any one of items 1 to 13 for the
production of hyaluronic acid (HA) having a molecular weight in the range of from about
30 20kDa to about 50kDa or from about 50kDa to about 1000 kDa.

Item 31: A method for producing hyaluronic acid comprising the steps of:

(a) culturing a recombinant yeast as defined in any one of items 1 and 3 to 12 in
a culture medium; and

(b) recovering the hyaluronic acid from said culture medium,
wherein the hyaluronic acid recovered in step (b) has a molecular weight controlled through the selection of:

- the nature and origin of the nucleic acid encoding the hyaluronidase of the recombinant yeast,
- the nature and origin of the promoter controlling the expression of the nucleic acid encoding the hyaluronidase(s) of the recombinant yeast,
- the presence of an anchoring and/or of a secretion signal associated to the encoded hyaluronidase(s) of the recombinant yeast,
- the pH of the culture medium during the step of culturing the recombinant yeast, and/or
- the duration of the culturing of the recombinant yeast.

Certain embodiments of the present invention may provide one or more of the following advantages:

- a process for producing Hyaluronic acid of controlled molecular weight;
- a process for producing Hyaluronic acid of controlled molecular weight in a *Saccharomyces* yeast cell; and
- a process for producing Hyaluronic acid of controlled molecular weight by varying genetic parameters (eg regulatory sequences) and/or process parameters (pH or fermentation time).

The details, examples and preferences provided in relation to any particular one or more of the stated aspects of the present invention will be further described herein and apply equally to all aspects of the present invention. Any combination of the embodiments, examples and preferences described herein in all possible variations thereof is encompassed by the present invention unless otherwise indicated herein, or otherwise clearly contradicted by context.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a schematic pathway for the production of hyaluronic acid
Figures 2A and 2B show agarose gels used to determine the molecular weight of the hyaluronic acid produced by strains according to the invention, (for example strain YA5235-1

(Figure 2A) and strain YA5359-10 (Figure 2B)). The agarose gels are obtained after running an aliquot of the supernatant of the considered strain and staining it with “stains all” (CAS Number 7423-31-6). HA molecular weight standards are present in each gel for reference.

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SUMMARY OF THE SEQUENCES

SEQ ID NO: 1 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus PBCV-1*

SEQ ID NO: 2 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus PBCV-1*

10 **SEQ ID NO: 3** is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Pasteurella multocida*

SEQ ID NO: 4 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Pasteurella multocida*

15 **SEQ ID NO: 5** is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Pasteurella multocida*

SEQ ID NO: 6 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Xenopus laevis*

SEQ ID NO: 7 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Streptococcus zooepidemicus*

20 **SEQ ID NO: 8** is an amino acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus PBCV-1*

SEQ ID NO: 9 is an amino acid sequence of hyaluronan synthase (HASA) originating from *Pasteurella multocida*

25 **SEQ ID NO: 10** is an amino acid sequence of hyaluronan synthase (HASA) originating from *Xenopus laevis*

SEQ ID NO: 11 is an amino acid sequence of hyaluronan synthase (HASA) originating from *Streptococcus zooepidemicus*

SEQ ID NO: 12 is a nucleic acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Arabidopsis thaliana*

30 **SEQ ID NO: 13** is a reencoded nucleic acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Chlorella virus PBCV-1*

- SEQ ID NO: 14** is a reencoded nucleic acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Chlorella virus PBCV-1*
- SEQ ID NO: 15** is a reencoded nucleic acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Streptococcus zooepidemicus*
- 5 **SEQ ID NO: 16** is an amino acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Arabidopsis thaliana*
- SEQ ID NO: 17** is an amino acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Chlorella virus PBCV1*
- SEQ ID NO: 18** is an amino acid sequence of UDP-Glucose dehydrogenase (HASB)
- 10 originating from *Streptococcus zooepidemicus*
- SEQ ID NO: 19** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Bothrops atrox* with a N-terminal secretion signal
- SEQ ID NO: 20** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Bothrops atrox* with a N-terminal secretion signal and a C-terminal anchoring signal
- 15 **SEQ ID NO: 21** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Cupiennius salei* with a N-terminal secretion signal
- SEQ ID NO: 22** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Cupiennius salei* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 23** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating
- 20 from *Hirudo Nipponia* with a N-terminal secretion signal
- SEQ ID NO: 24** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Hirudo Nipponia* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 25** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Loxosceles intermedia* with a N-terminal secretion signal
- 25 **SEQ ID NO: 26** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Loxosceles intermedia* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 27** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Tityus serrulatus* with a N-terminal secretion signal
- 30 **SEQ ID NO: 28** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Tityus serrulatus* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 29** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Vespa magnifica* with a N-terminal secretion signal

- SEQ ID NO: 30** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Vespa magnifica* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 31** is an amino acid sequence of hyaluronidase (HYAL) originating from *Bothrops atrox* with a N-terminal secretion signal
- 5 **SEQ ID NO: 32** is an amino acid sequence of hyaluronidase (HYAL) originating from *Bothrops atrox* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 33** is an amino acid sequence of hyaluronidase (HYAL) originating from *Cupiennius salei* with a N-terminal secretion signal
- SEQ ID NO: 34** is an amino acid sequence of hyaluronidase (HYAL) originating from
- 10 *Cupiennius salei* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 35** is an amino acid sequence of hyaluronidase (HYAL) originating from *Hirudo Nipponia* with a N-terminal secretion signal
- SEQ ID NO: 36** is an amino acid sequence of hyaluronidase (HYAL) originating from *Hirudo Nipponia* with a N-terminal secretion signal and a C-terminal anchoring signal
- 15 **SEQ ID NO: 37** is an amino acid sequence of hyaluronidase (HYAL) originating from *Loxosceles intermedia* with a N-terminal secretion signal
- SEQ ID NO: 38** is an amino acid sequence of hyaluronidase (HYAL) originating from *Loxosceles intermedia* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 39** is an amino acid sequence of hyaluronidase (HYAL) originating from *Tityus*
- 20 *serrulatus* with a N-terminal secretion signal
- SEQ ID NO: 40** is an amino acid sequence of hyaluronidase (HYAL) originating from *Tityus serrulatus* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 41** is an amino acid sequence of hyaluronidase (HYAL) originating from *Vespa magnifica* with a N-terminal secretion signal
- 25 **SEQ ID NO: 42** is an amino acid sequence of hyaluronidase (HYAL) originating from *Vespa magnifica* with a N-terminal secretion signal and a C-terminal anchoring signal
- SEQ ID NO: 43** is a nucleic acid sequence of the secretion sequence added in 5'
- SEQ ID NO: 44** is an amino acid sequence of the secretion sequence added in N-ter
- SEQ ID NO: 45** is a nucleic acid sequence of the anchoring sequence added in 3'
- 30 **SEQ ID NO: 46** is an amino acid sequence of the anchoring sequence added in C-ter
- SEQ ID NO: 47** is a nucleic acid sequence of glutamine-fructose-6-phosphate amidotransferase (GFA1) originating from *Saccharomyces cerevisiae*

- SEQ ID NO: 48** is a reencoded nucleic acid sequence of glutamine-fructose-6-phosphate amidotransferase (GFA1) originating from *Chlorella virus 1* (PBCV-1)
- SEQ ID NO: 49** is a reencoded nucleic acid sequence of glutamine-fructose-6-phosphate amidotransferase (GFA1) originating from *Chlorella virus 1* (PBCV-1)
- 5 **SEQ ID NO: 50** is an amino acid sequence of glutamine-fructose-6-phosphate amidotransferase (GFA1) originating from *Saccharomyces cerevisiae*
- SEQ ID NO: 51** is an amino acid sequence of glutamine-fructose-6-phosphate amidotransferase (GFA1) originating from *Chlorella virus 1* (PBCV-1)
- SEQ ID NO: 52** is a nucleic acid sequence of UDP-N-acetylglucosamine pyrophosphorylase (QRI1) originating from *Saccharomyces cerevisiae*
- 10 **SEQ ID NO: 53** is an amino acid sequence of UDP-N-acetylglucosamine pyrophosphorylase (QRI1) originating from *Saccharomyces cerevisiae*
- SEQ ID NO: 54** is a nucleic acid sequence of Phosphoglucomutase-1 (PGM1) originating from *Saccharomyces cerevisiae*
- 15 **SEQ ID NO: 55** is an amino acid sequence Phosphoglucomutase-1 (PGM1) originating from *Saccharomyces cerevisiae*
- SEQ ID NO: 56** is a nucleic acid sequence of UTP--glucose-1-phosphate uridylyltransferase (UGP1) originating from *Saccharomyces cerevisiae*
- SEQ ID NO: 57** is an amino acid sequence of UTP--glucose-1-phosphate uridylyltransferase (UGP1) originating from *Saccharomyces cerevisiae*
- 20 **SEQ ID NO: 58** is a nucleic acid sequence of Glucosamine 6-phosphate N-acetyltransferase (GNA1) originating from *Saccharomyces cerevisiae*
- SEQ ID NO: 59** is an amino acid sequence of Glucosamine 6-phosphate N-acetyltransferase (GNA1) originating from *Saccharomyces cerevisiae*
- 25 **SEQ ID NO: 60** is a nucleic acid sequence of phosphoacetylglucosamine mutase (PCM1) originating from *Saccharomyces cerevisiae*
- SEQ ID NO: 61** is an amino acid sequence of phosphoacetylglucosamine mutase (PCM1) originating from *Saccharomyces cerevisiae*
- SEQ ID NO: 62** is the nucleic acid sequence of promotor pTDH3
- 30 **SEQ ID NO: 63** is the nucleic acid sequence of promotor pTDH3.Sk
- SEQ ID NO: 64** is the nucleic acid sequence of promotor pTDH3-1.sba
- SEQ ID NO: 65** is the nucleic acid sequence of promotor pTDH3.Sar
- SEQ ID NO: 66** is the nucleic acid sequence of promotor pENO2

- SEQ ID NO: 67** is the nucleic acid sequence of promotor pTEF3
- SEQ ID NO: 68** is the nucleic acid sequence of promotor pTEF1
- SEQ ID NO: 69** is the nucleic acid sequence of promotor pTEF1.ago
- SEQ ID NO: 70** is the nucleic acid sequence of promotor pTEF1.Sba
- 5 **SEQ ID NO: 71** is the nucleic acid sequence of promotor pPDC1
- SEQ ID NO: 72** is the nucleic acid sequence of promotor pCCW12
- SEQ ID NO: 73** is the nucleic acid sequence of promotor pCCW12.Sm
- SEQ ID NO: 74** is the nucleic acid sequence of promotor pCCW12.Sk
- SEQ ID NO: 75** is the nucleic acid sequence of promotor pCCW12.Sba
- 10 **SEQ ID NO: 76** is the nucleic acid sequence of promotor pCCW12.Sar
- SEQ ID NO: 77** is the nucleic acid sequence of promotor pNUP57
- SEQ ID NO: 78** is the nucleic acid sequence of promotor pCCW10.ago
- SEQ ID NO: 79** is the nucleic acid sequence of promotor pCWP2
- SEQ ID NO: 80** is the nucleic acid sequence of promotor pRPLA1
- 15 **SEQ ID NO: 81** is the nucleic acid sequence of promotor pCUP1
- SEQ ID NO: 82** is the nucleic acid sequence of promotor pMET6
- SEQ ID NO: 83** is the nucleic acid sequence of promotor pMET25
- SEQ ID NO: 84** is the nucleic acid sequence of promotor pSAM1
- SEQ ID NO: 85** is the nucleic acid sequence of terminator tTPI1
- 20 **SEQ ID NO: 86** is the nucleic acid sequence of terminator tMET25
- SEQ ID NO: 87** is the nucleic acid sequence of terminator tDIT1
- SEQ ID NO: 88** is the nucleic acid sequence of terminator tRPL3
- SEQ ID NO: 89** is the nucleic acid sequence of terminator tRPL3.sm
- SEQ ID NO: 90** is the nucleic acid sequence of terminator tRPL3.sba
- 25 **SEQ ID NO: 91** is the nucleic acid sequence of terminator tRPL41B
- SEQ ID NO: 92** is the nucleic acid sequence of terminator tRPL15A
- SEQ ID NO: 93** is the nucleic acid sequence of terminator tRPL15A.sba
- SEQ ID NO: 94** is the nucleic acid sequence of terminator tIDP1
- SEQ ID NO: 95** is the nucleic acid sequence of terminator tTEF1.sba
- 30 **SEQ ID NO: 96** is a nucleic acid sequence of Glutamine synthetase (GLN1) originating from *Saccharomyces cerevisiae*
- SEQ ID NO: 97** is an amino acid sequence of Glutamine synthetase (GLN1) originating from *Saccharomyces cerevisiae*

SEQ ID NO: 98 is a nucleic acid sequence of Glutamate synthase (GLT1) originating from *Saccharomyces cerevisiae*

SEQ ID NO: 99 is an amino acid sequence of Glutamate synthase (GLT1) originating from *Saccharomyces cerevisiae*

5 **SEQ ID NO: 100** is the nucleic acid sequence of terminator tTDH3.

SEQ ID NO: 101 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CviK1*

SEQ ID NO: 102 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus IL-5-2s1*

10 **SEQ ID NO: 103** is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CZ-2*

SEQ ID NO: 104 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CVG-1*

15 **SEQ ID NO: 105** is an amino acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CviK1*

SEQ ID NO: 106 is an amino acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus IL-5-2s1*

SEQ ID NO: 107 is an amino acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CZ-2*

20 **SEQ ID NO: 108** is an amino acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CVG-1*

DETAILED DESCRIPTION OF THE INVENTION

25 The inventors have conceived genetically modified cells, and especially genetically modified yeasts, having the ability to produce hyaluronic acid, as compared to the parent cells, and especially as compared to the parent yeasts which are not naturally capable of doing so.

These genetically modified cells are described throughout the present specification.

Definitions

The term "Hyaluronic acid" also known as hyaluronan or HA, is polysaccharide composed of D-glucuronic acid and N-acetyl-D-glucosamine, linked via alternating β -(1 $\rightarrow$ 4) and β -(1 $\rightarrow$ 3) glycosidic bonds and having the chemical formula (C₁₄H₂₁NO₁₁)_n.

5 Hyaluronic acid may be produced in a recombinant cell.

As used herein, the term "recombinant", when used in reference to a cell, indicates that the cell has been modified by the introduction of an endogenous and/or heterologous nucleic acid or protein into the cell or the alteration of a native cell or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes or
10 nucleic acid that are not found within the native (non-recombinant) form of the cell or express native (eg endogenous) genes at a different level than their native level or express additional or supplementary copies of native (eg endogenous) at a different level than their native level.

As used herein, the term "recombinant", when used in reference to a nucleic acid or vector, are sequences formed/obtained by techniques of genetic engineering well known to
15 the man skilled in the art. The guidelines of the US National Institute for Health (NIH) accordingly indicate that :

"recombinant [...] nucleic acids are defined as:

i. molecules that (a) are constructed by joining nucleic acid molecules and (b) that can replicate in a living cell, i.e., recombinant nucleic acids;",

20 which reflects the conventional use of the word "recombinant" attached to nucleic acid sequences to mean recombined following the insertion of, or joining together with, another nucleic acid.

Proteins that result from the expression of recombinant DNA or recombinant vector within living cells are also termed recombinant proteins.

25 The term "recombinant" is accordingly synonymous with the term "genetically modified". The term "gene" is synonymous with the term "nucleic acid" or "nucleotide sequence".

A recombinant nucleic acid sequence for use in a recombinant cell, and in particular a recombinant yeast, of the invention may be provided in the form of a nucleic acid
30 construct. The term "nucleic acid construct" refers to as a nucleic acid molecule, either single- or double-stranded, which is isolated or derived from a native (eg endogenous) naturally occurring gene or is a heterologous nucleic acid or which has been modified to contain segments of nucleic acid which are combined and juxtaposed in a manner which would not

otherwise exist in nature. The term "nucleic acid construct" is synonymous with the term "expression cassette" or "heterologous nucleic acid expression cassette" when the nucleic acid construct contains one or more regulatory elements required for expression of a coding sequence, wherein said control sequences are operably linked to said coding sequence. Non-limiting examples of regulatory elements include promoters, enhancers, silencers, terminators, and poly-A signals.

A recombinant nucleic acid sequence for use in a recombinant cell of the invention may be provided in the form of an expression vector, wherein the polynucleotide sequence is operably linked to at least one control sequence for the expression of the polynucleotide sequence in a recombinant cell.

The terms "obtained from" or "originate from" or "originating from" a microorganism or animal generally means that a substance (e.g., a nucleic acid molecule or polypeptide) originating from the microorganism or animal is native to that microorganism or animal.

The terms "derived from" a microorganism or animal generally means that a substance (e.g., a nucleic acid molecule or polypeptide) derived from the microorganism or animal is the result of modifications brought to the substance native to (i.e. present as such) in that microorganism or animal. For example, regarding a nucleic acid sequence derived from a microorganism or animal, said nucleic acid sequence can correspond to a reencoded and/or truncated version of the native nucleic acid sequence from this microorganism or animal. Other modifications well known to the man skilled in the art can be brought to the native substance of a microorganism or animal leading to the substance used being "derived from" said microorganism or animal.

As used herein, the term "polypeptide" refers to a molecule comprising amino acid residues linked by peptide bonds and containing more than five amino acid residues. The amino acids are identified by either the single-letter or three-letter designations. The term "protein" as used herein is synonymous with the term "polypeptide" and may also refer to two or more polypeptides. Thus, the terms "protein", "peptide" and "polypeptide" can be used interchangeably. Polypeptides may optionally be modified (e.g., glycosylated, phosphorylated, acylated, farnesylated, prenylated, sulfonated, and the like) to add functionality. Polypeptides exhibiting activity may be referred to as enzymes. It will be

understood that, as a result of the degeneracy of the genetic code, a multitude of nucleotide sequences encoding a given polypeptide may be produced.

A polypeptide encoded by a recombinant nucleic acid for use in a recombinant cell, and in particular in a recombinant yeast, of the invention may comprise a signal peptide
5 and/or a propeptide sequence. In the event that a polypeptide expressed by a recombinant cell, and in particular a recombinant yeast, of the invention comprises a signal peptide and/or a propeptide, sequence identity may be calculated over the mature polypeptide sequence.

The term "operably linked" as used herein refers to two or more nucleic acid sequence elements that are physically linked and are in a functional relationship with each
10 other. For instance, a promoter is operably linked to a coding sequence if the promoter is able to initiate or regulate the transcription or expression of the coding sequence, in which case the coding sequence should be understood as being "under the control of" the promoter. Generally, when two nucleic acid sequences are operably linked, they will be in the same orientation and usually also in the same reading frame. They usually will be essentially
15 contiguous, although this may not be required.

The term "native" or "endogenous" as used herein with reference to molecules, and in particular enzymes and nucleic acids, indicates molecules that are expressed in the organism in which they originated or are found in nature.

The term "endogenous gene" means that the gene was present in the cell before
20 any genetic modification, in the wild-type strain. Endogenous genes may be overexpressed by introducing heterologous sequences in addition to, or to replace endogenous regulatory elements, or by introducing one or more additional or supplementary copies of the gene into the chromosome or a plasmid (said additional or supplementary copies being designated
25 "exogenous or heterologous genes" or "heterologous nucleotide sequences" or "heterologous nucleic acids" as defined herein). Endogenous genes may also be modified to modulate their expression and/or activity. For example, mutations may be introduced into the coding sequence to modify the gene product or heterologous sequences may be introduced in addition to or to replace endogenous regulatory elements. Modulation of an endogenous gene may result in the up-regulation and/or enhancement of the activity of the gene product, or
30 alternatively, in the down-regulation and/or attenuation of the activity of the endogenous gene product. Another way to enhance expression of endogenous genes is to introduce one or more additional or supplementary copies of the gene onto the chromosome or a plasmid (said

supplementary copies being designated “exogenous or heterologous genes” or “heterologous nucleotide sequences” or heterologous nucleic acids” as defined herein).

By “one or more additional or supplementary copies of a gene” according to the invention, it is for example understood in the present invention from 1 to 50 copies, in particular from 1 to 30 copies, more particularly from 1 to 20 copies, and preferably from 1 to 10 copies. Said copies may be inserted into the same locus or into different loci of a recombinant cell of the invention.

The term “exogenous gene” means that the gene was introduced into a cell, by means well known to the man skilled in the art, whereas this gene is or is not naturally occurring in the wild-type cell. Cells can express exogenous genes if these genes are introduced into the cell with all the elements allowing their expression in the cell. Transforming cells with exogenous DNA is a routine task for the man skilled in the art. Exogenous genes may be integrated into the host chromosome, or be expressed extra-chromosomally from plasmids or vectors. A variety of plasmids, which differ with respect to their origin of replication and their copy number in the cell, are all known in the art. The sequence of exogenous genes may be adapted for its expression in the cell. Indeed, the man skilled in the art knows the notion of codon usage bias and how to adapt nucleic sequences for a particular codon usage bias without modifying the deduced protein. In particular embodiments, codon optimized genes express native enzymes.

The term “heterologous gene” or heterologous nucleic acid sequence” refers to a gene or nucleic acid sequence not normally found in a given cell in nature. As such, a heterologous nucleic acid sequence may be: (a) foreign to its host cell (i.e. is “exogenous” to the cell); (b) naturally found in the host cell (i.e. “endogenous”) but present at an unnatural quantity in the cell (i.e., greater or lesser quantity than naturally found in the host cell); or (c) be naturally found in the host cell but positioned outside of its natural locus.

In the present application, all genes are referenced with their common names and with references to their nucleotide sequences and, the case arising, to their amino acid sequences. Using the references given in accession number for known genes, those skilled in the art are able to determine the equivalent genes in other organisms, bacterial strains, yeast, fungi, mammals, plants, etc. This routine work is advantageously done using consensus sequences that can be determined by carrying out sequence alignments with genes derived from other cells and designing degenerated probes to clone the corresponding gene in another organism.

The man skilled in the art knows different means to modulate, and in particular up-regulate or down-regulate, the expression of endogenous genes. For example, a way to enhance expression of, or over express, endogenous genes is to introduce one or more additional or supplementary copies of the gene onto the chromosome or a plasmid.

5 Another way is to replace the endogenous promoter of a gene with a stronger promoter. These promoters may be homologous or heterologous. Promoters particularly interesting in the present invention are described in more detail elsewhere in the present specification.

10 The nucleic acid expression construct may further comprise 5' and/or 3' recognition sequences and/or selection markers.

The term “inducible promoter” is used to qualify a promoter whose activity is induced, i.e. increased:

- in the presence of one or more particular metabolite(s). The higher the metabolite concentration in the medium, the stronger the promoter activity; or
- 15 - in the presence of a low concentration, or in the absence, of one or more metabolite(s). These metabolites are different from those whose increasing presence induces the activity of the promoter. The lower the metabolite concentration in the medium, the stronger the promoter activity.

20 The term “repressible promoter” is used to qualify a promoter whose activity is repressed, i.e. reduced:

- in the presence of one or more particular metabolite(s). The higher the metabolite concentration in the medium, the weaker the promoter activity; or
- 25 - in the presence of a low concentration, or in the absence, of one or more metabolite(s). These metabolites are different from those whose increasing presence represses the activity of the promoter. The lower the metabolite concentration in the medium, the weaker the promoter activity.

30 As used herein, the term “anchoring signal” when used in conjunction with a protein or polypeptide such as an enzyme (such as, for example hyaluronidase) means for example a first nucleic acid encoding a protein that is operably linked to a second nucleic acid encoding a protein or a polypeptide, or a first protein or polypeptide that is operably linked to a second protein or polypeptide, such as an enzyme (such as, for example hyaluronidase to form, for example a fusion protein), and that enables the cellular transport machinery of a cell,

in particular of a *S. cerevisiae* cell, to correctly anchor and/or position in the membrane of the cell the second protein operably linked to the first protein.

As used herein, the term “secretion signal” when used in conjunction with a protein or polypeptide such as an enzyme (such as, for example hyaluronidase) means for example a first nucleic acid encoding a peptide or protein that is operably linked to a second nucleic acid encoding a protein, or a first protein that is linked to a second protein, such as an enzyme (such as, for example hyaluronidase to form, for example a fusion protein), and that enables the cellular transport machinery of a cell, in particular of a *S. cerevisiae* cell, to locate at least the second protein to the membrane of the cell and to secrete the second protein outside of the cell, after the first protein has, for example, been cleaved from the second protein.

As used herein the terms “secretion signal” and “anchoring signal” when used in conjunction with a protein or polypeptide such as an enzyme (such as, for example, hyaluronidase), mean for example a first nucleic acid encoding a peptide or protein that is operably linked to a second nucleic acid encoding a protein, or a first protein that is operably linked to a second protein, such as an enzyme (such as, for example hyaluronidase), and that enables the cellular transport machinery of a cell, in particular of a *S. cerevisiae* cell, to locate at least the second protein to the membrane of the cell wherein if the second protein is also operably linked to an “anchoring signal” the second protein is not secreted but remains attached to the membrane of the cell. In some cases, a secretion-anchoring signal may provide a dual secretion signal and anchoring signal function.

Sequences of secretion and anchoring signals, methods for the expression, anchoring and/or secretion of heterologous proteins, such as enzymes (such as, for example, hyaluronidase) on the surface of a cell (such, as for example, a yeast cell) are well known in the art (see for example, Ast et al (2013) Cell 152: 1134-1145, Ast and Schuldiner (2013) Crit Rev Biochem Mol Biol 48(3) 273-288, Van der Vaart et al (1997) Applied Environmental Microbiology 63(2) 615-620 and the entire contents of each of these publications are incorporated herein by reference).

The “activity” of an enzyme is used interchangeably with the term “function” and designates, in the context of the invention, the capacity of an enzyme to catalyze a desired reaction. The amount of an enzyme in a host cell may be altered by modifying the transcription of the gene that encodes the enzyme. This can be achieved for example by modifying the copy number of the nucleotide sequence encoding the enzyme (e.g., by using a

higher or lower copy number expression vector comprising the nucleotide sequence, or by introducing additional copies of the nucleotide sequence into the genome of the host cell or by deleting or disrupting the nucleotide sequence in the genome of the host cell), by changing the order of coding sequences on a polycistronic mRNA of an operon or breaking up an operon
5 into individual genes each with its own control elements, or by increasing the strength of the promoter or operator to which the nucleotide sequence is operably linked.

Alternatively or in addition, the copy number of an enzyme in a host cell may be altered by modifying the level of translation of an mRNA that encodes the enzyme. This can be achieved for example by modifying the stability of the mRNA, modifying the sequence of
10 the ribosome binding site, modifying the distance or sequence between the ribosome binding site and the start codon of the enzyme coding sequence, modifying the entire intercistronic region located "upstream of" or adjacent to the 5' side of the start codon of the enzyme coding region, stabilizing the 3'-end of the mRNA transcript using hairpins and specialized sequences, modifying the codon usage of enzyme, altering expression of rare codon tRNAs
15 used in the biosynthesis of the enzyme, and/or increasing the stability of the enzyme, as, for example, via mutation of its coding sequence.

The activity of an enzyme in a host cell can be altered in a number of ways, including, but not limited to, expressing a modified form of the enzyme that exhibits increased or decreased solubility in the host cell, expressing an altered form of the enzyme
20 that lacks a domain through which the activity of the enzyme is inhibited, expressing a modified form of the enzyme that has a higher or lower K_{cat} or a lower or higher K_m for the substrate, or expressing an altered form of the enzyme that is more or less affected by feed back or feed-forward regulation by another molecule in the pathway.

The terms "encoding" or "coding for" refer to the process by which a
25 polynucleotide, through the mechanisms of transcription and translation, produces an amino-acid sequence.

The gene(s) encoding the enzyme(s) considered in the present invention can be exogenous or endogenous.

The methods implemented in the present invention preferably require the use of
30 one or more chromosomal integration constructs for the stable introduction of a heterologous nucleotide sequence into a specific location on a chromosome or for the functional disruption of one or more target genes in a genetically modified cell. In some embodiments, disruption of a target gene prevents the expression of the related functional protein. In some

embodiments, disruption of a target gene results in the expression of a non-functional protein from the disrupted gene.

Accordingly, a “disrupted endogeneous nucleic acid” in the present invention relates to an endogeneous nucleic acid, or gene, unable to encode the functional, or fully functional, protein or polypeptide it codes for before it was disrupted. The nucleic acid can for example be disrupted by the introduction of an integration construct into the nucleic acid, as illustrated in the examples. Said integration can for example prevent the expression of the related functional protein or polypeptide or result in the expression of a non-functional, or non-fully functional protein or polypeptide from the disrupted gene.

Parameters of chromosomal integration constructs that may be varied in the practice of the present invention include, but are not limited to, the lengths of the homologous sequences; the nucleotide sequence of the homologous sequences; the length of the integrating sequence; the nucleotide sequence of the integrating sequence; and the nucleotide sequence of the target locus. In some embodiments, an effective range for the length of each homologous sequence is 20 to 5,000 base pairs, preferentially 50 to 100 base pairs. In particular embodiments, the length of each homologous sequence is about 50 base pairs. For more information on the length of homology required for gene targeting, see D. Burke *et al.*, Methods in yeast Genetics – A cold spring harbor laboratory course Manual (2000).

In some embodiments, (a) disrupted gene(s) in which the above-mentioned DNA construct(s) is/are intended to be inserted may advantageously comprises one or more selectable markers useful for the selection of transformed cells. Preferably, said selectable marker(s) are comprised in the DNA construct(s) according to the present invention.

In some embodiments, the selectable marker is an antibiotic resistance marker. Illustrative examples of antibiotic resistance markers include, but are not limited to the, NAT1, AURI-C, HPH, DSDA, KAN<R>, and SH BLE gene products. The NAT 1 gene product from *S. noursei* confers resistance to nourseothricin; the AURI-C gene product from *Saccharomyces cerevisiae* confers resistance to Auerobasidin A (AbA); the HPH gene product of *Klebsiella pneumoniae* confers resistance to Hygromycin B; the DSDA gene product of *E. coli* allows cells to grow on plates with D-serine as the sole nitrogen source; the KAN<R> gene of the Tn903 transposon confers resistance to G418; and the SH BLE gene product from *Streptoalloteichus hindustanus* confers resistance to Zeocin (bleomycin).

In some embodiments, the antibiotic resistance marker is deleted after the genetically modified cell of the invention is isolated. The man skilled in the art is able to choose suitable marker in specific genetic context.

In a particular embodiment, a recombinant cell according to the invention is
5 devoid of any antibiotic resistance marker. This advantageously prevents the necessity to add antibiotics in the selection medium.

In some embodiments, the selectable marker rescues an auxotrophy (e.g., a nutritional auxotrophy) in the genetically modified cell. In such embodiments, a parent cell, and in particular a parent yeast, comprises a functional disruption in one or more gene
10 products that function in an amino acid or nucleotide biosynthetic pathway, such as, for example, the HIS3, LEU2, LYS1, LYS2, MET15, TRP1, ADE2, and URA3 gene products in yeast, which renders the parent cell incapable of growing in media without supplementation with one or more nutrients (auxotrophic phenotype). The auxotrophic phenotype can then be rescued by transforming the parent cell with a chromosomal integration encoding a functional
15 copy of the disrupted gene product (in some embodiments the functional copy of the gene may originate from close species, such as *Kluyveromyces*, *Candida* etc.), and the genetically modified cell generated can be selected based on the loss of the auxotrophic phenotype of the parent microbial cell.

For each of the nucleic acid sequences comprising a promoter sequence, a
20 coding sequence (e.g. an enzyme coding sequence), or a terminator sequence, reference sequences are described herein. The present description also encompasses nucleic acid sequences having specific percentages of nucleic acid identity with a reference nucleic acid sequence.

For each or the amino acid sequences of interest, reference sequences are
25 described herein. The present description also encompasses amino acid sequences (e.g. enzyme amino acid sequences), having specific percentages of amino acid identity with a reference amino acid sequence.

For obvious reasons, in all the present description, a specific nucleic acid sequence or a specific amino acid sequence which complies with, respectively, the considered
30 nucleotide or amino acid identity, should further lead to obtaining a protein (or enzyme) which displays the desired biological activity. As used herein, the "percentage of identity" between two nucleic acid sequences or between two amino acid sequences is determined by comparing both optimally aligned sequences through a comparison window.

The portion of the nucleotide or amino-acid sequence in the comparison window may thus include additions or deletions (for example "gaps") as compared to the reference sequence (which does not include these additions or these deletions) so as to obtain an optimal alignment between both sequences.

5 The terms "sequence homology" or "sequence identity" or "homology" or "identity" are used interchangeably herein. For the purpose of the invention, it is defined here that in order to determine the percentage of sequence homology or sequence identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes. In order to optimize the alignment between the two sequences gaps
10 may be introduced in any of the two sequences that are compared. Such alignment can be carried out over the full length of the sequences being compared. Alternatively, the alignment may be carried out over a shorter length, for example over about 20, about 50, about 100 or more nucleic acids/bases or amino acids. The sequence identity is the percentage of identical matches between the two sequences over the reported aligned region.

15 A comparison of sequences and determination of percentage of sequence identity between two sequences can be accomplished using a mathematical algorithm. The skilled person will be aware of the fact that several different computer programs are available to align two sequences and determine the identity between two sequences (Kruskal, J. B. (1983) An overview of sequence comparison In D. Sankoff and J. B. Kruskal, (ed.), Time
20 warps, string edits and macromolecules: the theory and practice of sequence comparison, pp. 1-44 Addison Wesley).

The percent sequence identity between two amino acid sequences or between two nucleotide sequences may be determined using the Needleman and Wunsch algorithm for the alignment of two sequences. (Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol.
25 48, 443-453). Both amino acid sequences and nucleotide sequences can be aligned by the algorithm. The Needleman-Wunsch algorithm has been implemented in the computer program NEEDLE.

For the purpose of the invention, the NEEDLE program from the EMBOSS package was used (version 2.8.0 or higher, EMBOSS: The European Molecular Biology Open
30 Software Suite (2000) Rice, P. Longden J. and Bleasby, A. Trends in Genetics 16, (6) pp276—277, <http://emboss.bioinformatics.nl/>). For protein sequences EBLOSUM62 is used for the substitution matrix. For nucleotide sequence, EDNAFULL is used. The optional parameters used are a gap opening penalty of 10 and a gap extension penalty of 0.5. No end gap penalty

is added. In the Output section, Yes has been indicated in response to the question “Brief identity and similarity” and “SRS pairwise” indicated as Output alignment format.

After alignment by the program NEEDLE as described above the percentage of sequence identity between a query sequence and a sequence of the invention is calculated as follows: Number of corresponding positions in the alignment showing an identical amino acid or identical nucleotide in both sequences divided by the total length of the alignment after subtraction of the total number of gaps in the alignment. The identity defined as herein can be obtained from NEEDLE by using the NOBRIEF option and is labeled in the output of the program as "longest-identity".

The similarity of nucleotide and amino acid sequences, i.e. the percentage of sequence identity, can be determined via sequence alignments using several other art-known algorithms, preferably with the mathematical algorithm of Karlin and Altschul (Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5877), with hmalign (HMMER package, <http://hmmer.wustl.edu/>) or with the CLUSTAL algorithm (Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) Nucleic Acids Res. 22, 4673-80) available e.g. on <https://www.ebi.ac.uk/Tools/msa/clustalo/> or the GAP program (mathematical algorithm of the University of Iowa) or the mathematical algorithm of Myers and Miller (1989 - Cabios 4: 11-17) or Clone Manager 9. Preferred parameters used are the default parameters as they are set on <https://www.ebi.ac.uk/Tools/msa/clustalo/>.

The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX). A similar algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al (1990) J. Mol. Biol. 215, 403-410. BLAST polynucleotide searches are performed with the BLASTN program, score = 100, word length = 12, to obtain polynucleotide sequences that are homologous to those nucleic acids which encode the relevant protein.

BLAST protein searches are performed with the BLASTP program, score = 50, word length = 3, to obtain amino acid sequences homologous to the SHC polypeptide. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al (1997) Nucleic Acids Res. 25, 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used. Sequence matching analysis may be supplemented by established homology mapping techniques like Shuffle-LAGAN (Brudno M., Bioinformatics 2003b, 19 Suppl 1: 154-162) or

Markov random fields. When percentages of sequence identity are referred to in the present application, these percentages are calculated in relation to the full length of the longer sequence, if not specifically indicated otherwise.

In particular embodiments, % identity between two sequences is determined
5 using CLUSTAL O (version 1.2.4).

The "fermentation" or "culture" is generally conducted in fermenters with an appropriate culture medium adapted to the cell being cultivated, containing at least one simple carbon source, and if necessary co-substrates.

10 The term "fermentation composition" refers to a composition which comprises genetically modified host cells and products or metabolites produced by the genetically modified host cells. An example of a fermentation composition is a whole cell broth, which can be the entire contents of a vessel (e.g., a flasks, plate, or fermentor), including cells, aqueous phase, and compounds produced from the genetically modified host cells.

15 The term "medium" refers to a culture medium or cultivation medium or fermentation medium.

For maximal production of hyaluronic acid, the recombinant cells used as production hosts preferably have a high rate of carbohydrate utilization. These characteristics
20 may be conferred by mutagenesis and selection, genetic engineering, or may be natural. Fermentation media, or "culture medium" or "cultivation medium", for the present cells may contain at least about 10 g/L of glucose and/or sucrose. Additional carbon substrates may include but are not limited to monosaccharides such as fructose, mannose, xylose and arabinose; oligosaccharides such as lactose, maltose, galactose, or sucrose; polysaccharides
25 such as starch or cellulose or mixtures thereof and unpurified mixtures from renewable feedstocks such as cheese whey permeate cornsteep liquor, sugar beet molasses, and barley malt. Other carbon substrates may include glycerol, acetate and/or ethanol.

Hence, it is contemplated that the source of carbon utilized in the present invention may encompass a wide variety of carbon containing substrates and will only be
30 limited by the choice of cell, and in particular of yeast.

Although it is contemplated that all of the above-mentioned carbon substrates and mixtures thereof are suitable in the present invention, preferred carbon substrates are glucose, fructose, and sucrose, or mixtures of these with C5 sugars such as xylose and/or

arabinose for cells, and in particular yeasts, modified to use C5 sugars, and more particularly glucose.

Preferred carbon substrates are glucose or sucrose.

In addition to an appropriate carbon source, fermentation media may contain
5 suitable minerals, salts, cofactors, buffers and other components, known to those skilled in the art, suitable for the growth of the cultures and promotion of the enzymatic pathway necessary for the production of the desired product.

Besides, additional genetic modifications suitable for the growth of recombinant cells according to the invention may be considered.

10 The terms "Aerobic conditions" refers to concentrations of oxygen in the culture medium that are sufficient for an aerobic or facultative anaerobic cell, and in particular yeast, to use di-oxygen as a terminal electron acceptor.

"Microaerobic condition" refers to a culture medium in which the concentration of oxygen is less than that in air, i.e. oxygen concentration up to 6% O₂.

15 An "appropriate culture medium" designates a medium (e.g. a sterile, liquid medium) comprising nutrients essential or beneficial to the maintenance and/or growth of the cell such as carbon sources or carbon substrate, nitrogen sources, for example, peptone, yeast extracts, meat extracts, malt extracts, urea, ammonium sulfate, ammonium chloride, ammonium nitrate and ammonium phosphate; phosphorus sources, for example,
20 monopotassium phosphate or dipotassium phosphate; trace elements (e.g., metal salts), for example magnesium salts, cobalt salts and/or manganese salts; as well as growth factors such as amino acids, vitamins, growth promoters, and the like. The term "carbon source" or "carbon substrate" or "source of carbon" according to the present invention denotes any source of carbon that can be used by those skilled in the art to support the normal growth of a
25 cell, including hexoses (such as glucose, galactose or lactose), pentoses, monosaccharides, oligosaccharides, disaccharides (such as sucrose, cellobiose or maltose), molasses, starch or its derivatives, cellulose, hemicelluloses and combinations thereof.

Culture mediums that are particularly appropriate to produce recombinant cells of the invention, and in particular recombinant yeasts of the invention, are described in greater
30 details further below in the text.

As used herein, the term "about" refers to a reasonable range about a value as determined by the practitioner of skill. In certain embodiments, the term about refers to $\pm$ one,

two, or three standard deviations. In certain embodiments, the term about refers to $\pm 5\%$, 10%, 20%, or 25%. In certain embodiments, the term about refers to ± 0.1 , 0.2, or 0.3 logarithmic units, e.g. pH units.

5 General features of genetic modifications introduced according to the invention

- All the genome modifications are inserted in recombinant cells, and in particular recombinant yeasts, according to known genetic engineering techniques:

- The successive nucleic acid sequences included in a gene construct that is introduced in the recombinant cell genome according to the invention are of the following structure:

Prom₁-ORF₁-term₁-ORF₂-gene₂-term₂ - .../... -Prom_n-ORF_n-term_n, wherein:

- Prom₁ is a sequence regulating the expression of the coding sequence ORF₁,

- ORF₁ is a nucleic acid sequence encoding a desired protein PROT₁, and especially a desired enzyme PROT₁,

- Term₁ is a transcription terminator sequence that mediates transcriptional termination by providing signals in the newly synthesized mRNA that trigger processes which release the mRNA from the transcriptional complex, and

- “1”, “2”, .../... “n” may or may not describe the same ORF (Open Reading Frame), promoter or terminator. The order of the nucleic acid sequences does not matter. “n” is an integer usually ranging from 5 and 20. These constructs are inserted in one of the recombinant cell chromosome at a controlled location. In some embodiments, the insertion site is neither essential for the functionality of the inserted construct, nor for the viability of the resulting genetically modified cell.

As will be understood by those of skill in the art, it can be advantageous to modify a coding sequence to enhance its expression in a particular host. The genetic code is redundant with 64 possible codons, but most organisms typically use a subset of these codons. The codons that are utilized most often in a species are called optimal codons, and those not utilized very often are classified as rare or low-usage codons. Codons can be substituted to reflect the preferred codon usage of the host, in a process sometimes called “codon optimization” or “controlling for species codon bias.” Codon optimization for other host cells can be readily determined using codon usage tables or can be performed using commercially available software, such as CodonOp (www.idtdna.com/CodonOptfrom) from Integrated DNA Technologies. Optimized coding sequences containing codons preferred by a particular

prokaryotic or eukaryotic host (Murray et al, 1989 , Nucl Acids Res . 17: 477-508) can be prepared, for example, to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced from a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, typical stop codons for *S. cerevisiae* and mammals are UAA and UGA, respectively. The typical stop codon for monocotyledonous plants is UGA, whereas insects and *E. coli* commonly use UAA as the stop codon (Dalphin et al, 1996, Nucl Acids Res. 24: 216-8).

- When the recombinant cell is a yeast cell, and in particular is *Saccharomyces cerevisiae* yeast cell, nucleic acid sequences introduced in the yeast genome and originating from other organisms than *Saccharomyces cerevisiae* are generally “transcoded” (generally “codon-optimized”), meaning that these nucleic acid sequences are synthesized with an optimal codon usage for expression in *S. cerevisiae*. The nucleotide sequence (and not the protein sequence) of some nucleic acid sequences from *S. cerevisiae* has also been modified (“transcoded”) to minimize recombination with an endogenous copy of the said gene.

- Genes may be deleted through standard procedures used in cell genetic engineering. In some embodiments, the genes targeted for deletion may be interrupted by insertion of one of the above-described gene constructs, or alternatively the genes targeted for deletion are replaced by a short stretch of nucleotide.

- A nucleic acid sequences may be rendered “inducible or repressible” by deleting an endogenous copy of the nucleic acid sequences (if necessary) and placing a new copy of the ORF under the control of an inducible or repressible promoter. An inducible or repressible promoter is a promoter which activity is modulated or controlled, i.e. either increased or decreased, upon a change in the environmental conditions or external stimuli. Induction or repression may be artificially controlled, which encompasses induction or repression by abiotic factors such as chemical compounds not found naturally in the cell, and in particular yeast, of interest, light, oxygen levels, heat or cold. A list and sequences of inducible or repressible promoters are described elsewhere in the present specification.

Recombinant cells according to the invention

The inventors have conceived recombinant cells, in particular recombinant yeasts, having an ability of producing hyaluronic acid.

The present invention relates to recombinant cells, and in particular recombinant yeasts, having the ability to produce hyaluronic acid, and wherein this ability to produce hyaluronic acid is obtained through a plurality of alterations that have been introduced in the genome thereof, by genetic engineering methods.

5 The present invention pertains to a recombinant yeast cell producing hyaluronic acid (HA), wherein the recombinant cell comprises:

(a) one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity;

(b) one or more recombinant nucleic acids encoding a polypeptide having UDP-
10 Glucose dehydrogenase (UDP-GlcDH or HASB) activity;

(c) one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity wherein the polypeptide having hyaluronidase activity comprises a secretion signal so that hyaluronic acid, in particular of a desired molecular weight (HAMW), is produced by the recombinant yeast cell, and

15 (d) (i) one or more recombinant nucleic acids encoding a polypeptide having a glutamine synthetase (GLN1) activity; and/or

(ii) one or more disrupted endogeneous nucleic acids encoding a glutamate synthase (GLT1);

wherein said recombinant yeast cell belongs to the *Saccharomyces* genus, or to
20 the *Candida* genus, or to the *Kluyveromyces* genus, or to the *Ogataea* genus, or to the *Yarrowia* genus, or to the *Debaryomyces* genus, or to the *Ashbya* genus, and is in particular selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Saccharomyces bayanus*, *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces castelli*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*,
25 *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces polysporus*, *Kluyveromyces thermotolerans*, *Ogataea polymorpha*, *Yarrowia lipolytica*, *Debaryomyces hansenii*, and *Ashbya gossypii*, and is preferably *Saccharomyces cerevisiae*.

The present invention further relates to a recombinant host cell producing hyaluronic acid (HA) wherein the recombinant host cell comprises:

30 (a) one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity;

(b) one or more recombinant nucleic acids encoding a polypeptide having UDP-
Glucose dehydrogenase (UDP-GlcDH or HASB) activity;

(c) one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity wherein the polypeptide having hyaluronidase activity comprises a secretion signal and an anchoring signal so that hyaluronic acid, in particular of a desired molecular weight (HAMW) is produced by the host cell; and

5 (d) (i) one or more recombinant nucleic acids encoding a polypeptide having a glutamine synthetase (GLN1) activity; and/or

(ii) one or more disrupted endogeneous nucleic acids encoding a glutamate synthase (GLT1).

The inventors have found that an ability to produce hyaluronic acid by cells, and
10 in particular yeast cells, may be reached by introducing in the genome of these cells a plurality of genetic alterations.

The production of hyaluronic acid by cells of the invention, and in particular by yeast cells of the invention, has been achieved by optimizing the endogenous metabolism of UDP-Glucose, and optionally UDP-N-Acetyl-glucosamine, and direct the subsequent
15 artificially modified metabolic pathway mainly towards hyaluronic acid production while in the same time maintaining an optimal viability of the resulting genetically modified cells.

It has been determined that a hyaluronic acid production by recombinant cells according to the invention may be increased by increasing the conversion of glucose-6-phosphate into the successive intermediate metabolites (i) glucose-1-phosphate, UDP-glucose,
20 UDP-glucuronate and hyaluronic acid and (ii) fructose-6-phosphate, glucosamine-6-phosphate, N-acetyl-glucosamine-6-phosphate, N-acetyl-glucosamine-1-phosphate, UDP-N-acetyl-glucosamine and hyaluronic acid, while maintaining a metabolic balance allowing a good viability of the resulting recombinant cells.

Indeed, in order to obtain a viable recombinant cell of the invention, many
25 different constructs were tested in order to obtain a viable and efficient recombinant cell, and in particular a viable recombinant yeast. In particular such recombinant yeast were difficult to obtain because the temporary accumulation of some intermediates appeared to be toxic for yeasts.

Unexpected technical difficulties were encountered in establishing the
30 conditions suitable for the preparation of a recombinant cell able to produce hyaluronic acid, and in particular to produce hyaluronic acid with a controlled molecular weight.

By "controlled" molecular weight of a hyaluronic acid of the invention is intended to mean that at least 80%, in particular at least 85%, of the hyaluronic acid produced

by a method of the invention has a molecular weight comprised within a certain molecular weight's range through the regulation of at least one parameter of the method and/or of the recombinant cell of the invention, such as for example:

- 5 - the nature and origin of the nucleic acid encoding the hyaluronidase of the recombinant cell,
- the nature and origin of the promoter controlling the expression of the nucleic acid encoding the hyaluronidase(s) of the recombinant cell,
- the presence of an anchoring and/or of a secretion signal associated to the encoded hyaluronidase(s) of the recombinant cell,
- 10 - the pH of the culture medium during the step of culturing the recombinant cell, and/or
- the duration of the culturing of the recombinant cell.

Indeed, after much research and experimental trial, the inventors discovered that it was possible to cultivate a recombinant cell, and in particular a recombinant yeast cell, more particularly a recombinant *Saccharomyces cerevisiae* yeast cell, able to produce hyaluronic acid having a controlled molecular weight controlled using the following parameters:

- 20 - the selection of the nature and origin of the nucleic acid sequences encoding the polypeptide having hyaluronidase activity of the recombinant cell, in particular the recombinant yeast, of the invention; and/or
- the nature and origin of the promoter controlling the expression of the nucleic acid sequences encoding the polypeptide having hyaluronidase activity of the recombinant cell, in particular the recombinant yeast, of the invention; and/or
- 25 - the optional presence of an anchoring signal, in addition to a secretion signal, associated to the encoded polypeptide having hyaluronidase activity of the recombinant cell, in particular the recombinant yeast, according to the invention; and/or
- the pH of the culture medium during the step of culturing the recombinant cell, in particular the recombinant yeast cell, according to the invention; and/or
- the duration of the culturing of the recombinant cell, in particular the recombinant yeast cell, according to the invention.
- 30

To the inventors' knowledge, this has never been achieved before.

The molecular weight of the hyaluronic acid (HA) can be in the range of less than 50kDa, preferably in the range of about 20kDa to about 50kDa.

Alternatively, the molecular weight of the hyaluronic acid (HA) can be in the range of greater than 50kDa, preferably in the range of about 50kDa to about 250kD.

In another alternative, the molecular weight of the HA can be in the range of greater than 100kDa, preferably in the range of about 100kDa to about 1500kDa.

5 The nucleic acid encoding a polypeptide having a glutamine synthetase activity may be obtained or derived from *Saccharomyces cerevisiae*.

The nucleic acid encoding a polypeptide having hyaluronidase activity in a recombinant cell of the invention may be obtained or derived from at least one of *Cupiennius salei*, *Loxosceles intermedia*, *Hirudo nipponia*, *Bothrops atrox* or *Tityus serrulatus*.

10 The nucleic acid encoding a polypeptide having hyaluronan synthase activity in a recombinant cell of the invention may be obtained or derived from at least one of *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Chlorella virus CviK1*, *Chlorella virus IL-5-2s1*, *Chlorella virus CZ-2*, *Chlorella virus CVG-1*, *Xenopus laevis* or *Pasteurella multocida*, and is in particular obtained or derived from *Streptococcus zooepidemicus*,
15 *Chlorella virus PBCV1*, *Chlorella virus CviK1*, *Chlorella virus IL-5-2s1*, *Chlorella virus CZ-2*, *Chlorella virus CVG-1* or *Xenopus laevis*.

The nucleic acid encoding a polypeptide having UDP-Glucose dehydrogenase activity in a recombinant cell of the invention may be obtained or derived from at least one of *Arabidopsis thaliana*, *Chlorella virus PBCV1* or *Streptococcus zooepidemicus*, and in
20 particular from *Arabidopsis thaliana* or *Chlorella virus PBCV1*.

The recombinant cell according to the invention can comprise a recombinant nucleic acid encoding one or more of:

- (i) a polypeptide having glutamine-fructose-6-phosphate amidotransferase (GFA1) activity; and/or
- 25 (ii) a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase (QRI1) activity.

The recombinant cell according to the invention can comprise at least one recombinant nucleic acid encoding one or more of:

- (i) a polypeptide having Phosphoglucomutase-1 (PGM1) activity; and/or
- 30 (ii) a polypeptide having UTP-glucose-1-phosphate uridylyltransferase (UGP1) activity; and/or
- (iii) a polypeptide having Glucosamine-6-phosphate N-acetyltransferase (GNA1) activity; and/or

(iv) a polypeptide having phosphoacetylglucosamine mutase (PCM1) activity.

The recombinant yeast cell according to the invention can be in particular selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Saccharomyces bayanus*, *Saccharomyces paradoxus*, *Saccharomyces mikatae*,
5 *Saccharomyces castelli*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces polysporus*, *Kluyveromyces thermotolerans*, *Ogataea polymorpha*, *Yarrowia lipolytica*, *Debaryomyces hansenii*, and *Ashbya gossypii*, and is preferably *Saccharomyces cerevisiae*.

The recombinant host cell according to the invention can belong to the
10 *Saccharomycetales* order and is in particular selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Saccharomyces bayanus*, *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces castelli*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces polysporus*, *Kluyveromyces thermotolerans*, *Ogataea polymorpha*,
15 *Yarrowia lipolytica*, *Debaryomyces hansenii*, and *Ashbya gossypii*, and is preferably *Saccharomyces cerevisiae*.

Another object of the invention relates to a method of producing hyaluronic acid (HA) of a desired molecular weight (HAMW) comprising:

(a) cultivating a recombinant cell as defined above, i.e. a recombinant yeast cell
20 or a recombinant host cell of the invention, in a cultivation medium for a time sufficient to produce hyaluronic acid (HA) of the desired molecular weight; and

(b) optionally isolating or recovering the hyaluronic acid (HA) from the recombinant cell and/or from the cultivation medium.

In the method of the invention, the HA may have a molecular weight of from
25 about 20 kDa to about 50 kDa, and preferably from 20 kDa to 30 kDa.

In the method of the invention, the HA may alternatively have a molecular weight of from 30 kDa to 50 kDa.

In the method of the invention, the HA may alternatively have a molecular weight of from about 50 kDa to about 150 kDa.

30 In the method of the invention, the HA may alternatively have a molecular weight of from about 150 kDa to about 1500 kDa.

In a method of the invention, the nucleic acid encoding a polypeptide having hyaluronidase activity may be obtained or derived from *Cupiennius salei*, *Loxosceles intermedia*, *Hirudo nipponia*, *Bothrops atrox* or *Tityus serrulatus*.

In a method of the invention, the nucleic acid encoding a polypeptide having
5 hyaluronan synthase activity may be obtained or derived from at least one of *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Chlorella virus CviK1*, *Chlorella virus IL-5-2s1*, *Chlorella virus CZ-2*, *Chlorella virus CVG-1*, *Xenopus laevis* or *Pasteurella multocida*, and is in particular obtained or derived from at least one of *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Chlorella virus CviK1*, *Chlorella virus IL-5-2s1*, *Chlorella virus CZ-2*,
10 *Chlorella virus CVG-1*, or *Xenopus laevis*.

In a method of the invention, the nucleic acid encoding a polypeptide having UDP-Glucose dehydrogenase activity may be obtained or derived from at least one of *Arabidopsis thaliana*, *Chlorella virus PBCV1* or *Streptococcus zooepidemicus*, and in particular obtained or derived from *Arabidopsis thaliana* or *Chlorella virus PBCV1*.

15 In the method of the invention, the recombinant cell may comprise at least one recombinant nucleic acid encoding one or more of:

(i) a polypeptide having glutamine-fructose-6-phosphate amidotransferase (GFA1) activity; and/or

(ii) a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase (QRI1)
20 activity.

In the method of the invention, the recombinant cell may comprise at least one recombinant nucleic acid encoding one or more of:

(i) a polypeptide having Phosphoglucomutase-1 (PGM1) activity;

(ii) a polypeptide having UTP-glucose-1-phosphate uridylyltransferase (UGP1)
25 activity;

(iii) a polypeptide having Glucosamine-6-phosphate N-acetyltransferase (GNA1) activity; and/or

(iv) a polypeptide having phosphoacetylglucosamine mutase (PCM1) activity.

In the method of the invention, the recombinant cell may be a member of the
30 genus *Saccharomyces*, and in particular is *Saccharomyces cerevisiae*.

In the method of the invention, the molecular weight of the hyaluronic acid may be controlled by the fermentation time.

In the method of the invention, the time sufficient to produce hyaluronic acid (HA) of a desired molecular weight may be a period of from about 35 hours to about 50 hours, preferably from about 40 hours to about 50 hours, preferably about 48 hours.

In the method of the invention, the molecular weight of the hyaluronic acid may
5 be controlled by the pH of the cultivation medium.

In the method of the invention, the molecular weight of the hyaluronic acid may be controlled by regulating the pH of the cultivation medium during the cultivation step (a) of a method of the invention.

In the method of the invention, the molecular weight of the hyaluronic acid may
10 be controlled by removing the biomass from the cultivation medium.

The method of the invention can be carried out on an industrial scale, preferably where the cultivation medium is at least about 100L, more preferably in the range of about 1000L to about 3000L, even more preferably about 10,000L or even more preferably about 100,000L, or even about 250,000L.

15

Another object of the invention relates to hyaluronic acid (HA) obtained or obtainable from a recombinant cell of the invention or from the method of the invention.

A further object of the invention relates to a cultivation medium comprising the hyaluronic acid (HA) according to the invention.

20 The present invention further relates to a composition comprising the hyaluronic acid (HA) according to the invention.

The present invention moreover relates to an industrial product or a consumer product or a consumable comprising (i) the HA having a molecular weight in the range of greater than 100kDa, preferably in the range of about 100kDa to about 1500kDa, (ii) the
25 cultivation medium of the invention or (iii) the composition of the invention.

The present invention also relates to use of a recombinant cell according to the invention for the production of hyaluronic acid (HA) having a molecular weight in the range of from about 20kDa to about 50kDa or from about 50kDa to about 1000 kDa.

Another object of the present invention relates to a method for producing
30 hyaluronic acid comprising the steps of:

(a) culturing a recombinant yeast according to the invention in a culture medium; and

(b) recovering the hyaluronic acid from said culture medium,

wherein the hyaluronic acid recovered in step (b) has a molecular weight controlled through the selection of:

- the nature and origin of the nucleic acid encoding the hyaluronidase of the recombinant yeast,
- 5 - the nature and origin of the promoter controlling the expression of the nucleic acid encoding the hyaluronidase(s) of the recombinant yeast,
- the presence of an anchoring and/or of a secretion signal associated to the encoded hyaluronidase(s) of the recombinant yeast,
- the pH of the culture medium during the step of culturing the recombinant
- 10 yeast, and/or
- the duration of the culturing of the recombinant yeast.

More particularly, the invention pertains to a hyaluronic acid-producing recombinant yeast, wherein the recombinant yeast comprises:

- (A) one or more recombinant nucleic acids encoding a polypeptide having
 - 15 glutamine-fructose-6-phosphate amidotransferase (GFA1) activity;
 - (B) one or more recombinant nucleic acids encoding a polypeptide having a UDP-N-acetylglucosamine pyrophosphorylase (QRI1) activity;
 - (C) one or more recombinant nucleic acids encoding a polypeptide having a UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity;
 - 20 (D) one or more recombinant nucleic acids encoding a polypeptide having a hyaluronan synthase (HASA) activity; and
 - (E) one or more recombinant nucleic acids encoding a polypeptide having a hyaluronidase activity wherein the polypeptide having hyaluronidase activity comprises a secretion signal so that hyaluronic acid, in particular of a desired molecular weight (HAMW),
 - 25 is produced by the recombinant yeast cell,
- said recombinant yeast being *Saccharomyces cerevisiae*.

In a particular embodiment, the recombinant cell according to the invention, and in particular the recombinant yeast according to the invention, comprises one recombinant

30 nucleic acid encoding a polypeptide having glutamine synthetase (GLN1) activity.

In a particular embodiment, the recombinant cell according to the invention, and in particular the recombinant yeast according to the invention, is such that at least one, and in

particular all, its endogeneous nucleic acids encoding a glutamate synthase (GLT1) are disrupted.

In a particular embodiment, the recombinant cell according to the invention, and in particular the recombinant yeast according to the invention:

- 5 - comprises one recombinant nucleic acid encoding a polypeptide having glutamine synthetase (GLN1) activity, and in particular a polypeptide having glutamine synthetase (GLN1) activity obtained or derived from *Saccharomyces cerevisiae*; and
- is such that at least one, and in particular all, its endogeneous nucleic acids encoding a glutamate synthase (GLT1) are disrupted.

- 10 In a particular embodiment, the recombinant cell according to the invention, and in particular the recombinant yeast according to the invention comprises only one recombinant nucleic acid encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase (GFA1) activity.

- In a particular embodiment, the recombinant cell according to the invention, and
- 15 in particular the recombinant yeast comprises between 5 and 10 recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase (QRI1) activity.

- In a particular embodiment, the recombinant cell according to the invention, and in particular the recombinant yeast comprises between 3 and 7 recombinant nucleic acids
- 20 encoding a polypeptide having UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity.

- In another embodiment, the one or more nucleic acids encoding a polypeptide having a UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity is obtained or derived from at least one of *Arabidopsis thaliana*, *Chlorella virus PBCVI* or *Streptococcus*
- 25 *zooepidemicus*, and in particular obtained or derived from *Arabidopsis thaliana* or *Chlorella virus PBCVI*.

- In a particular embodiment, the recombinant cell according to the invention, and in particular the recombinant yeast comprises between 4 and 8 recombinant nucleic acids encoding a polypeptide having hyaluronan synthase (HASA) activity.

- 30 In another embodiment, the one or more recombinant nucleic acids encoding a polypeptide having a hyaluronan synthase (HASA) activity is obtained or derived from at least one of *Streptococcus zooepidemicus*, *Chlorella virus PBCVI*, *Chlorella virus CviKI*, *Chlorella virus IL-5-2s1*, *Chlorella virus CZ-2*, *Chlorella virus CVG-1*, *Xenopus laevis* or

Pasteurella multocida, and is in particular obtained or derived from *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Chlorella virus CviK1*, *Chlorella virus IL-5-2s1*, *Chlorella virus CZ-2*, *Chlorella virus CVG-1* or *Xenopus laevis*.

In a particular embodiment, the recombinant cell according to the invention, and
5 in particular the recombinant yeast of the invention only comprises one recombinant nucleic acid encoding a polypeptide having hyaluronidase activity.

In another embodiment, the one or more recombinant nucleic acids encoding a polypeptide having a hyaluronidase activity is obtained or derived from *Cupiennius salei*, *Loxosceles intermedia*, *Hirudo nipponia*, *Bothrops atrox* or *Tityus serrulatus*.

10 In another embodiment, the recombinant cell according to the invention, and in particular the recombinant yeast cell may comprise at least one recombinant nucleic acid encoding one or more of:

- (A) a polypeptide having Phosphoglucomutase-1 (PGM1) activity; and/or
- (B) a polypeptide having UTP-glucose-1-phosphate uridylyltransferase
15 (UGP1) activity; and/or
- (C) a polypeptide having Glucosamine-6-phosphate N-acetyltransferase (GNA1) activity; and/or
- (D) a polypeptide having phosphoacetylglucosamine mutase (PCM1)
activity.

20 In particular, the recombinant cell according to the invention, and in particular the recombinant yeast cell of the invention comprises at least two, in particular at least three, and more particularly all of the modifications indicated above.

In a particular embodiment, the nucleic acid encoding a polypeptide having Phosphoglucomutase-1 (PGM1) activity, the nucleic acid encoding a polypeptide having
25 UTP-glucose-1-phosphate uridylyltransferase (UGP1) activity, the nucleic acid encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase (GFA1) activity, the nucleic acid encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase (GNA1) activity, the nucleic acid encoding a polypeptide having phosphoacetylglucosamine mutase (PCM1) activity and the nucleic acid encoding a polypeptide having UDP-N-
30 acetylglucosamine pyrophosphorylase (QRI1) activity are nucleic acids originating or derivating from a yeast, preferably from *Saccharomyces cerevisiae*.

In a particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide defined above and comprised in a recombinant cell according to the invention,

and in particular comprised in the recombinant yeast of the invention are under the control of a promoter selected from the group consisting of pPDC1, pTDH3, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTEF1, pENO2, pRPLA1, pNUP57 and pTEF3.

5 In a particular embodiment, the inducible or repressible promoters mentioned in the present specification are selected from the group consisting of promoters inducible or repressible with copper or promoters inducible or repressible with methionine, in particular selected from the group consisting of pMET6, pMET25 and pSAM1.

The invention further relates to a method for producing hyaluronic acid as previously described, and comprising the steps of:

10 (a) culturing a recombinant cell according to the invention, and in particular a recombinant yeast as defined herein in a culture medium; and

(b) recovering the hyaluronic acid from said culture medium,

wherein the hyaluronic acid recovered in step (b) has a molecular weight controlled through the selection of:

- 15 - the nature and origin of the nucleic acid encoding the hyaluronidase of the recombinant cell according to the invention, and in particular the recombinant yeast according to the invention, and/or
- the nature and origin of the promoter controlling the expression of the nucleic acid encoding the hyaluronidase(s) of the recombinant cell according to the invention, and in particular the recombinant yeast, and/or
- 20 - the presence of an anchoring and/or of a secretion signal associated to the encoded hyaluronidase(s) of the recombinant cell according to the invention, and in particular the recombinant yeast, and/or
- the pH of the culture medium during the step of culturing the recombinant cell according to the invention, and in particular the recombinant yeast; and/or
- 25 - the duration of the culturing of the recombinant cell according to the invention, and in particular the recombinant yeast.

Recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity

30 A recombinant cell according to the invention, and in particular a recombinant yeast of the invention, comprises one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity.

A polypeptide having hyaluronan synthase activity according to the invention means a polypeptide that converts the intermediate metabolites UDP-Glucuronate and UDP-N-acetylglucosamine (UDP-GlcNAc) into hyaluronic acid ((β -D-1,3-GlcNAc- β -D-1,4-GlcA)_n).

5 In an embodiment, one or more of the recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity are under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention, such as for example the inducible or repressible promoters pCUP1 or pMET25.

One or more of the recombinant nucleic acids encoding a polypeptide having
10 hyaluronan synthase activity may be under the control of a promoter selected from the group consisting of pCCW12, pCCW12.Sm, pTDH3-1.Sba and pTDH3.Sar.

The said one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity may originate or be derived from at least one of *Streptococcus zooepidemicus* (sz), *Chlorella virus PBCV1* (Vir), *Chlorella virus CviK1* (Vir), *Chlorella virus IL-5-2s1* (Vir), *Chlorella virus CZ-2* (Vir), *Chlorella virus CVG-1* (Vir), *Xenopus laevis* (xl)
15 or *Pasteurella multocida* (pm), and may originate or derive in particular from *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Chlorella virus CviK1*, *Chlorella virus IL-5-2s1*, *Chlorella virus CZ-2*, *Chlorella virus CVG-1* or *Xenopus laevis*, as shown in the examples herein.

20 A recombinant cell according to the invention, and in particular a recombinant yeast according to the invention, may comprise between 2 and 8, in particular between 4 and 8 recombinant nucleic acids encoding a polypeptide having hyaluronan synthase (HASA) activity. A recombinant cell according to the invention, and in particular a recombinant yeast according to the invention, may for example comprise 2 or 6, and in particular 6, recombinant
25 nucleic acids encoding a polypeptide having hyaluronan synthase (HASA) activity.

Illustratively, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity may be inserted within the JLP1 gene and/or within the SAM3 gene and/or within the TRP1 gene and/or within the LYP1 gene of the recombinant
30 cell, and in particular of the recombinant yeast, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast, of the invention comprises:

- between 2 and 8, in particular between 4 and 8 recombinant nucleic acids encoding a polypeptide having hyaluronan synthase (HASA) activity;

- said one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity originating or being derived from at least one of *Streptococcus zooepidemicus* (sz), *Chlorella virus PBCV1* (Vir), *Xenopus laevis* (xl) or *Pasteurella multocida* (pm), and originating or being derived in particular from at least one of *Streptococcus zooepidemicus*, *Chlorella virus PBCV1* or *Xenopus laevis*; and

- said one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity being under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention, such as for example the inducible or repressible promoters pCUP or pMET25 and/or under the control of a promoter selected from the group consisting of pCCW12, pCCW12.Sm, pTDH3-1.Sba and pTDH3.Sar.

Recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity

A recombinant cell according to the invention, and in particular a recombinant yeast according to the invention, comprises one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity.

A polypeptide having UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity according to the invention means a polypeptide that converts the intermediate metabolite Uridine-Diphosphate-Glucose (UDP-Glucose) into UDP-Glucuronate.

In an embodiment, one or more of the recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity are under the control of an inducible or repressible promoter that is functional in recombinant cells of the invention, such as for example the inducible or repressible promoters pMET25 or pMET6.

One or more of the recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity may be under the control of a promoter selected from the group consisting of pCCW12, in particular the pCCW12.sk and the pCCW12.sba promoters; pTEF1.Sba and pTDH3.Sk.

The one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity may originate or be derived from at least one of *Arabidopsis*

thaliana, *Chlorella virus PBCV1* or *Streptococcus zooepidemicus*, and in particular may originate or derive from *Arabidopsis thaliana* or *Chlorella virus PBCV1*.

A recombinant cell according to the invention, and in particular a recombinant yeast according to the invention, may comprise between 2 and 7, in particular between 3 and 5 7 recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity. A recombinant cell according to the invention, and in particular a recombinant yeast according to the invention, may for example comprise 2 or 5 recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity.

Illustratively, the one or more recombinant nucleic acids encoding a polypeptide 10 having UDP-Glucose dehydrogenase activity may be inserted within the JLP1 gene and/or within the TRP1 gene, and/or within the LYP1 gene of the recombinant cell, and in particular of the recombinant yeast, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast, of the invention comprises:

- 15 - between 2 and 7, and in particular between 3 and 7 recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity;
- said one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity originating or being derived from at least one of *Arabidopsis thaliana*, *Chlorella virus PBCV1* or *Streptococcus zooepidemicus*, and 20 originating or being derived in particular from *Arabidopsis thaliana* or *Chlorella virus PBCV1*; and
- said one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity being under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention, such as for example the 25 inducible or repressible promoters pMET25 or pMET6 and/or under the control of a promoter selected from the group consisting of pCCW12, in particular the pCCW12.sk and the pCCW12.sba promoters; pTEF1.Sba and pTDH3.Sk.

Recombinant nucleic acids encoding a polypeptide having Hyaluronidase activity

30 A recombinant cell according to the invention, and in particular a recombinant yeast according to the invention, comprises one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity.

A polypeptide having hyaluronidase activity according to the invention means a polypeptide that degrades hyaluronic acid, i.e. that converts a hyaluronic acid of a given molecular weight into a hyaluronic acid of a lower molecular weight.

As previously indicated, polypeptides having hyaluronidase activity of the present invention comprise a secretion signal.

In an embodiment, polypeptide having hyaluronidase activity comprise both a secretion signal and an anchoring signal.

In an embodiment, the said one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity are under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention.

One or more of the recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may be under the control of a promoter selected from the group consisting of pTEF1, pCCW12, pCCW12.sba, pCCW12.Sar, pPDC1, pTEF3, pTDH3, pNUP57, pCWP2 and pCCW10.ago.

The said one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may originate or be derived from at least one of *Cupiennius salei* (Csa), *Loxosceles intermedia* (Li), *Hirudo nipponia* (Hn), *Bothrops atrox* (Ba) or *Tityus serrulatus* (Ts) as shown in the examples herein.

A recombinant cell according to the invention, and in particular a recombinant yeast according to the invention, may comprise only one recombinant nucleic acid encoding a polypeptide having hyaluronidase activity.

Illustratively, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may be inserted within the JLP1 gene and/or within the LYP1 gene, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast, of the invention comprises:

- only one recombinant nucleic acid encoding a polypeptide having hyaluronidase activity;
- said recombinant nucleic acid encoding a polypeptide having hyaluronidase activity originating or being derived from *Cupiennius salei* (Csa), *Loxosceles intermedia* (Li), *Hirudo nipponia* (Hn), *Bothrops atrox* (Ba) or *Tityus serrulatus* (Ts);

- said recombinant nucleic acid encoding a polypeptide having hyaluronidase activity comprising either (i) a secretion signal and no anchoring signal or (ii) a secretion signal and an anchoring signal; and

- said recombinant nucleic acid encoding a polypeptide having hyaluronidase activity being under the control of a promoter selected from the group consisting of pTEF1, pCCW12, pCCW12.sba, pCCW12.Sar, pPDC1, pTEF3, pTDH3, pNUP57, pCWP2 and pCCW10.ago.

Recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity

10 A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, may comprise one or more recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity.

A polypeptide having glutamine synthetase activity according to the invention means a polypeptide that converts glutamate into glutamine while consuming one ATP and one NH_4^+ .

In an embodiment, one or more of the recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity are under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention.

One or more of the recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity may be under the control of a promoter selected from the group consisting of pTEF1 and pTEF1.Ago.

The said one or more recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity can originate or be derived from *Saccharomyces cerevisiae*, as shown in the examples herein.

25 A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, may comprise only one recombinant nucleic acid encoding a polypeptide having glutamine synthetase activity.

Illustratively, the one or more recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity may be inserted within the LYP1 or GLT1 gene of the recombinant cell, and in particular of the recombinant yeast cell, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast cell, of the invention comprises:

- only one recombinant nucleic acid encoding a polypeptide having glutamine synthetase activity;

5 - said recombinant nucleic acid encoding a polypeptide having glutamine synthetase activity originating or being derived from *Saccharomyces cerevisiae*; and

- said recombinant nucleic acid encoding a polypeptide having glutamine synthetase activity being under the control of a promoter selected from the group consisting of pTEF1 and pTEF1.Ago.

10 In a further embodiment of the invention, a recombinant cell, and in particular a recombinant yeast cell, of the invention comprises:

- only one recombinant nucleic acid encoding a polypeptide having glutamine synthase activity;

15 - said recombinant nucleic acid encoding a polypeptide having glutamine synthetase activity originating or being derived from *Saccharomyces cerevisiae*;

- said recombinant nucleic acid encoding a polypeptide having glutamine synthetase activity being under the control of a promoter selected from the group consisting of pTEF1 and pTEF1.Ago; and

20 - said recombinant nucleic acid encoding a polypeptide having glutamine synthetase activity being inserted within the LYP1 or GLT1 gene of the recombinant cell, and in particular of the recombinant yeast cell.

25 Recombinant nucleic acids encoding a polypeptide having Glutamine-fructose-6-phosphate amidotransferase activity

A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, may comprise one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity.

30 A polypeptide having glutamine-fructose-6-phosphate amidotransferase activity according to the invention means a polypeptide that converts fructose-6-phosphate into glucosamine-6-phosphate.

In an embodiment, one or more of the recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity are under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention.

5 One or more of the recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity may be under the control of a promoter selected from the group consisting of pTEF1 and pTEF1.Ago.

 The said one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity can originate or be derived from
10 *Saccharomyces cerevisiae*, as shown in the examples herein.

A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, may comprise only one recombinant nucleic acid encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity.

 Illustratively, the one or more recombinant nucleic acids encoding a polypeptide
15 having glutamine-fructose-6-phosphate amidotransferase activity may be inserted within the SAM3 gene of the recombinant cell, and in particular of the recombinant yeast cell, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast cell, of the invention comprises:

20 - only one recombinant nucleic acid encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity;

 - said recombinant nucleic acid encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity originating or being derived from *Saccharomyces cerevisiae*; and

25 - said recombinant nucleic acid encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity being under the control of a promoter selected from the group consisting of pTEF1 and pTEF1.Ago.

Recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine
30 pyrophosphorylase activity

A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, can comprise one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity.

A polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity according to the invention means a polypeptide that can convert N-acetyl-glucosamine into UDP-N-acetyl-glucosamine.

In an embodiment, one or more of the recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity are under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention, such as for example the inducible or repressible promoters pMET6 or pCUP1.

One or more of the recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity may be under the control of the promoter pTDH3.

The said one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity can originate or be derived from *Saccharomyces cerevisiae*, as shown in the examples herein.

A recombinant cell according to the invention, and in particular a recombinant yeast according to the invention, may comprise between 5 and 10 recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity. A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, may for example comprise 5, 7 or 8 recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity.

Illustratively, the one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity may be inserted within the HIS3 gene and/or within the JLP1 gene and/or within the SAM3 gene of the recombinant cell, and in particular of the recombinant yeast, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast cell, of the invention comprises:

- between 5 and 10 recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity;

- said one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity originating or being derived from *Saccharomyces cerevisiae*;

- said one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity being under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention, such as for example the inducible or repressible promoters pMET6 or pCUP1 and/or under the control of the promoter pTDH3.

Recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 activity

A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, may comprise one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 (PGM1) activity.

A polypeptide having Phosphoglucomutase-1 (PGM1) activity according to the invention means a polypeptide that converts Glucose-6-phosphate into the intermediate metabolite Glucose-1-phosphate.

In an embodiment, one or more of the recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 (PGM1) activity are under the control of an inducible or repressible promoter that is functional in recombinant cells of the invention, such as for example the inducible or repressible promoters.

One or more of the recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 (PGM1) activity may be under the control of the promoter pPDC1.

The one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 (PGM1) activity may originate or be derived from *Saccharomyces cerevisiae*, as shown in the examples herein.

A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, may comprise only one recombinant nucleic acid encoding a polypeptide having Phosphoglucomutase-1 (PGM1) activity.

Illustratively, the one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 activity may be inserted within the SAM3 gene of the recombinant cell, and in particular of the recombinant yeast, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast, of the invention comprises:

- only one recombinant nucleic acid encoding a polypeptide having Phosphoglucomutase-1 activity;

- said recombinant nucleic acid encoding a polypeptide having Phosphoglucomutase-1 activity originating or being derived from *Saccharomyces cerevisiae*; and
- said recombinant nucleic acid encoding a polypeptide having Phosphoglucomutase-1 activity being under the control of the promoter pPDC1.

Recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity

A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, comprises one or more recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase (UGP1) activity.

A polypeptide having UTP-glucose-1-phosphate uridylyltransferase (UGP1) activity according to the invention means a polypeptide that converts the intermediate metabolite Glucose-1-Phosphate into the intermediate metabolite UDP-glucose.

In an embodiment, one or more of the recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity are under the control of an inducible or repressible promoter that is functional in recombinant cells of the invention, such as for example the inducible or repressible promoters pSAM1 or pCUP1.

One or more of the recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity may be under the control of a promoter selected from the group consisting of pPDC1 and pENO2.

The one or more recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity can originate or be derived from *Saccharomyces cerevisiae*, as shown in the examples herein.

A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, can comprise between 5 and 10 recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity. A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, may for example comprise 5, 7 or 8 recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity.

Illustratively, the one or more recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity gene may be inserted within the

HIS3 gene and/or within the JLP1 gene and/or within the SAM3 gene of the recombinant cell, and in particular of the recombinant yeast, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast cell, of the invention comprises:

- 5 - between 5 and 10 recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity;
- said one or more recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity originating or being derived from *Saccharomyces cerevisiae*; and
- 10 - said one or more recombinant nucleic acids encoding a polypeptide having being under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention, such as for example the inducible or repressible promoters pSAM1 or pCUP1 and/or under the control of a promoter selected from the group consisting of pPDC1 and pENO2.

15

Recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity

A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, can comprise one or more recombinant nucleic acids
20 encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase (GNA1) activity.

A polypeptide having Glucosamine-6-phosphate N-acetyltransferase (GNA1) activity according to the invention means a polypeptide that can convert Glucosamine-6-phosphate into N-acetyl-glucosamine-6-phosphate.

25

In an embodiment, one or more of the recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity are under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention.

One or more of the recombinant nucleic acids encoding a polypeptide having
30 Glucosamine-6-phosphate N-acetyltransferase activity may be under the control of the promoter pCWP2.

The said one or more recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity may originate or be derived from *Saccharomyces cerevisiae*, as shown in the examples herein.

5 A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, can comprise only one recombinant nucleic acid encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity.

10 Illustratively, the one or more recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity may be inserted within the SAM3 gene of the recombinant cell, and in particular of the recombinant yeast, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast, of the invention comprises:

- only one recombinant nucleic acid encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity;
- 15 - said recombinant nucleic acid encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity originating or being derived from *Saccharomyces cerevisiae*; and
- said recombinant nucleic acid encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity being under the control of the promoter pCWP2.

20

Recombinant nucleic acids encoding a polypeptide having Phosphoacetylglucosamine mutase activity

25 A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, can comprise one or more recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase (PCM1) activity.

A polypeptide having phosphoacetylglucosamine mutase (PCM1) activity according to the invention means a polypeptide that can convert N-acetyl-glucosamine-6-phosphate N-acetyl-glucosamine-1-phosphate.

30 In an embodiment, one or more of the recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity are under the control of an inducible or repressible promoter that is functional in the recombinant cells of the invention.

One or more of the recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity may be under the control a promoter selected from the group consisting of pTEF3 and pTEF1.

The said one or more recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity can originate or be derived from *Saccharomyces cerevisiae*, as shown in the examples herein.

A recombinant cell according to the invention, and in particular a recombinant yeast cell according to the invention, may comprise only one recombinant nucleic acid encoding a polypeptide having phosphoacetylglucosamine mutase activity.

Illustratively, the one or more recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity may be inserted within the SAM3 gene of the recombinant cell, and in particular of the recombinant yeast, as it is shown in the examples herein.

In an embodiment of the invention, a recombinant cell, and in particular a recombinant yeast cell, of the invention comprises:

- only one recombinant nucleic acid encoding a polypeptide having phosphoacetylglucosamine mutase activity;
- said recombinant nucleic acid encoding a polypeptide having phosphoacetylglucosamine mutase activity originating or being derived from *Saccharomyces cerevisiae*; and
- said recombinant nucleic acid encoding a polypeptide having phosphoacetylglucosamine mutase activity being under the control of the promoter a promoter selected from the group consisting of pTEF3 and pTEF1.

25 HYALURONAN SYNTHASE (HASA)

The hyaluronan synthase enzyme is a protein which is described in the art for catalyzing the conversion of UDP-glucuronate or UDP-N-acetyl-glucose into hyaluronic acid. The hyaluronan synthase originating from *Streptococcus zooepidemicus*, *Chlorella virus PBCVI*, *Xenopus laevis* or *Pasteurella multocida* may be termed HASA.

A method implemented to measure the activity level of a polypeptide having hyaluronan synthase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method of colorimetric determination after treatment with concentrated sulfuric acid and carbazole described by Bitter and Muir (Analytical Biochemistry, 4, 330-334, 1962).

Preferred polypeptide having hyaluronan synthase activity in the present
5 invention is an enzyme having an EC number of n° 2.4.1.212.

According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity may originate or be derived from organisms preferably selected in a group consisting of prokaryotic organisms and eukaryotic organisms. In some embodiments, the one or more recombinant nucleic acids
10 encoding a polypeptide having hyaluronan synthase activity may originate or be derived from archaeobacteria. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity may originate or be derived from bacteria, and especially from *Streptococcus zooepidemicus* (Sz) or from *Pasteurella multocida* (Pm), from *Chlorella virus PBCV1* (Vir), *Chlorella virus CviK1* (Vir), *Chlorella virus IL-5-2s1*
15 (Vir), *Chlorella virus CZ-2* (Vir), *Chlorella virus CVG-1* (Vir) or from *Xenopus laevis* (Xl).

According to a yet preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 65%, advantageously at least 70%, preferably at least 80%, nucleic acid identity with a nucleic acid sequence as set forth as
20 sequence SEQ ID NO: 1 (Vir), SEQ ID NO: 2 (Vir), SEQ ID NO: 3 (Pm), SEQ ID NO: 4 (Pm), SEQ ID NO: 5 (Pm), SEQ ID NO: 6 (Xl), SEQ ID NO: 7 (sz), SEQ ID NO: 101 (Vir), SEQ ID NO: 102 (Vir), SEQ ID NO: 103 (Vir) or SEQ ID NO: 104 (Vir) and (ii) a biological activity of the same nature as the nucleic acid sequence having, respectively, the nucleic acid sequence as set forth as sequence SEQ ID NO: 1 (Vir), SEQ ID NO: 2 (Vir), SEQ ID NO: 3
25 (Pm), SEQ ID NO: 4 (Pm), SEQ ID NO: 5 (Pm), SEQ ID NO: 6 (Xl), SEQ ID NO: 7 (sz), SEQ ID NO: 101 (Vir), SEQ ID NO: 102 (Vir), SEQ ID NO: 103 (Vir) or SEQ ID NO: 104 (Vir).

A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that converts UDP-glucuronate or UDP-N-acetyl-glucose into hyaluronic acid.
30

As described herein, a nucleic acid sequence having at least 65% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%,

81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

As described herein, a nucleic acid sequence having at least 70% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

For the amino acid sequence of the polypeptide having hyaluronan synthase activity originating from *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Xenopus laevis* or *Pasteurella multocida*, the one skilled in the art may refer, respectively, to the accession numbers B4U0D4, Q84419, P13563, Q7BLV3, M1GZS8, M1H5V3, M1H2Q1 and M1HN86 in the UniProt database, or to SEQ ID NO: 8 (Vir), SEQ ID NO: 9 (Pm) SEQ ID NO: 10 (XI), SEQ ID NO:11 (Sz), SEQ ID NO: 105 (Vir), SEQ ID NO: 106 (Vir), SEQ ID NO: 107 (Vir) or SEQ ID NO: 108 (Vir) described herein, in particular to to SEQ ID NO: 8 (Vir), SEQ ID NO: 9 (Pm) SEQ ID NO: 10 (XI), SEQ ID NO:11 (Sz), SEQ ID NO: 105 (Vir), SEQ ID NO: 106 (Vir) or SEQ ID NO: 108 (Vir), and more particularly to SEQ ID NO: 8 (Vir), SEQ ID NO: 9 (Pm) SEQ ID NO: 10 (XI), SEQ ID NO:11 (Sz).

According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 50%, advantageously at least 65%, preferably at least 80%, amino acid identity with the amino acid sequence set forth as SEQ ID NO: 8 (Vir), SEQ ID NO: 9 (Pm) SEQ ID NO: 10 (XI), SEQ ID NO:11 (Sz), SEQ ID NO: 105 (Vir), SEQ ID NO: 106 (Vir), SEQ ID NO: 107 (Vir) or SEQ ID NO: 108 (Vir), and also a biological activity of the same nature as the amino acid sequence set forth as SEQ ID NO: 8 (Vir), SEQ

ID NO: 9 (Pm) SEQ ID NO: 10 (XI), SEQ ID NO:11 (Sz), SEQ ID NO: 105 (Vir), SEQ ID NO: 106 (Vir), SEQ ID NO: 107 (Vir) or SEQ ID NO: 108 (Vir).

A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the conversion of UDP-glucuronate or UDP-N-acetyl-glucose into hyaluronic acid.

As described herein, an amino acid sequence having at least 50% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 65% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 80% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As above-mentioned, the expression level of the one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity in the present invention is regulated by at least one promoter and at least one terminator, such as herein after defined more in details, which are present in 5' and 3' position respectively of the one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity.

30 **UDP-GLUCOSE DEHYDROGENASE (UDP-GlcDH OR HASB)**

The UDP-Glucose dehydrogenase is a protein which is known in the art to catalyze the conversion of UDP-glucose into UDP-glucuronate. The UDP-Glucose

dehydrogenase originating from the genome of *Arabidopsis thaliana*, *Chlorella virus PBCVI* or *Streptococcus zooepidemicus* may be termed HASB.

A method implemented to measure the activity level of a polypeptide having UDP-Glucose dehydrogenase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method described by Oka and Jigami (FEBS Journal 273, 2645-2657, 2006).

Preferred polypeptide having UDP-Glucose dehydrogenase activity in the present specification is an enzyme having an EC number 1.1.1.22.

According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity may originate or be derived from organisms preferably selected in a group comprising prokaryotic organisms and eukaryotic organisms. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity may originate or be derived from archaeobacteria. In some preferred embodiments, the one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity may originate or be derived from yeast, and especially from *Arabidopsis thaliana*, *Chlorella virus PBCVI* or *Streptococcus zooepidemicus haromyces*.

According to a yet preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 65%, advantageously at least 70%, preferably at least 80%, nucleic acid identity with a nucleic acid as set forth as sequence SEQ ID NO: 12 (At), SEQ ID NO. 13 (Vir), SEQ ID NO. 14 (Vir) or SEQ ID NO: 15 (Sz), and (ii) a biological activity of the same nature as the nucleic acid as set forth as sequence SEQ ID NO: 12 (At), SEQ ID NO. 13 (Vir), SEQ ID NO. 14 (Vir) or SEQ ID NO: 15 (Sz). The nucleic acids set forth as sequences SEQ ID NO: 12 (At), SEQ ID NO: 13 (Vir), SEQ ID NO. 14 (Vir) and SEQ ID NO: 15 (Sz) encode a polypeptide having UDP-Glucose dehydrogenase activity originating, respectively, from *Arabidopsis thaliana* (At), *Chlorella virus PBCVI* (Vir) or *Streptococcus zooepidemicus* (Sz), that may herein also be collectively termed HASB.

A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that converts UDP-glucose into UDP-glucuronate.

As described herein, a nucleic acid sequence having at least 65% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

As described herein, a nucleic acid sequence having at least 70% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequences, and also a biological activity of the same nature as the said reference nucleic acid sequences.

As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequences.

For the amino acid sequence of the polypeptide having UDP-Glucose dehydrogenase activity from *Arabidopsis thaliana*, *Chlorella virus PBCV1* or *Streptococcus zooepidemicus*, the one skilled in the art may refer to the accession numbers NP_173979.1, NP_048965 or KIS19289, respectively, in the UniProt database, or to the sequences as set forth in SEQ ID NO: 16 (At), SEQ ID NO: 17 (Vir) and SEQ ID NO: 18 (Sz) described herein.

According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity may be nucleic acid(s) encoding a polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 55%, advantageously at least 65%, preferably at least 80%, amino acid identity with the amino acid sequence of SEQ ID NO: 16 (At), SEQ ID NO: 17 (Vir) and SEQ ID NO: 18 (Sz), and also a biological activity of the same nature as the amino acid sequence of SEQ ID NO: 16 (At), SEQ ID NO: 17 (Vir) and SEQ ID NO: 18 (Sz).

A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the conversion of UDP-glucose into UDP-glucuronate.

As described herein, an amino acid sequence having at least 55% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 65% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 80% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As above-mentioned, the expression level of the one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity in the present invention is regulated by at least one promoter and at least one terminator, such as herein after defined more in details, which are present in 5' and 3' position respectively of the one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase activity.

25

HYALURONIDASE (HYAL)

The hyaluronidase enzyme is a protein which is described in the art for catalyzing the degradation of hyaluronic acid molecules into smaller hyaluronic acid molecules. The hyaluronidase originating from *Cupiennius salei*, *Loxosceles intermedia*, *Hirudo nipponia*, *Bothrops atrox*, *Tityus serrulatus* or *Vespa magnifica* may be termed HYAL.

The polypeptide having hyaluronidase activity of the invention may possess both a secretion signal and an anchoring signal or a secretion signal and no anchoring signal or a

secretion-anchor signal with a dual secretion and anchoring function. When the encoded polypeptide having hyaluronidase activity possesses both a secretion signal and an anchoring signal, it may be termed HYAL-31 as represented in the examples. When the encoded polypeptide having hyaluronidase activity possesses a secretion signal and no anchoring
5 signal, it may be termed HYAL-3 as represented in the examples.

A method implemented to measure the activity level of a polypeptide having hyaluronidase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously monitor the molecular weight of the obtained hyaluronic acid on an agarose gel.

10 Preferred polypeptide having hyaluronidase activity in the present specification is an enzyme having an EC number of n° EC 3.2.1.35.

According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may originate or be derived from organisms preferably selected in a group comprising prokaryotic organisms and eukaryotic
15 organisms. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may originate or be derived from archaeobacteria. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may originate or be derived from organisms preferably selected from yeasts. In some other preferred embodiments, the one or more recombinant nucleic acids
20 encoding a polypeptide having hyaluronidase activity may originate or be derived from *Cupiennius salei* (Csa), *Loxosceles intermedia* (Li), *Hirudo nipponia* (Hn), *Bothrops atrox* (Ba), *Tityus serrulatus* (Ts) or *Vespa magnifica* (Vm), and in particular from *Cupiennius salei* (Csa), *Loxosceles intermedia* (Li), *Hirudo nipponia* (Hn), *Bothrops atrox* (Ba) and *Tityus serrulatus* (Ts).

25 According to a yet preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may be selected from the group consisting of nucleic acid sequences having at least 65%, advantageously at least 70%, preferably at least 80%, nucleic acid identity with a nucleic acid of SEQ ID NO: 19 (Ba), SEQ ID NO: 25 (Li), SEQ ID NO: 21 (Csa), SEQ ID NO: 27 (Ts), SEQ ID NO: 23 (Hn), or
30 SEQ ID NO: 29 (Vm) and also a biological activity of the same nature as the nucleic acid of SEQ ID NO: 19 (Ba), SEQ ID NO: 25 (Li), SEQ ID NO: 21 (Csa), SEQ ID NO: 27 (Ts), SEQ ID NO: 23 (Hn), or SEQ ID NO: 29 (Vm). The nucleic acid of SEQ ID NO: 19 (Ba), SEQ ID NO: 25 (Li), SEQ ID NO: 21 (Csa), SEQ ID NO: 27 (Ts), SEQ ID NO: 23 (Hn) or SEQ ID

NO: 29 (Vm) encode a polypeptide having hyaluronidase activity, comprise a secretion signal and no anchoring signal, and originate or derive from *Bothrops atrox*, *Loxosceles intermedia*, *Cupiennius salei*, *Tityus serrulatus*, *Hirudo nipponia* or *Vespa magnifica*, respectively.

According to another preferred embodiment, the one or more recombinant
5 nucleic acids encoding a polypeptide having hyaluronidase activity may be selected from the group consisting of nucleic acid sequences having at least 65%, advantageously at least 70%, preferably at least 80%, nucleic acid identity with a nucleic acid of SEQ ID NO: 22 (Csa), SEQ ID NO: 26 (Li), SEQ ID NO: 24 (Hn), SEQ ID NO: 20 (Ba), SEQ ID NO: 28 (Ts) or
10 SEQ ID NO: 30 (Vm), and also a biological activity of the same nature as the nucleic acid of SEQ ID NO: 22 (Csa), SEQ ID NO: 26 (Li), SEQ ID NO: 24 (Hn), SEQ ID NO: 20 (Ba), SEQ ID NO: 28 (Ts) or SEQ ID NO: 30 (Vm). The nucleic acid of SEQ ID NO: 22 (Csa), SEQ ID NO: 26 (Li), SEQ ID NO: 24 (Hn), SEQ ID NO: 20 (Ba) SEQ ID NO: 28 (Ts) or
15 SEQ ID NO: 30 (Vm) encode a polypeptide having hyaluronidase activity, comprise a secretion signal and an anchoring signal, and originate or derive from *Cupiennius salei*, *Loxosceles intermedia*, *Hirudo nipponia*, *Bothrops atrox*, *Tityus serrulatus* or *Vespa magnifica*, respectively.

In a particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may be selected from the group consisting of nucleic acid sequences (i) having at least 65%, advantageously at least 70%, preferably at
20 least 80%, nucleic acid identity with a nucleic acid of SEQ ID NO: 19 (Ba), SEQ ID NO: 25 (Li), SEQ ID NO: 21 (Csa), SEQ ID NO: 27 (Ts), or SEQ ID NO: 23 (Hn) and (ii) having a biological activity of the same nature as the nucleic acid of SEQ ID NO: 19 (Ba), SEQ ID NO: 25 (Li), SEQ ID NO: 21 (Csa), SEQ ID NO: 27 (Ts), or SEQ ID NO: 23 (Hn).

In a particular embodiment, the one or more recombinant nucleic acids encoding
25 a polypeptide having hyaluronidase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 65%, advantageously at least 70%, preferably at least 80%, nucleic acid identity with a nucleic acid of SEQ ID NO: 22 (Csa), SEQ ID NO: 26 (Li), SEQ ID NO: 24 (Hn), SEQ ID NO: 20 (Ba), or SEQ ID NO: 28 (Ts) and (ii) having a biological activity of the same nature as the nucleic acid of SEQ ID NO: 22 (Csa), SEQ ID
30 NO: 26 (Li), SEQ ID NO: 24 (Hn), SEQ ID NO: 20 (Ba), or SEQ ID NO: 28 (Ts).

A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that catalyzes the degradation of hyaluronic acid molecules into smaller hyaluronic acid molecules.

As described herein, a nucleic acid sequence having at least 65% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

As described herein, a nucleic acid sequence having at least 70% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

For the amino acid sequence of the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity from *Cupiennius salei*, *Loxosceles intermedia*, *Hirudo nipponia*, *Bothrops atrox* or *Tityus serrulatus*, the one skilled in the art may refer to the accession numbers A0A0S4JYH2, R4J7Z9, X4Y2L4, A0A2H4Z8F4 or P85841, respectively in the UniProt database, or to SEQ ID NO: 33 (Csa), SEQ ID NO: 37 (Li), SEQ ID NO: 35 (Hn), SEQ ID NO: 31 (Ba) SEQ ID NO: 39 (Ts) or SEQ ID NO: 41 (Vm), described herein.

According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 50%, advantageously at least 65%, preferably at least 80%, amino acid identity with the amino acid sequence of SEQ ID NO: 33 (Csa), SEQ ID NO: 37 (Li), SEQ ID NO: 35 (Hn), SEQ ID NO: 31 (Ba), SEQ ID NO: 39 (Ts) or SEQ ID NO: 41 (Vm) which comprise a secretion signal and no anchoring signal, and also a biological activity of the same nature as the amino acid sequence SEQ ID NO: 33 (Csa), SEQ ID NO: 37 (Li), SEQ ID NO: 35 (Hn), SEQ ID NO: 31 (Ba), SEQ ID NO: 39 (Ts) or SEQ ID NO: 41 (Vm).

According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 55%, advantageously at least 65%, preferably at least 80%, amino acid identity with the amino acid sequence of SEQ ID NO: 34 (Csa), SEQ ID NO: 38 (Li), SEQ ID NO: 36 (Hn), SEQ ID NO: 32 (Ba), SEQ ID NO: 40 (Ts) or SEQ ID NO: 42 (Vm) which comprise a secretion signal and an anchoring signal, and also a biological activity of the same nature as the amino acid sequence of SEQ ID NO: 34 (Csa), SEQ ID NO: 38 (Li), SEQ ID NO: 36 (Hn), SEQ ID NO: 32 (Ba), SEQ ID NO: 40 (Ts) or SEQ ID NO: 42 (Vm).

10 In a particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may be nucleic acid(s) encoding a polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 55%, advantageously at least 65%, preferably at least 80%, amino acid identity with the amino acid sequence of SEQ ID NO: 33 (Csa), SEQ ID NO: 37 (Li), SEQ ID NO: 35 (Hn), SEQ ID NO: 31 (Ba), or SEQ ID NO: 39 (Ts), and also a biological activity of the same nature as the amino acid sequence of SEQ ID NO: 33 (Csa), SEQ ID NO: 37 (Li), SEQ ID NO: 35 (Hn), SEQ ID NO: 31 (Ba), or SEQ ID NO: 39 (Ts).

In another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity may be nucleic acid(s) encoding a polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 55%, advantageously at least 65%, preferably at least 80%, amino acid identity with the amino acid sequence of SEQ ID NO: 34 (Csa), SEQ ID NO: 38 (Li), SEQ ID NO: 36 (Hn), SEQ ID NO: 32 (Ba) or SEQ ID NO: 40 (Ts), and also a biological activity of the same nature as the amino acid sequence of SEQ ID NO: 34 (Csa), SEQ ID NO: 38 (Li), SEQ ID NO: 36 (Hn), SEQ ID NO: 32 (Ba) or SEQ ID NO: 40 (Ts).

A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the degradation of hyaluronic acid molecules into smaller hyaluronic acid molecules.

As described herein, an amino acid sequence having at least 55% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid

identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 65% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 80% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As above-mentioned, the expression level of the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity in the present invention is regulated by at least one promoter and at least one terminator, such as herein after defined more in details, which are present in 5' and 3' position respectively of the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity.

GLUTAMINE SYNTHETASE (GLN1)

The glutamine synthetase enzyme is a protein which is described in the art for catalyzing the conversion of glutamate into glutamine. The glutamine synthetase originating from *Saccharomyces cerevisiae* may be termed GLN1.

A method implemented to measure the activity level of a polypeptide having glutamine synthetase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method described by Legrain et al. (1982) European Journal of Biochemistry 123, 611-616.

Preferred polypeptide having glutamine synthetase activity in the present invention is an enzyme having an EC number of n° EC 6.3.1.2.

According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity may originate or be derived from organisms preferably selected in a group comprising prokaryotic organisms and eukaryotic organisms. In some embodiments, the one or more recombinant nucleic acids

encoding a polypeptide having glutamine synthetase activity may originate or be derived from archaeobacteria. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity may originate or be derived from a yeast, and especially from *Saccharomyces cerevisiae*.

5 According to a yet preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 65%, advantageously at least 70%, preferably at least 80%, nucleic acid identity with the nucleic acid sequence as set forth as sequence SEQ ID NO: 96 (Sc), and (ii) a biological activity of the same nature as the
10 nucleic acid sequence as set forth as sequence SEQ ID NO: 96 (Sc). The nucleic acid as set forth as sequence SEQ ID NO: 96 encodes a polypeptide having glutamine synthetase activity originating from *Saccharomyces cerevisiae*, that may also be termed GLN1.

 A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that converts glutamate into glutamine, in
15 particular through the consumption of one ATP and one NH_4^+ .

 As described herein, a nucleic acid sequence having at least 65% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
20 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

 As described herein, a nucleic acid sequence having at least 70% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%,
25 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

 As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at
30 least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

For the amino acid sequence of the polypeptide having glutamine synthetase activity originating from *Saccharomyces cerevisiae*, the one skilled in the art may refer to the accession number P32288 in the UniProt database, or to the sequence SEQ ID NO: 97 described herein.

5 According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 35%, advantageously at least 65%, preferably at least 80%, amino acid identity with the amino acid sequence of SEQ ID NO: 97, and also a
10 biological activity of the same nature as the amino acid sequence of SEQ ID NO: 97.

A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the conversion of glutamate into glutamine.

As described herein, an amino acid sequence having at least 35% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at
15 least 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a
20 biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 65% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
25 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 80% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,
30 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As above-mentioned, the expression level of the polypeptide having glutamine synthetase activity in the present invention is regulated by at least one promoter and at least

one terminator, such as herein after defined more in details, which are present in 5' and 3' position respectively of one or more recombinant nucleic acids encoding a polypeptide having glutamine synthetase activity.

5 **GLUTAMATE SYNTHASE (GLT1)**

The glutamate synthase enzyme is a protein which is described in the art for catalyzing the conversion of glutamine into glutamate. The glutamate synthase originating from *Saccharomyces cerevisiae* may be termed GLT1.

10 A method implemented to measure the activity level of a polypeptide having glutamate synthase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method described by Roon et al. (1974) Journal of bacteriology 118,89-95.

Preferred polypeptide having glutamate synthase activity in the present invention is an enzyme having an EC number of n° EC 1.4.1.14 (SEQ ID NO: 98).

15 For the amino acid sequence of the polypeptide having glutamate synthase activity from *Saccharomyces cerevisiae*, the one skilled in the art may refer to the accession number Q12680 in the UniProt database, or to the sequence SEQ ID NO: 99 described herein.

As above-mentioned, the expression level of a polypeptide having glutamate synthase activity may be reduced in a recombinant cell, and in particular in a recombinant yeast according to the invention as compared to the said cell, and in particular yeast, in its non-recombined form, i.e. at least one endogeneous gene of the recombinant cell, and in particular of the recombinant yeast, is disrupted.

GLUTAMINE-FRUCTOSE-6-PHOSPHATE AMIDOTRANSFERASE (GFA1)

25 The glutamine-fructose-6-phosphate amidotransferase enzyme is a protein which is described in the art for catalyzing the conversion of fructose-6-phosphate into glucosamine-6-phosphate. The glutamine-fructose-6-phosphate amidotransferase originating from *Saccharomyces cerevisiae* may be termed GFA1.

30 A method implemented to measure the activity level of a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method described by Shiga Shibatan and Hiroaki Kitazawa (Plant Biotechnology 26, 149-152, 2009).

Preferred polypeptide having glutamine-fructose-6-phosphate amidotransferase activity in the present invention is an enzyme having an EC number of n° EC 2.6.1.16.

According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity may originate or be derived from organisms preferably selected in a group comprising prokaryotic organisms and eukaryotic organisms. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity may originate or be derived from archaeobacteria. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity may originate or be derived from organisms preferably selected from *Bacillus subtilis*, and yeasts. In some other preferred embodiments, the one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity may originate or be derived from a yeast, and especially from *Saccharomyces cerevisiae*.

According to a yet preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 65%, advantageously at least 70%, preferably at least 80%, nucleic acid identity with the nucleic acid sequence as set forth as sequence SEQ ID NO: 47 (Sc), and (ii) a biological activity of the same nature as the nucleic acid sequence as set forth as sequence SEQ ID NO: 47 (Sc). The nucleic acid as set forth as sequence SEQ ID NO: 47 encodes a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity originating from *Saccharomyces cerevisiae*, that may also be termed GFA1.

According to yet another embodiment, the one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 65%, advantageously at least 70%, preferably at least 80%, nucleic acid identity with the nucleic acid sequence as set forth as sequence SEQ ID NO: 48 or SEQ ID NO: 49, and (ii) a biological activity of the same nature as the nucleic acid sequence as set forth as sequence SEQ ID NO: 48 or SEQ ID NO: 49. The nucleic acid sequence as set forth as sequence SEQ ID NO: 48 or SEQ ID NO: 49 encodes a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity originating from *Chlorella virus PBCV1*.

A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that converts fructose-6-phosphate into glucosamine-6-phosphate.

As described herein, a nucleic acid sequence having at least 65% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

As described herein, a nucleic acid sequence having at least 70% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

For the amino acid sequence of the polypeptide having glutamine-fructose-6-phosphate amidotransferase activity originating from *Saccharomyces cerevisiae*, the one skilled in the art may refer to the accession number NP8012818 in the UniProt database, or to the sequence SEQ ID NO: 50 described herein.

For the amino acid sequence of the polypeptide having glutamine-fructose-6-phosphate amidotransferase activity originating from *Chlorella virus PBCV1*, the one skilled in the art may also refer to the accession number NP_048448 in the UniProt database, or to the sequence SEQ ID NO: 51 described herein.

According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 35%, advantageously at least 65%, preferably at least 80%, amino acid identity with the amino acid

sequence of SEQ ID NO: 50 or with SEQ ID NO: 51, and also a biological activity of the same nature as the amino acid sequence of SEQ ID NO: 50 or with SEQ ID NO: 51.

A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the conversion of of fructose-6-phosphate into
5 glucosamine-6-phosphate.

As described herein, an amino acid sequence having at least 35% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%,
10 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 65% amino acid
15 identity with a reference amino acid sequence encompasses amino acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 80% amino acid
20 identity with a reference amino acid sequence encompasses amino acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As above-mentioned, the expression level of the polypeptide having glutamine-fructose-6-phosphate amidotransferase activity in the present invention is regulated by at least one promoter and at least one terminator, such as herein after defined more in details, which are present in 5' and 3' position respectively of one or more recombinant nucleic acids encoding a polypeptide having glutamine-fructose-6-phosphate amidotransferase activity.
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30

UDP-N-ACETYLGLUCOSAMINE PYROPHOSPHORYLASE (QR11)

The UDP-N-acetylglucosamine pyrophosphorylase enzyme is a protein which is described in the art for catalyzing the conversion of N-acetyl-glucosamine-6-phosphate into

UDP-N-acetyl-glucose. The UDP-N-acetylglucosamine pyrophosphorylase originating from *Saccharomyces cerevisiae* may be termed QRI1.

A method implemented to measure the activity level of a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method described by Mio et al. (The Journal of Biological Chemistry, Vol. 273, No 23, June 5, 1998, 14392-14397) except that UDP-N-acetyl-glucosamine is detected by LC MS/MS using a Synergi RP Fusion column.

Preferred polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity in the present invention is an enzyme having an EC number of n° EC 2.7.7.23

According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity may originate or be derived from organisms preferably selected in a group consisting of prokaryotic organisms and eukaryotic organisms. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity may originate or be derived from archaeobacteria. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity may originate or be derived from organisms preferably selected from *Bacillus subtilis*, and yeasts. In some other preferred embodiments, the one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity may originate or be derived from yeasts, and especially from *Saccharomyces cerevisiae*.

According to a yet preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 65%, advantageously at least 70%, preferably at least 80%, nucleic acid identity with a nucleic acid sequence as set forth as SEQ ID NO: 52, and (ii) a biological activity of the same nature as the nucleic acid sequence as set forth as SEQ ID NO: 52. The nucleic acid sequence set forth as SEQ ID NO: 52 encodes a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity originating from *Saccharomyces cerevisiae*, that may also be termed QRI1.

A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that converts N-acetyl-glucosamine-6-phosphate into UDP-N-acetyl-glucose.

As described herein, a nucleic acid sequence having at least 65% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

As described herein, a nucleic acid sequence having at least 70% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

For the amino acid sequence of the polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity originating from *Saccharomyces cerevisiae*, the one skilled in the art may refer to the accession number NP_010180 in the UniProt database, or to SEQ ID NO. 53 described herein.

According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 35%, advantageously at least 45%, preferably at least 80%, amino acid identity with the amino acid sequence set forth as sequence SEQ ID NO. 53, and also a biological activity of the same nature as the amino acid sequence set forth as sequence SEQ ID NO. 53.

A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the conversion of N-acetyl-glucosamine-6-phosphate into UDP-N-acetyl-glucose.

As described herein, an amino acid sequence having at least 35% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 45% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As described herein, an amino acid sequence having at least 80% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As above-mentioned, the expression level of the one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity in the present invention is regulated by at least one promoter and at least one terminator, such as herein after defined more in details, which are present in 5' and 3' position respectively of the one or more recombinant nucleic acids encoding a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase activity.

PHOSPHOGLUCOMUTASE-1 (PGM1)

The Phosphoglucomutase-1 enzyme is a protein which is described in the art for catalyzing the conversion of Glucose-6-phosphate into Glucose-1-phosphate. The Phosphoglucomutase-1 originating from *Saccharomyces cerevisiae* may be termed PGM1.

A method implemented to measure the activity level of a polypeptide facing
5 Phosphoglucomutase-1 activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method described by Tiwari and Bhat (Biochemical and Biophysical Research Communications 366, 340-345, 2008).

Preferred polypeptide having Phosphoglucomutase-1 activity in the present
10 invention is an enzyme having an EC number of n° 5.4.2.2.

According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 activity may originate from organisms preferably selected in a group consisting of prokaryotic organisms and eukaryotic organisms. In some embodiments, the one or more recombinant nucleic acids encoding a
15 polypeptide having Phosphoglucomutase-1 activity may originate or be derived from archaeobacteria. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 activity may originate or be derived from organisms preferably selected from bacteria. In a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 activity
20 may originate or be derived from *Saccharomyces cerevisiae*.

According to an embodiment, the one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 activity may be selected from the group consisting of nucleic acid sequences having (i) at least 80% nucleic acid identity with the nucleic acid sequence set forth as sequence SEQ ID NO: 54, which originates from
25 *Saccharomyces cerevisiae*, and (ii) a biological activity of the same nature as the nucleic acid sequence set forth as sequence SEQ ID NO: 54.

A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that converts Glucose-6-phosphate into Glucose-1-phosphate.

30 As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

For the amino acid sequence of the peptide having Phosphoglucomutase-1 activity from *Saccharomyces cerevisiae*, the one skilled in the art may refer to the accession
5 number NP33401 in the UniProt database, or to SEQ ID NO: 55 described herein.

According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 80% amino acid identity with the amino acid
10 sequence set forth as sequence SEQ ID NO: 55, and also a biological activity of the same nature as the amino acid sequence set forth as sequence SEQ ID NO: 55.

A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the conversion of Glucose-6-phosphate into Glucose-1-phosphate.

15 As described herein, an amino acid sequence having at least 80% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

20 As above-mentioned, the expression level of the one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1 activity in the present invention is regulated by at least one promoter and at least one terminator, such as herein after defined more in details, which are present in 5' and 3' position respectively of the one or more recombinant nucleic acids encoding a polypeptide having Phosphoglucomutase-1
25 activity.

UTP-GLUCOSE-1-PHOSPHATE URIDYLTRANSFERASE (UGP1)

The UTP-glucose-1-phosphate uridylyltransferase enzyme is a protein which is described in the art for catalyzing the conversion of Glucose-1-Phosphate into UDP-glucose.

30 The UTP-glucose-1-phosphate uridylyltransferase originating from *Saccharomyces cerevisiae* may be termed UGP1.

A method implemented to measure the activity level of a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method
5 described by Roeben (J. Mol. Biol 364, 551-560, 2006).

Preferred polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity in the present invention is an enzyme having an EC number of n° 2.7.7.9.

According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity
10 may originate or be derived from organisms preferably selected in a group consisting of prokaryotic organisms and eukaryotic organisms. In some embodiments, the one or more recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity may originate or be derived from archaeobacteria. In some
15 UTP-glucose-1-phosphate uridylyltransferase activity may originate or be derived from organisms preferably selected from bacteria. In a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity may originate or be derived from *Saccharomyces cerevisiae*.

According to a particular embodiment, the one or more recombinant nucleic
20 acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 80% nucleic acid identity with a nucleic acid as set forth as sequence SEQ ID NO: 56, originating from *Saccharomyces cerevisiae*, and (ii) a biological activity of the same nature as the nucleic acid as set forth as sequence SEQ ID NO: 56.

25 A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that converts Glucose-1-phosphate into UDP-glucose.

As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at
30 least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

For the amino acid sequence of the polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity originating from *Saccharomyces cerevisiae*, the one skilled in the art may refer to the accession number NP_32861 in the UniProt database, or to the sequence set forth as sequence SEQ ID NO: 57 described herein.

5 According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 80% amino acid identity with the amino acid sequence set forth as SEQ ID NO: 57, and also a biological activity of the same
10 nature as the amino acid sequence set forth as SEQ ID NO: 57.

A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the conversion of Glucose-1-phosphate into UDP-glucose.

As described herein, an amino acid sequence having at least 80% amino acid
15 identity with a reference amino acid sequence encompasses amino acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As above-mentioned, the expression level of the one or more recombinant
20 nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity in the present invention is regulated by at least one promoter and at least one terminator, such as herein after defined more in details, which are present in 5' and 3' position respectively of the one or more recombinant nucleic acids encoding a polypeptide having UTP-glucose-1-phosphate uridylyltransferase activity.

25

GLUCOSAMINE-6-PHOSPHATE N-ACETYLTRANSFERASE (GNA1)

The Glucosamine-6-phosphate N-acetyltransferase enzyme is a protein which is described in the art for catalyzing the conversion of Glucosamine-6-phosphate into N-acetyl-glucosamine-6-phosphate. The Glucosamine-6-phosphate N-acetyltransferase originating
30 from *Saccharomyces cerevisiae* may be termed GNA1.

A method implemented to measure the activity level of a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method described by Li et al. (Anal. Biochem. 370, 142-146, 2007).

Preferred polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity in the present invention is an enzyme having an EC number of n° 2.3.1.4.

5 According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity may originate or be derived from organisms preferably selected in a group consisting of prokaryotic organisms and eukaryotic organisms. In some preferred embodiments, the one or more recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-
10 acetyltransferase activity may originate or be derived from a yeast, and especially from *Saccharomyces cerevisiae*.

According to a particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 80%
15 nucleic acid identity with a nucleic acid sequence as set forth as sequence SEQ ID NO: 58, and (ii) a biological activity of the same nature as the nucleic acid sequence as set forth as sequence SEQ ID NO: 58. The nucleic acid sequence as set forth as sequence SEQ ID NO: 58 encodes a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity originating from *Saccharomyces cerevisiae*, that may also be termed GNA1.

20 A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that converts Glucosamine-6-phosphate into N-acetyl-glucosamine-6-phosphate.

As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at
25 least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

For the amino acid sequence of the polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity originating from *Saccharomyces cerevisiae*, the one
30 skilled in the art may refer to the accession number NP_116637 in the UniProt database, or to SEQ ID NO. 59 described herein.

According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase

activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 80% amino acid identity with the amino acid sequence set forth as SEQ ID NO. 59, and also a biological activity of the same nature as the amino acid sequence set forth as SEQ ID NO. 59.

5 A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the conversion of Glucosamine-6-phosphate into N-acetyl-glucosamine-6-phosphate.

As described herein, an amino acid sequence having at least 80% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at
10 least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As above-mentioned, the expression level of the one or more recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase
15 activity in the present invention is regulated by at least one promoter and at least one terminator, such as herein after defined more in details, which are present in 5' and 3' position respectively of the one or more recombinant nucleic acids encoding a polypeptide having Glucosamine-6-phosphate N-acetyltransferase activity.

20 **PHOSPHOACETYLGLUCOSAMINE MUTASE (PCM1)**

The phosphoacetylglucosamine mutase enzyme is a protein which is described in the art for catalyzing the conversion of N-acetyl-glucosamine-6-phosphate into N-acetyl-glucosamine-1-phosphate. The phosphoacetylglucosamine mutase originating from *Saccharomyces cerevisiae* may be termed PCM1.

25 A method implemented to measure the activity level of a polypeptide having phosphoacetylglucosamine mutase activity belongs to the general knowledge of the one skilled in the art.

In this regard, the one skilled in the art may advantageously refer to the method described by Bandini et al. (Molecular Microbiology 85(3), 513-534, 2012).

30 Preferred polypeptide having phosphoacetylglucosamine mutase activity in the present invention is an enzyme having an EC number of n° 5.4.2.3.

According to a preferred embodiment, the one or more recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity may originate

or be derived from organisms preferably selected in a group consisting of prokaryotic organisms and eukaryotic organisms. In some preferred embodiments, the one or more recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity may originate or be derived from a yeast, and especially from *Saccharomyces cerevisiae*.

According to a particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity may be selected from the group consisting of nucleic acid sequences having (i) at least 80% nucleic acid identity with a nucleic acid sequence set forth as SEQ ID NO: 60, and (ii) a biological activity of the same nature as the nucleic acid sequence set forth as SEQ ID NO: 60. The nucleic acid set forth as SEQ ID NO: 60 encodes a polypeptide having phosphoacetylglucosamine mutase activity originating from *Saccharomyces*, that may also be termed PCM1.

A biological activity of the same nature regarding this sequence is, as previously explained, the capacity to code for a polypeptide that converts N-acetyl-glucosamine-6-phosphate into N-acetyl-glucosamine-1-phosphate.

As described herein, a nucleic acid sequence having at least 80% nucleotide identity with a reference nucleic acid sequence encompasses nucleic acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% nucleotide identity with the said reference nucleic acid sequence, and also a biological activity of the same nature as the said reference nucleic acid sequence.

For the amino acid sequence of the polypeptide having phosphoacetylglucosamine mutase activity originating from *Saccharomyces cerevisiae*, the one skilled in the art may refer to the accession number NP_010856 in the UniProt database, or to SEQ ID NO. 61 described herein.

According to another particular embodiment, the one or more recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity may be nucleic acid(s) encoding polypeptide having an amino acid sequence selected from the group consisting of sequences having at least 80% amino acid identity with the amino acid sequence set forth as SEQ ID NO. 61, and also a biological activity of the same nature as the amino acid sequence set forth as SEQ ID NO. 61.

A biological activity of the same nature regarding this sequence is as described previously, i.e. the capacity to catalyze the conversion of N-acetyl-glucosamine-6-phosphate into N-acetyl-glucosamine-1-phosphate.

As described herein, an amino acid sequence having at least 80% amino acid identity with a reference amino acid sequence encompasses amino acid sequences having at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% amino acid identity with the said reference amino acid sequence, and also a biological activity of the same nature as the said reference amino acid sequence.

As above-mentioned, the expression level of the one or more recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity in the present invention is regulated by at least one promoter and at least one terminator, such as herein after defined more in detail, which are present in 5' and 3' position respectively of the one or more recombinant nucleic acids encoding a polypeptide having phosphoacetylglucosamine mutase activity.

15

PROMOTERS

As disclosed herein, the expression of the genes of interest that have been genetically engineered for obtaining a recombinant cell according to the invention comprise appropriate regulatory sequences that are functional in the recombinant cells of the invention, and in particular in recombinant yeast cells of the invention, including in particular *Saccharomyces cerevisiae*.

Various promoters may be used for the desired expression of the coding sequences of interest.

Promoters according to the invention can be selected from the group consisting of the following promoters:

- pTDH3 (SEQ ID NO: 62),
- pTDH3.sk (SEQ ID NO: 63),
- pTDH3-1.Sba (SEQ ID NO: 64),
- pTDH3.Sar (SEQ ID NO: 65),
- pENO2 (SEQ ID NO: 66),
- pTEF3 (SEQ ID NO: 67),
- pTEF1 (SEQ ID NO: 68),
- pTEF1.ago (SEQ ID NO: 69),

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- pTEF1.sba (SEQ ID NO: 70),
- pPDC1 (SEQ ID NO: 71),
- pCCW12 (SEQ ID NO: 72),
- pCCW12.Sm (SEQ ID NO: 73),
- 5 • pCCW12.sk (SEQ ID NO: 74),
- pCCW12.sba (SEQ ID NO: 75),
- pCCW12.sar (SEQ ID NO: 76),
- pNUP57 (SEQ ID NO: 77),
- pCCW10.ago (SEQ ID NO: 78),
- 10 • pCWP2 (SEQ ID NO: 79), and
- pRPLA1 (SEQ ID NO: 80).

Promoters more particularly interesting in the present invention may be selected from the group consisting of:

- 15 • pTDH3 (SEQ ID NO: 62),
- pTDH3.sk (SEQ ID NO: 63),
- pTDH3-1.Sba (SEQ ID NO: 64),
- pTDH3.Sar (SEQ ID NO: 65),
- pENO2 (SEQ ID NO: 66),
- 20 • pTEF3 (SEQ ID NO: 67),
- pTEF1 (SEQ ID NO: 68),
- pTEF1.ago (SEQ ID NO: 69),
- pTEF1.sba (SEQ ID NO: 70),
- pPDC1 (SEQ ID NO: 71),
- 25 • pCCW12 (SEQ ID NO: 72),
- pCCW12.Sm (SEQ ID NO: 73),
- pCCW12.sk (SEQ ID NO: 74),
- pCCW12.sba (SEQ ID NO: 75), and
- pCCW12.sar (SEQ ID NO: 76),

30 Said promoters can in particular be selected from the group consisting of pTDH3, pTDH3-1.Sba, pTDH3.Sar, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, and pCCW12.sar.

Alternatively, promoters interesting in the present invention may be selected from the group consisting of:

- pNUP57 (SEQ ID NO: 77), and
- pCCW10.ago (SEQ ID NO: 78).

5 Other promoters of interest in the present invention may be:

- pCWP2 (SEQ ID NO: 79); and
- pRPLA1 (SEQ ID NO: 80).

As previously mentioned, inducible or repressible promoters are promoters whose activity is controlled by the presence or absence of biotic or abiotic factors and also by
10 the quantity of said factor. Accordingly, for some promoters, their activity will in particular be induced and thus increased when the quantity of a given factor increases or is increased, and, accordingly, the activity of these same promoters can be repressed and thus reduced when the quantity of said factor diminishes or is reduced. The quantity of said factor(s) in the culture medium of a recombinant yeast cell of the invention comprising inducible or
15 repressible promoters can be decided and thus controlled by the man skilled in the art.

For example, increasing the quantity of copper in a culture medium of a recombinant yeast cell according to the invention comprising a pCUP-1 promoter will induce and thus increase transcription of the gene under the control of this promoter. On the contrary, reducing the quantity of copper in said culture medium will lead to a repression, and thus a
20 reduced, transcription of the gene under the control of this promoter.

In another example, increasing the quantity of methionine in a culture medium of a recombinant yeast cell according to the invention comprising a pMET6 promoter will repress and thus decrease transcription of the gene under the control of this promoter. On the contrary, reducing the quantity of methionine in said culture medium will lead to an induced,
25 and thus an increased, transcription of the gene under the control of this promoter.

For this reason, the following promoters are referred to in the present text as being inducible or repressible promoters.

According to a first embodiment, inducible or repressible promoters according to the invention may be selected from the group comprising promoters inducible or repressible
30 with copper, promoters inducible or repressible with methionine and promoters inducible or repressible with threonine, and are in particular is CUP1 – copper inducible or repressible (SEQ ID NO: 81).

According to this embodiment, the inducible or repressible promoter according to the invention can in particular be pCUP1.

The activity of these promoters is thus induced by the increasing presence of methionine, copper or threonine as indicated above, and their activity diminishes, i.e. is repressed, when the quantity of methionine, copper or threonine is reduced.

According to a second embodiment, inducible or repressible promoters according to the invention may be selected from the group comprising promoters inducible or repressible with copper, promoters inducible or repressible with lysine and promoters inducible or repressible with methionine, and in particular selected from the group consisting of:

- pMET6 – methionine inducible or repressible (SEQ ID NO: 82),
- pMET25 – methionine inducible or repressible (SEQ ID NO: 83), and
- pSAM1 – methionine inducible or repressible (SEQ ID NO: 84),

According to this particular embodiment, the inducible or repressible promoter according to the invention may, be selected from the group consisting of pMET6, pMET25 and pSAM1.

The activity of these promoters is thus repressed by the increasing presence of methionine, copper, lysine or glucose as indicated above, and their activity increases, i.e. is induced, when the quantity of methionine, copper, lysine or glucose is reduced.

In a particular embodiment, inducible or repressible promoters according to the invention may be selected from the group comprising promoters inducible or repressible with copper, promoters inducible or repressible with glucose, promoters inducible or repressible with lysine, promoters inducible or repressible with methionine and promoters inducible or repressible with threonine.

In a more particular embodiment, the inducible or repressible promoter according to the invention may be selected from the group consisting of pCUP1, pMET6, pSAM1, and pMET25.

Synthetic promoters as described in Blazeck & Alper (2013) Biotechnol. J. 8 46-58 can also be used.

The promoters of the invention can originate from any organism from the Saccharomycetes class and can in particular originate from an organism selected from the group consisting of at least one of *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Saccharomyces castellii*, *Saccharomyces bayanus*, *Saccharomyces arboricola*, *Saccharomyces*

kudriavzevii, *Saccharomyces mikatae*, *Ashbya gossypii*, *Kluyveromyces lactis*, *Pichia pastoris*, *Candida glabrata*, *Candida tropicalis*, *Debaryomyces castellii*, *Yarrowia lipolitica*, and *Cyberlindnera jadinii*, .

The promoters of the invention can preferably originate from an organism
5 selected from the group consisting of *Saccharomyces cerevisiae* (*sc*), *Saccharomyces mikatae* (*Sm*), *Saccharomyces kudriavzevii* (*sk*), *Saccharomyces bayanus* (*sba*), and *Saccharomyces arboricola* (*Sar*).

TERMINATORS

10 As it is disclosed herein, the expression of the genes of interest that have been genetically engineered for obtaining a recombinant cell according to the invention, and in particular a recombinant yeast according to the invention, comprise appropriate transcription terminator sequences that are functional in recombinant cells of the invention, and in particular in recombinant yeast cells of the invention, in particular in *Saccharomyces*
15 *cerevisiae*.

Said transcription terminators, identical or different, may be found in literature Yamanishi *et al.*, (2013) ACS synthetic biology 2, 337-347.

Terminators more particularly interesting in the present invention may be selected from the group comprising:

- 20 • tTPI1 from the gene encoding for the Triose Phosphate Isomerase (SEQ ID NO: 85),
- tMET25 from the gene encoding for the O-acetyl homoserine-O-acetyl serine
sulfhydrylase (SEQ ID NO: 86),
- tDIT1 (SEQ ID NO: 87),
- 25 • tRPL3 (SEQ ID NO: 88),
- tRPL3.sm (SEQ ID NO: 89),
- tRPL3.sba (SEQ ID NO: 90),
- tRPL41B (SEQ ID NO: 91),
- tRPL15A (SEQ ID NO: 92),
- 30 • tRPL15A.sba (SEQ ID NO: 93),
- tIDP1 (SEQ ID NO: 94),
- tTEF1.sba (SEQ ID NO: 95), and
- tTDH3 (SEQ ID NO: 100).

In particular, said terminator may be selected from the group consisting of tTPI1, tMET25, tDIT1, tRPL3, tRPL3.sm, tRPL3.sba, tRPL41B, tRPL15A, tRPL15A.sba, tIDP1, tTEF1.sba and tTDH3.

The terminators of the invention can originate from any organism from the
5 Saccharomycetes class and can in particular originate from an organism selected from the group consisting of *Saccharomyces cerevisiae* and *Saccharomyces Bayanus*.

RECOMBINANT CELLS

Recombinant cells of the invention can be selected from the group consisting of
10 yeasts and bacteria.

Recombinant cells of the invention, such as a recombinant host cell of the invention, are preferably recombinant yeast cells.

Generally, yeast can grow rapidly and can be cultivated at higher density as compared with bacteria, and does not require an aseptic environment in the industrial setting.
15 Furthermore, yeast cells can be more easily separated from the culture medium compared to bacterial cells, greatly simplifying the process for product extraction and purification.

A recombinant cell of the invention, and in particular a recombinant yeast cell of the invention is preferably a *Saccharomycetales* cell.

A recombinant cell of the invention, and in particular a recombinant yeast of the
20 invention can in particular belong to the *Saccharomyces* genus, or to the *Candida* genus, or to the *Kluyveromyces* genus, or to the *Ogataea* genus, or to the *Yarrowia* genus, or to the *Debaryomyces* genus, or to the *Ashbya* genus.

A recombinant cell of the invention belonging to the *Saccharomyces* genus can be selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces boulardii*,
25 *Saccharomyces bayanus*, *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces castelli*, *Saccharomyces cariocanus*, *Saccharomyces kudriavzevii*, *Saccharomyces arboricolus*, *Saccharomyces pastorianus*, *Saccharomyces uvarum* and *Saccharomyces delbrueckii*.

A recombinant cell of the invention belonging to the *Candida* genus can be
30 selected from the group consisting of *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida dubliniensis*, *Candida parapsilosis*, *Candida lusitanae* and *Candida guilliermondii*.

A recombinant cell of the invention belonging to the *Kluyveromyces* genus can be selected from the group consisting of *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces polysporus*, *Kluyveromyces thermotolerens*, *Kluyveromyces dobzhanskii* and *Kluyveromyces wickerhamii*.

5 A recombinant cell of the invention belonging to the *Ogataea* genus can be selected from the group consisting of *Ogataea polymorpha*, *Ogataea histriana*, *Ogataea deakii*, *Ogataea kolombanensis*, *Ogataea philodendra*, *Ogataea siamensis*, *Ogataea angusta*, *Ogataea parapolyomorpha*, *Ogataea minuta*, *Ogataea nonfermentans* and *Ogataea kodamae*.

10 A recombinant cell of the invention belonging to the *Yarrowia* genus can be selected from the group consisting of *Yarrowia lipolytica*, *Yarrowia parophonii*, *Yarrowia galli*, *Yarrowia oslonensis*, *Yarrowia alimentaria*, *Yarrowia hollandica* and *Yarrowia yakushimensis*.

A recombinant cell of the invention belonging to the *Debaryomyces* genus can be selected from the group consisting of *Debaryomyces hansenii*, *Debaryomyces carsonii*,
15 *Debaryomyces castellii*, *Debaryomyces maramba*, *Debaryomyces occidentalis*, *Debaryomyces oviformis*, *Debaryomyces nepalensis*, *Debaryomyces coudertii*, *Debaryomyces udenii*, *Debaryomyces psychrosporus* and *Debaryomyces yamadae*.

A recombinant cell of the invention belonging to the *Ashbya* genus can be selected from the group consisting of *Ashbya gossypii* and *Ashbya aceri*.

20

A recombinant cell of the invention, and in particular a recombinant yeast cell of the invention, can in particular be selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Saccharomyces bayanus*, *Saccharomyces paradoxus*, *Saccharomyces mikatae*, *Saccharomyces castelli*, *Candida albicans*, *Candida glabrata*,
25 *Candida tropicalis*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces polysporus*, *Kluyveromyces thermotolerens*, *Ogataea polymorpha*, *Yarrowia lipolytica*, *Debaryomyces hansenii*, and *Ashbya gossypii*, and is preferably *Saccharomyces cerevisiae*.

In a particular embodiment, the recombinant yeast cell according to the invention is of the genus *Saccharomyces*, *Kluyveromyces*, or *Eremothecium*, more particularly
30 is of the species selected from the group consisting of *Saccharomyces cerevisiae*, *Kluyveromyces Marxianus*, *Ogataea polymorpha* and *Ashbya gossypii*.

In an embodiment, a recombinant host cell of the invention is a yeast selected belonging to the *Saccharomycetales* order, in particular to the *Saccharomycetaceae* family,

and is in particular selected from the group consisting of *Yarrowia lipolitica*, *Kluyveromyces Marxianus*, *Ogataea polymorpha*, *Ashbya gossypii* and *Saccharomyces cerevisiae*.

A recombinant cell of the invention can most preferably be a *Saccharomyces cerevisiae* cell.

5 As above-mentioned, a recombinant cell according to the invention has the ability to produce hyaluronic acid by insertion of the one or more recombinant nucleic acids according to the present invention. In certain embodiments, a recombinant yeast according to the invention has the ability to produce hyaluronic acid having a controlled size (controlled molecular weight) by insertion of the one or more recombinant nucleic acids according to the
10 present invention.

Methods implemented to insert a specific DNA construct within a gene belong to the general knowledge of a man skilled in the art. A related method is described in more details in the herein after examples.

15 However, unexpected technical difficulties were encountered because the consequences of the insertion of DNA constructs within the genome of a cell, and in particular within the genome of a yeast, such as for example in the genome of *Saccharomyces cerevisiae*, are unpredictable. In particular, the survival rate of the cells, and in particular of the yeasts, and their ability to grow and produce the desired hyaluronic acid is also unpredictable.

20 In order to obtain a recombinant cell, and in particular a recombinant yeast, of the invention, many different constructs were tested by the inventors in order to obtain a viable and efficient recombinant cell, and in particular yeast.

CULTURE CONDITIONS

25 The present invention also relates to the use of a recombinant cell of the invention, for the production of hyaluronic acid, in particular of hyaluronic acid of controlled molecular weight.

The present invention further relates to a method of producing hyaluronic acid (HA) of a desired molecular weight (HAMW) comprising:

30 (a) cultivating a recombinant cell of the present invention in a cultivation medium for a time sufficient to produce hyaluronic acid (HA) of the desired molecular weight; and

(b) optionally isolating or recovering the hyaluronic acid (HA) from the recombinant cell and/or from the cultivation medium.

Typically, cells of the invention, and in particular yeasts of the invention, are grown at a temperature in the range of about 20°C to about 37°C, preferably at a temperature ranging from 27 to 34°C, in an appropriate culture medium.

Suitable growth media for cells of the invention, and in particular for yeasts of the invention, are common commercially prepared media such as broth that includes yeast nitrogen base, ammonium sulfate, and dextrose as the carbon/energy source or YPD Medium, a blend of peptone, yeast extract, and dextrose in optimal proportions for growing most. Other defined or synthetic growth media may also be used and the appropriate medium for growth of the particular cell, and in particular yeast, will be known by one skilled in the art of microbiology or fermentation science.

A particular medium that is suitable herein is the SY medium which comprises the following elements:

KH₂PO₄: 100 mM; MgSO₄ 7H₂O: 2,8 mM; K₂SO₄: 11,5 mM; Na₂SO₄: 1,1 mM; NaCl: 2,6 mM; CaCl₂ 2H₂O: 0,7 mM; CuSO₄ 5H₂O: 15 µM; KI: 6 µM; FeCl₃: 30 µM; ZnSO₄ 7H₂O: 61 µM; MnSO₄ H₂O: 25 µM; H₂SO₄: 110 µM; Panthotenic Acids hemicalcium salt: 42 µM; Thiamin hydrochloride: 59 µM; Pyridoxin hydrochloride: 49 µM; Myo-Inositol (C₆H₁₂O₆): 555 µM; Nicotinic acid (C₆H₅NO₂): 29 µM; D-Biotine: 0,82 µM; Ammonium citrate tribasic: 33 mM; and glucose or sucrose 2-30%.

Carbon sources that may be used in the culture medium include fructose, mannose, xylose and arabinose; oligosaccharides such as lactose, maltose, galactose, or sucrose; polysaccharides such as starch or cellulose or mixtures thereof; and unpurified mixtures from renewable feedstocks such as cheese whey permeate cornsteep liquor, sugar beet molasses, and barley malt.

Nitrogen sources that may be included in the culture medium include peptone, yeast extracts, meat extracts, malt extracts, urea, ammonium sulfate, ammonium chloride, ammonium nitrate, ammonium phosphate, ammonium citrate and combinations thereof.

The culture medium may further comprise trace elements (e.g., metal salts), for example magnesium salts, cobalt salts and/or manganese salts; as well as growth factors such as amino acids, vitamins, growth promoters, and the like.

Examples of vitamins that may be included are Panthotenic Acids hemicalcium, Thiamin hydrochloride; Pyridoxin hydrochloride; Myo-Inositol; Nicotinic acid; D-Biotine, folic acid, p-aminobenzoïque acid, riboflavin.

A culture medium of the invention may further include rare elements, such as
5 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, KI, FeCl_3 , $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, or H_2SO_4 , MgCl_2 , CaCl_2 , NaCl, K_2HPO_4 , KH_2PO_4 , ZnCl, H_3BO_3 , MnSO_4 , Na_2MoO_4 .

The term “appropriate culture medium” is above-defined.

Examples of known culture media for a recombinant cell according to the present invention are known to the person skilled in the art, and are presented in the following
10 publication D. Burke et al., Methods in yeast Genetics – A cold spring harbor laboratory course Manual (2000).

Suitable pH ranges for the fermentation may be between pH 3.0 to pH 7.5, where pH 4 to pH 6 is preferred as the initial condition.

As mentioned elsewhere in this specification, the pH value of the culture
15 medium can be regulated during the cultivation step of a method of the invention in order to modulate the activity of the polypeptide facing hyaluronidase activity, which will impact the molecular weight of the hyaluronic acid molecules produced by the recombinant cell of the invention, and in particular by the recombinant yeast of the invention.

In particular, the pH of the culture medium can be modified according to the
20 hyaluronic acid that is meant to be produced by the recombinant yeast. For example, the pH of the culture medium may remain at a pH of 4, of 5,5 or of 6 during the culture time.

In a particular embodiment, the pH of the culture medium may change or be changed during the time length of the culture of the recombinant cell of the invention, in particular of the recombinant yeast of the invention. As previously mentioned,
25 *Saccharomyces cerevisiae* acidifies the medium in which it is cultivated and thus reduces the pH of its culture medium. For example, the pH of the culture medium may begin at 6, may be taken down to 4 and then brought back up to 6. In another example, the pH of the culture medium may begin at 6, remain or be maintained at 6, and then may be lowered to 4.

In a particular embodiment, the pH of the culture medium may be modulated
30 during the cultivating step (a) of a method of the invention so that at the end of the cultivating step of a method of the invention, the pH of the cultivation medium is the same as the pH at the beginning of said cultivation step (a).

In another embodiment, the pH of the culture medium may remain the same during the time length of the culture of the recombinant cell of the invention, and in particular the recombinant yeast cell of the invention.

Said time length of the culture of a recombinant cell according to the invention, and in particular of the recombinant yeast of the invention, can vary depending on the molecular weight of the hyaluronic acid of interest. The longer said time length, the lower the molecular weight of hyaluronic acid in a given culture medium of a recombinant cell of the invention, and in particular a recombinant yeast cell of the invention.

The time length of the culture time of a recombinant cell of the invention, and in particular a recombinant yeast cell in the present invention can be a period of from about 35 hours to about 50 hours, preferably from about 40 hours to about 50 hours, and is in particular about 48 hours.

Fermentations may be performed under aerobic conditions or micro-aerobic conditions.

The amount of hyaluronic acid product in the fermentation medium can be determined using a number of methods known in the art, for example, high performance liquid chromatography (HPLC) or gas chromatography (GC).

The present process may employ a batch method of fermentation. A classical batch fermentation is a closed system where the composition of the medium is set at the beginning of the fermentation and not subject to artificial alterations during the fermentation. Thus, at the beginning of the fermentation, the medium is inoculated with the desired organism or organisms, and fermentation is permitted to occur without adding anything to the system. Typically, however, a "batch" fermentation method or system is batch with respect to the addition of carbon source and attempts are often made at controlling factors such as temperature, pH and oxygen concentration. In batch systems, the metabolite and biomass compositions of the system change constantly up to the time when the fermentation is stopped. Within batch cultures cells progress through a static lag phase to a high growth log phase and finally to a stationary phase where growth rate is diminished or halted. If untreated, cells in the stationary phase will eventually die. Cells in log phase generally are responsible for the bulk of production of end product or intermediate.

A Fed-Batch system may also be used in the present invention. A Fed-Batch system is similar to a typical batch system with the exception that the carbon source substrate is added in increments as the fermentation progresses. Fed-Batch systems are useful when

catabolite repression (e.g. glucose repression) is apt to inhibit the metabolism of the cells and where it is desirable to have limited amounts of substrate in the media. Measurement of the actual substrate concentration in Fed-Batch systems is difficult and is therefore estimated on the basis of the changes of measurable factors such as pH, dissolved oxygen and the partial
5 pressure of waste gases such as CO₂.

Batch and Fed-Batch culturing methods are common and well known in the art and examples may be found in *Biotechnology: A Textbook of Industrial Microbiology*, Crueger, Crueger, and Brock, Second Edition (1989) Sinauer Associates, Inc., Sunderland, MA, or Deshpande, Mukund V., *Appl. Biochem. Biotechnol.*, 36, 227, (1992). Although the
10 present invention is performed in batch mode it is contemplated that the method would be adaptable to continuous fermentation.

Continuous fermentation is an open system where a defined fermentation medium is added continuously to a bioreactor and an equal amount of conditioned media is removed simultaneously for processing. Continuous fermentation generally maintains the
15 cultures at a constant high density where cells are primarily in log phase growth.

Continuous fermentation allows for the modulation of one factor or any number of factors that affect cell growth or end product concentration. For example, one method will maintain a limiting nutrient such as the carbon source or nitrogen level at a fixed rate and allow all other parameters to vary. In other systems a number of factors affecting growth can
20 be altered continuously while the cell concentration, measured by media turbidity, is kept constant. Continuous systems strive to maintain steady state growth conditions and thus the cell loss due to the medium being drawn off must be balanced against the cell growth rate in the fermentation. Methods of modulating nutrients and growth factors for continuous fermentation processes as well as techniques for maximizing the rate of product formation are
25 well known in the art of industrial microbiology.

It is contemplated that the present invention may be practiced using either batch, fed- batch or continuous processes and that any known mode of fermentation would be suitable. Additionally, it is contemplated that cells may be immobilized on a substrate as whole cell catalysts and subjected to fermentation conditions for production.

30 In order to still improve the hyaluronic acid production, a particular embodiment may consist of culturing the recombinant cells of the invention, in particular the recombinant yeast cells of the invention, in an appropriate culture medium, such as above-mentioned,

wherein the said culture medium comprises an optimal amount of carbon source, especially glucose or sucrose.

In preferred embodiments, the carbon source comprised in said optimal culture medium consists of glucose and/or sucrose. In preferred embodiments, the said optimal culture medium comprises 1% w/w or more glucose and/or sucrose, in particular comprises 5% w/w or more glucose and/or sucrose, in particular comprises 10% w/w or more glucose and/or sucrose, in particular comprises 15% w/w or more glucose and/or sucrose. In preferred embodiments, the said optimal culture medium comprises at most 40% w/w glucose, which includes at most 35% w/w glucose.

10 In a preferred embodiment, the method of the invention is carried out on an industrial scale.

More particularly, the cultivation medium of the method according to the invention can be at least about 100L, more preferably in the range of about 1000L to about 3000L, even more preferably about 10,000L, even more preferably 100,000L, or even about 250,000L.

The invention further relates to a method for producing hyaluronic acid as previously described, and comprising the steps of:

- (a) culturing a recombinant cell of the invention in a culture medium; and
- (b) recovering the hyaluronic acid from said culture medium,

20 wherein the hyaluronic acid recovered in step (b) has a molecular weight controlled through the selection of:

- the nature and origin of the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity of the recombinant cell of the invention, and in particular of the recombinant yeast of the invention,
- 25 - the nature and origin of the promoter controlling the expression of the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity of the recombinant cell of the invention, and in particular of the recombinant yeast of the invention,
- the presence or absence of an anchoring signal associated to the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity of the recombinant cell of the invention, and in particular of the recombinant yeast of the invention,
- 30

- the pH of the culture medium during the step of culturing the recombinant cell of the invention, and in particular of the recombinant yeast of the invention, and/or
- the duration of the culturing of the recombinant cell of the invention, and in particular of the recombinant yeast of the invention.

The molecular weight of the hyaluronic acid can be a particular molecular weight or more preferably a specific range of molecular weights such as, for example, less than 50 kDa, in the range of about 20 kDa to about 50 kDa, greater than or equal to 50 kDa, in the range of about 50 kDa to about 150kDa, in the range of about 50 kDa to about 250kDa, greater than or equal to 100 kDa, in the range of about 100kDa to about 1500 kDa, in the range of about 150kDa to about 1500 kDa, greater than 1000 kDa or greater than 1500kDa.

The present invention also relates to the use of a recombinant cell according to the invention, and in particular a recombinant yeast cell of the invention, for the production of hyaluronic acid (HA) having a molecular weight in the range of from about 20kDa to about 50kDa or from about 50kDa to about 1000 kDa.

The one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity of a recombinant cell of the invention can for example be selected from those originating or being derived from at least one of *Cupiennius salei* (Csa), *Loxosceles intermedia* (Li), *Hirudo nipponia* (Hn), *Bothrops atrox* (Ba), *Tityus serrulatus* (Ts) or *Vespa magnifica* (Vm), in particular from at least one of *Cupiennius salei* (Csa), *Loxosceles intermedia* (Li), *Hirudo nipponia* (Hn), *Bothrops atrox* (Ba) or *Tityus serrulatus* (Ts), more particularly from those of sequences set forth as sequences SEQ ID NO: 19 (Ba), SEQ ID NO: 25 (Li), SEQ ID NO: 21 (Csa), SEQ ID NO: 27 (Ts) or SEQ ID NO: 23 (Hn) (in the presence of a secretion signal and in the absence of an anchoring signal).

The one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity of a recombinant cell of the invention can for example be selected from those originating or being derived from at least one of *Cupiennius salei* (Csa), *Loxosceles intermedia* (Li), *Hirudo nipponia* (Hn), *Bothrops atrox* (Ba), *Tityus serrulatus* (Ts) or *Vespa magnifica* (Vm), in particular from *Cupiennius salei* (Csa), *Loxosceles intermedia* (Li), *Hirudo nipponia* (Hn), *Bothrops atrox* (Ba) or *Tityus serrulatus* (Ts), in particular from those of sequences set forth as sequences SEQ ID NO: 22 (Csa), SEQ ID NO: 26 (Li), SEQ ID NO:

24 (Hn), SEQ ID NO: 20 (Ba) or SEQ ID NO: 28 (Ts) (in the presence of both a secretion signal and an anchoring signal).

The one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity of a recombinant cell of the invention can for example be placed under the control of a promoter selected from the group consisting of pTEF1, pCCW12, pCCW12.sba, pCCW12.Sar, pPDC1, pTEF3, pTDH3, pNUP57 and pCCW10.ago.

In particular, the promoters of the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity of a recombinant cell of the invention, and in particular of a recombinant yeast of the invention, may originate or be derived from *Saccharomyces Bayanus*, *Saccharomyces kudriavzevii*, *Saccharomyces mikatae*, *Saccharomyces arboricola* or other saccharomycetales, or *Abishia gossypii*.

The secretion signal of the present invention may for example have:

- the nucleic acid sequence set forth as SEQ ID NO: 43; and/or
- the amino acid sequence set forth as SEQ ID NO: 44.

The anchoring signal of the present invention may for example have:

- the nucleic acid sequence set forth as SEQ ID NO: 45; and/or
- the amino acid sequence set forth SEQ ID NO: 46.

The secretion signal may be fused to the polypeptide having hyaluronidase activity by creating a chimeric nucleic acid starting by a nucleic acid sequence encoding the signal peptide followed by a recombinant nucleic acid encoding a polypeptide having hyaluronidase activity as previously defined.

The secretion signal and anchoring signal may be fused to the polypeptide having hyaluronidase activity by creating a chimeric nucleic acid starting by a nucleic acid sequence encoding the signal peptide followed by a recombinant nucleic acid encoding a polypeptide having hyaluronidase activity as previously defined, followed by a nucleic acid sequence encoding for the anchoring signal.

Such chimeric nucleic acid sequences may be obtained by techniques known by the man skilled in the art such as chemical synthesis of nucleic acids or any recombination techniques such as cloning or PCR.

The invention further relates to a method as previously described, for producing hyaluronic acid having a molecular weight less than or equal to about 50 kDa, and in particular less than 50 kDa and greater than or equal to about 20 kDa, wherein:

(a) the pH of the medium is superior to 4, and is in particular in the range of from about 5 to about 7;

(b) the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity integrated in the genome of the recombinant cell of the invention
5 originates or is derived:

(i) from *Tityus serrulatus*, possesses a secretion signal but is devoid of an anchoring signal, and is under the control of a promoter selected from the group consisting of pTDH3, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTDH3-1.Sba and pTDH3.Sar;

10 (ii) from *Cupiennius salei*, possesses a secretion signal but is devoid of an anchoring signal, and is under the control of a promoter selected from the group consisting of pNUP57 and pCCW10.ago;

(iii) from *Cupiennius salei*, possesses a secretion signal and an anchoring signal, and is under the control of the promoter pCWP2;

15 (iv) from *Loxosceles intermedia* possesses a secretion signal but is devoid of an anchoring signal, and is under the control of a promoter selected from the group consisting of pTDH3, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTDH3-1.Sba and pTDH3.Sar; or

(v) from *Hirudo nipponia*, possesses a secretion signal but is devoid of
20 an anchoring signal, and is under the control of a promoter selected from the group consisting of pNUP57 and pCCW10.ago; or

(vi) from *Hirudo nipponia*, possesses both a secretion signal and an anchoring signal, and is under the control of a promoter selected from the group consisting of pTDH3, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTDH3-1.Sba and pTDH3.Sar; and
25

(c) the step of recovering the hyaluronic acid from the culture medium is performed about 48 hours after the beginning of the culturing of the recombinant cell of the invention, in particular the recombinant yeast cell of the invention;

the recombinant cell being in particular a recombinant yeast cell, and more
30 particularly a *Saccharomyces cerevisiae* cell.

The invention further relates to a method as previously described, for producing hyaluronic acid having a molecular weight greater than or equal to about 50 kDa and less than or equal to about 1000 kDa, wherein:

(a) the pH of the medium is between about 4 and about 6 and is optionally varied
5 from about 6 to about 4 during the culturing step of the recombinant cell, in particular the recombinant yeast cell, of the invention;

(b) the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity integrated in the genome of the recombinant cell of the invention originates or is derived:

10 (i) from *Tityus serrulatus*, possesses a secretion signal but is devoid of an anchoring signal, and is under the control of a promoter selected from the group consisting of pTDH3, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTDH3-1.Sba and pTDH3.Sar;

(ii) from *Tityus serrulatus*, possesses both a secretion signal and an
15 anchoring signal, and is under the control of a promoter selected from the group consisting of pTDH3, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTDH3-1.Sba and pTDH3.Sar;

(iii) from *Loxosceles intermedia*, possesses both a secretion signal and an anchoring signal, and is under the control of a promoter selected from the group consisting of
20 pNUP57 and pCCW10.ago;

(iv) from *Loxosceles intermedia*, possesses a secretion signal but is devoid of an anchoring signal, and is under the control of a promoter selected from the group consisting of pTDH3, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTDH3-1.Sba and pTDH3.Sar,

25 (v) from *Cupiennius salei*, possesses both a secretion signal and an anchoring signal, and is under the control of a promoter selected from the group consisting of pNUP57 and pCCW10.ago,

(vi) from *Cupiennius salei*, possesses a secretion signal but is devoid of an anchoring signal, and is under the control of a promoter selected from the group consisting
30 of pNUP57 and pCCW10.ago, or

(vii) from *Hirudo nipponia*, possesses both a secretion signal and an anchoring signal, and is under the control of a promoter selected from the group consisting of

pTDH3, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTDH3-1.Sba and pTDH3.Sar; and

(c) the step of recovering the hyaluronic acid from the culture medium is performed about 48 hours after the beginning of the culturing of the recombinant cell of the invention, in particular the recombinant yeast cell of the invention;

the recombinant cell being in particular a recombinant yeast cell, and more particularly a *Saccharomyces cerevisiae* cell.

The invention further relates to a method as previously described, for producing hyaluronic acid having a molecular weight greater than about 1000 kDa, and in particular having a molecular weight from about 1000 kDa to about 1.5 Million Da, wherein:

(a) the pH of the medium is from about 4 to about 6 and is optionally varied from about 6 to about 4 during the culturing step of the recombinant cell, in particular recombinant yeast, of the invention;

(b) the one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity integrated in the genome of the recombinant cell of the invention originates or is derived:

(i) from *Tityus serrulatus*, possesses a secretion signal but is devoid of an anchoring signal, and is under the control of a promoter selected from the group consisting of pNUP57 and pCCW10.ago;

(ii) from *Loxosceles intermedia*, possesses a secretion signal but is devoid of an anchoring signal, and is under the control of a promoter selected from the group consisting of pNUP57 and pCCW10.ago;

(iii) from *Bothrops atrox*, possesses a secretion signal but is devoid of an anchoring signal, and is under the control of a promoter selected from the group consisting of pTDH3, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTDH3-1.Sba and pTDH3.Sar; or

(iv) from *Bothrops atrox*, possesses both a secretion signal and an anchoring signal, and is under the control of a promoter selected from the group consisting of pTDH3, pENO2, pTEF3, pTEF1, pPDC1, pCCW12, pCCW12.Sm, pCCW12.sk, pCCW12.sba, pCCW12.sar, pTDH3-1.Sba and pTDH3.Sar;

and

(c) the step of recovering the hyaluronic acid from the culture medium is performed about 48 hours after the beginning of the culturing of the recombinant cell of the invention, in particular the recombinant yeast cell of the invention;

the recombinant cell being in particular a recombinant yeast, and more particularly a *Saccharomyces cerevisiae* cell.

Another aspect of the present invention relates to hyaluronic acid (HA) obtained or obtainable from a recombinant cell of the invention or from a method according to the invention.

A further aspect of the invention is a cultivation medium comprising the hyaluronic acid of the invention.

The invention further relates to a composition comprising the hyaluronic acid according to the invention.

The invention also relates to an industrial product or a consumer product or a consumable comprising (i) the hyaluronic acid of the invention, (ii) the cultivation medium comprising hyaluronic acid of the invention or (iii) the composition comprising hyaluronic acid of the invention.

In particular, said industrial product or consumer product or consumable according to the invention can be a cosmetic product, a flavour product, a fragrance product, a food product, a food, a beverage, a texturant, a pharmaceutical composition, a dietary supplement, a nutraceutical, a cleaning product and/or a dental and/or an oral hygiene composition.

PURIFICATION OF HYALURONIC ACID

According to a specific aspect of the invention, the fermentative production of hyaluronic acid preferably comprises a step of isolation of the hyaluronic acid produced from the culture medium. Recovering the hyaluronic acid from the culture medium is a routine task for a man skilled in the art. It may be achieved by a number of techniques well known in the art including but not limiting to pervaporation, selective precipitation, filtration, centrifugation, spray drying, lyophilisation or liquid extraction. The expert in the field knows how to adapt parameters of each technique dependant on the characteristics of the material to be separated.

The yeast as model of a cell in the present invention is preferred in that the synthesized hyaluronic acid is/are entirely exported outside the cells, thus simplifying the purification process.

Gas stripping is achieved with a stripping gas chosen among helium, argon,
5 carbon dioxide, hydrogen, nitrogen or mixture thereof.

Liquid extraction is achieved with organic solvent as the hydrophobic phase such as pentane, hexane, heptane or dodecane. Renewal solvents may also be used.

Those of skill in the art will recognize that, due to the degenerate nature of the genetic code, a variety of DNA molecules differing in their nucleotide sequences can be used
10 to encode a given enzyme of the disclosure. The native DNA sequence encoding the biosynthetic enzymes described above are referenced herein merely to illustrate an embodiment of the disclosure, and the disclosure includes DNA molecules of any sequence that encode the amino acid sequences of the polypeptides and proteins of the enzymes utilized in the methods of the disclosure. In similar fashion, a polypeptide can typically tolerate one or
15 more amino acid substitutions, deletions, and insertions in its amino acid sequence without loss or significant loss of a desired activity. The disclosure includes such polypeptides with different amino acid sequences than the specific proteins described herein so long as the modified or variant polypeptides have the enzymatic anabolic or catabolic activity of the reference polypeptide. Furthermore, the amino acid sequences encoded by the DNA
20 sequences shown herein merely illustrate embodiments of the disclosure.

Described herein are specific genes and proteins useful in the methods, compositions and organisms of the disclosure; however it will be recognized that absolute identity to such genes is not necessary. For example, changes in a particular gene or polynucleotide comprising a sequence encoding a polypeptide or enzyme can be performed
25 and screened for activity. Typically such changes comprise conservative mutations and silent mutations. Such modified or mutated polynucleotides and polypeptides can be screened for expression of a functional enzyme using methods known in the art.

Due to the inherent degeneracy of the genetic code, other polynucleotides which encode substantially the same or functionally equivalent polypeptides can also be used to
30 clone and express the polynucleotides encoding such enzymes

Techniques known to those skilled in the art may be suitable to identify additional homologous genes and homologous enzymes. Generally, analogous genes and/or

analogous enzymes can be identified by functional analysis and will have functional similarities.

Techniques known to those skilled in the art may be suitable to identify analogous genes and analogous enzymes or any biosynthetic pathway genes, proteins, or enzymes, techniques may include, but are not limited to, cloning a gene by PCR using primers based on a published sequence of a gene/enzyme of interest, or by degenerate PCR using degenerate primers designed to amplify a conserved region among a gene of interest. Further, one skilled in the art can use techniques to identify homologous or analogous genes, proteins, or enzymes with functional homology or similarity. Techniques include examining a cell or cell culture for the catalytic activity of an enzyme through *in vitro* enzyme assays for said activity (e.g . as described herein or in Kiritani, K., Branched-Chain Amino Acids Methods Enzymology, 1970), then isolating the enzyme with said activity through purification, determining the protein sequence of the enzyme through techniques such as Edman degradation, design of PCR primers to the likely nucleic acid sequence, amplification of said DNA sequence through PCR, and cloning of said nucleic acid sequence. To identify homologous or similar genes and/or homologous or similar enzymes, analogous genes and/or analogous enzymes or proteins, techniques also include comparison of data concerning a candidate gene or enzyme with databases such as BRENDA, KEGG, or MetaCYC. The candidate gene or enzyme may be identified within the above mentioned databases in accordance with the teachings herein.

DERIVATIVES

The term “Hyaluronic acid (HA)”, as used herein, is also intended to cover derivatives of Hyaluronic acid, such as but not limited to acetylated or sulfated Hyaluronic acid useful for applications in a cosmetic product, flavor product a fragrance product, a food product, a food, a beverage, a texturant, a pharmaceutical composition, a dietary supplement, a nutraceutical, a cleaning product and/or a dental and/or an oral hygiene composition or combinations thereof

Hyaluronic acid may be prepared as a composition.

FORMULATIONS AND PRODUCTS

A composition of the invention may be incorporated into a formulation/product, such as a nutraceutical, pharmaceutical, veterinary, oenological or cosmetic formulation/product.

Thus, the present invention provides a formulation comprising a composition of the invention. For example, the present invention may provide a cosmetic formulation comprising the composition of the invention.

The present invention also provides a product comprising a composition of the invention. For example, the present invention may provide a cosmetic product comprising the composition of the invention.

A “cosmetic product” is intended to mean any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition, correcting body odours and/or combinations thereof.

A “substance” is intended to mean a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.

A “mixture” is intended to mean a mixture or solution composed of two or more substances.

The present invention also provides the use of a composition of the invention in a nutraceutical, pharmaceutical, veterinary, oenological or cosmetic formulation/product.

Such formulations or products are hereinafter referred to as the “formulations or products of the invention”.

The nutraceutical, pharmaceutical, veterinary, oenological or cosmetic formulation/product may optionally further comprise pharmaceutically/veterinary/cosmetic (including cosmetic active) ingredients, such as excipients, carriers and mixtures thereof as appropriate.

“Cosmetic” or “Cosmetic Active Ingredients” means any and all natural, naturally occurring, nature identical, synthetic synthetically produced, biosynthetically produced, sustainable, renewable and/or biodegradable compounds, ingredients,

intermediates, molecules, substances, raw materials or products individually or as part of a mixture of compounds, ingredients, intermediates, molecules, substances, raw materials or products, blends, compositions, formulations (including but not limited to skin moisturizers, creams, balms, serums, oils, eye, facial makeup, wash off hair products, leave-on hair products, hair colorants (including but not limited to natural hair colorants) and/or combinations thereof), finished products and related technologies including, but not limited to components, incorporated, for example, into a cosmetic formulation (such as but not limited to natural colorants, preservatives, emulsifiers, anti-oxidants and the like which do not, for example, have an activity on the skin, hair, scalp and the like but play a role in the formulation of the finished product), delivery systems, marketing aids (such as, for example, coloured unispheres applied to translucent formulations) and methods of making anything related thereto useful in/used for/intended for:

- application by rubbing, pouring, sprinkling, spraying or otherwise directly on or to a human or animal body and/or by placing in contact with the various external and/or on surface parts of a human or animal body (including but not limited to the skin, hair, body hair, the hair system, scalp, nails, lips, external genitalia, teeth, oral and/or nasal mucosa and the like); and/or application indirectly on or to a human or animal body such as for example, application as part of a textile or application to a textile as part of a delivery device (such as a capsule) or a delivery system (such as blends or formulations) applied to a textile; and/or
- cleansing, caring, cooling, beautifying, conditioning, treating, soothing, texturizing, promoting attractiveness, protecting, maintaining, improving, enhancing, altering and/or changing an external part and/or surface of a human or animal body (such as, but not limited to the scalp) or the aesthetic appearance of a human or animal body; and/or with a view to mainly cleaning or perfuming, or protecting or maintaining in good condition or combating body odour or changing the appearance of or correcting or repairing a state of imbalance in skin, oral mucosa, scalp or hair by providing a calming, healing, repairing, or revitalization, hydration of the skin or in order to provide relief to, lubricate, moisten, tone, heal, sterilize, relieve, correct and/or remedy states of dryness, irritation, injury or fatigue, and/or with a view to correcting pigmentation disorders or providing a non-pharmaceutical prevention and/or treatment of dandruff, acne, irritation and/or inflammation and the like and/or rebalancing the bacterial flora (such as, for example, the microbiome) on the surface of the skin (such as, for example, by promoting the level of beneficial bacterial flora on the skin surface) and/or for the purposes of keeping a human or animal body in good condition for

health and/or wellbeing purposes and/or for improving the appearance of a human or animal body by, for example, improving the appearance of a product applied to a human or body; and/or

- providing a cosmetic and/or dermatological function and/or benefit with a biological activity benefit (but without affecting a body's structure or function. For the avoidance of doubt, a Cosmetic or Cosmetic Active Ingredient or any part thereof may also qualify as a Functional Ingredient and/or Nutraceutical.

"Functional Ingredient" means a food ingredient or part of a food that provides medicinal or health benefits, including any of the following: a carotenoid, dietary fiber, fatty acid, saponin, antioxidant, flavonoid, isothiocyanate, phenol, polyphenol (such as resveratrol), plant sterol or stanol (phytosterols and phytostanols), a polyol, a prebiotic, a phytoestrogen, soy protein, sulfides/thiol, a vitamin, glucosamine, preservatives, hydration agents, edible gelling ingredients, edible gel mixes and gel compositions, long chain primary aliphatic saturated alcohols, colour agents, texturizing agents, emulsifiers and combinations thereof.

"Nutraceutical" means any and all natural, naturally occurring, sustainable, synthetically-produced and bio-synthetically-produced compounds, mixtures of compounds, Functional Ingredients, molecules, compositions, raw materials, and intermediates (including components and delivery devices (such as capsules) related thereto, delivery systems thereof (such as blends or formulations) and methods of making the foregoing) that are associated with health and/or cosmetic benefits, as well as improving or maintaining the appearance of the human body. For the avoidance of doubt, Nutraceuticals includes compounds that can be used as supplements to food or beverage, whether a solid formulation, capsule, tablet, liquid formulation, solution or suspension.

Alternatively, the nutraceutical, pharmaceutical, veterinary, oenological or cosmetic formulation/product may consist or consist essentially of the composition of the invention.

The cosmetic formulation/product may be an anti-aging formulation.

As used herein, references to pharmaceutically, veterinary or cosmetically acceptable excipients may refer to pharmaceutically, veterinary or cosmetically acceptable adjuvants, diluents and/or carriers as known to those skilled in the art.

By "pharmaceutically/veterinary/cosmetically acceptable" we mean that the additional components of the composition are generally safe, non-toxic, and neither biologically nor otherwise undesirable. For example, the additional components may be

generally sterile and pyrogen free. Such components must be “acceptable” in the sense of being compatible with the composition of the invention and not deleterious to the recipients thereof. Thus, “pharmaceutically acceptable excipients” includes any compound(s) used in forming a part of the formulation that is intended to act merely as an excipient, i.e. not
5 intended to have biological activity itself.

The nutraceutical, pharmaceutical, veterinary, oenological or cosmetic formulation/product may be in the form of a liquid or a solid.

Liquid dosage formulations/products for oral administration include solutions, emulsions, aqueous or oily suspensions, syrups and elixirs.

10 Formulations and products (e.g. pharmaceutical, veterinary or cosmetic formulations/products) described herein, such as those intended for oral administration, may be prepared according to methods known to those skilled in the art, such as by mixing the components of the formulation/product together.

The formulation or product (e.g. pharmaceutical, veterinary or cosmetic
15 formulation/product) may contain one or more additional ingredients, such as pharmaceutical ingredients and excipients, such as sweetening agents, flavouring agents, colouring agents and preserving agents.

The formulation or product (e.g. pharmaceutical, veterinary or cosmetic formulation/product) may also contain one or more additional active ingredients, such as
20 cosmetic or pharmaceutical active ingredients, such as hyaluronic acid, centella asiatica extract, peptides such as Matrixyl® and Argireline®, and mixtures thereof.

The formulation or product of the invention may contain the active ingredient(s) in admixture with non-toxic pharmaceutically acceptable excipients (or ingredients). These excipients (or ingredients) may, for example, be: inert diluents, such as calcium carbonate,
25 sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, maltodextrin or alginic acid; binding agents, for example, starch, gelatine or acacia; or lubricating agents, for example magnesium stearate, stearic acid, talc and mixtures thereof.

Liquid formulations or products (e.g. pharmaceutical, veterinary or cosmetic
30 formulations/products) may be contained within a capsule, which may be uncoated or coated as defined above.

Suitable pharmaceutical or veterinary carriers include inert solid diluents or fillers, sterile aqueous solutions and various organic solvents. Examples of liquid carriers are

syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene and water.

Moreover, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

Suitable pharmaceutical carriers include inert sterile aqueous solutions and various organic solvents. Examples of liquid carriers are syrup, vegetable oils, phospholipids, fatty acids, fatty acid amines, polyoxyethylene and water. Moreover, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

Suitable cosmetic carriers are typically those that are suitable for topical administration to the outer surface of the human body, such as the skin and/or hair and/or scalp.

Typically, such carriers are dermatologically acceptable.

The phrase "dermatologically acceptable carrier" means that the carrier is suitable for topical application to the keratinous tissue, has good aesthetic properties, is compatible with the actives in the composition, and will not cause any unreasonable safety or toxicity concerns.

The carrier can be in a wide variety of forms. In some instances, the solubility or dispersibility of the components (e.g. extracts, sunscreen active, additional components) may dictate the form and character of the carrier. Non-limiting examples include simple solutions (e.g. aqueous or anhydrous), dispersions, emulsions, and solid forms (e.g. gels, sticks, flowable solids, or amorphous materials).

The dermatologically acceptable carrier may be in the form of an emulsion. An emulsion may be generally classified as having a continuous aqueous phase (e.g. oil-in-water and water-in-oil-in-water) or a continuous oil phase (e.g. water-in-oil or oil-in-water). The oil phase of the present invention may comprise silicone oils, non-silicone oils such as hydrocarbon oils, esters, ethers, and the like, and mixtures thereof. The aqueous phase typically comprises water and water-soluble ingredients (e.g. water-soluble moisturizing agents, conditioning agents, anti-microbials, humectants and/or other skin care actives). However, in some instances, the aqueous phase may comprise components other than water, including but not limited to watersoluble moisturizing agents, conditioning agents, antimicrobials, humectants and/or other watersoluble skin care actives. In some instances, the

non-water component of the composition comprises a humectant, such as glycerin and/or other polyol(s). Emulsions may also contain an emulsifier. Emulsifiers may be non-ionic, anionic or cationic.

The carrier may contain one or more dermatologically acceptable, hydrophilic diluents. As used herein, "diluent" includes materials in which the composition of the invention can be dispersed, dissolved, or otherwise incorporated. Hydrophilic diluents include water, organic hydrophilic diluents, such as lower monovalent alcohols (e.g., C1-C4), and low molecular weight glycols and polyols, including propylene glycol, polyethylene glycol, polypropylene glycol, glycerol, butylene glycol, 1,2,4-butanetriol, sorbitol esters, 1,2,6-hexanetriol, ethanol, isopropanol, sorbitol esters, butanediol, ether propanol, ethoxylated ethers, propoxylated ethers and combinations thereof.

The cosmetic formulation/product may optionally include one or more additional ingredients commonly used in cosmetic compositions (e.g., colorants, skin tone agents, skin anti-aging agents, anti-inflammatory agents, sunscreen agents, combinations of these and the like), provided that the additional ingredients do not undesirably alter the anti-glycation benefits provided by the composition.

In some instances, it may be desirable to select skin tone agents that function via different biological pathways so that the actives do not interfere with one another, which could reduce the efficacy of both agents. The additional ingredients, when incorporated into the composition, should be suitable for use in contact with human skin tissue without undue toxicity, incompatibility, instability, allergic response, and the like.

The term "carrier" as used herein, may also refer to a natural product or a product originating from nature that has been transformed or modified so that it is distinct from the natural product from which it originated, such as maltodextrin.

The amount of the composition of the invention present in nutraceutical, pharmaceutical, veterinary, oenological or cosmetic formulations or products will vary depending on the application.

Typically, the amount of composition of the invention that may be present in nutraceutical, pharmaceutical, veterinary, oenological or cosmetic formulations or products will be from about 0.001 to about 50% by weight, such as from about 0.01% to about 30% or from about 1% to about 20% of the nutraceutical, pharmaceutical, veterinary, oenological or cosmetic formulations or products, such as from about 0.01 to about 20%, or from about 0.1 to 10% or from about 1 to about 5% by weight of the formulation or product.

Those of skill in the art will recognize that, due to the degenerate nature of the genetic code, a variety of DNA molecules differing in their nucleotide sequences can be used to encode a given enzyme of the disclosure. The native DNA sequence encoding the biosynthetic enzymes described above are referenced herein merely to illustrate an embodiment of the disclosure, and the disclosure includes DNA molecules of any sequence that encode the amino acid sequences of the polypeptides and proteins of the enzymes utilized in the methods of the disclosure. In similar fashion, a polypeptide can typically tolerate one or more amino acid substitutions, deletions, and insertions in its amino acid sequence without loss or significant loss of a desired activity. The disclosure includes such polypeptides with different amino acid sequences than the specific proteins described herein so long as the modified or variant polypeptides have the enzymatic anabolic or catabolic activity of the reference polypeptide. Furthermore, the amino acid sequences encoded by the DNA sequences shown herein merely illustrate embodiments of the disclosure.

Described herein are specific genes and proteins useful in the methods, compositions and organisms of the disclosure; however it will be recognized that absolute identity to such genes is not necessary. For example, changes in a particular gene or polynucleotide comprising a sequence encoding a polypeptide or enzyme can be performed and screened for activity. Typically such changes comprise conservative mutations and silent mutations. Such modified or mutated polynucleotides and polypeptides can be screened for expression of a functional enzyme using methods known in the art.

Due to the inherent degeneracy of the genetic code, other polynucleotides which encode substantially the same or functionally equivalent polypeptides can also be used to clone and express the polynucleotides encoding such enzymes.

Techniques known to those skilled in the art may be suitable to identify additional homologous genes and homologous enzymes. Generally, analogous genes and/or analogous enzymes can be identified by functional analysis and will have functional similarities.

Techniques known to those skilled in the art may be suitable to identify analogous genes and analogous enzymes or any biosynthetic pathway genes, proteins, or enzymes, techniques may include, but are not limited to, cloning a gene by PCR using primers based on a published sequence of a gene/enzyme of interest, or by degenerate PCR using degenerate primers designed to amplify a conserved region among a gene of interest. Further,

one skilled in the art can use techniques to identify homologous or analogous genes, proteins, or enzymes with functional homology or similarity. Techniques include examining a cell or cell culture for the catalytic activity of an enzyme through *in vitro* enzyme assays for said activity (e.g . as described herein or in Kiritani, K., Branched-Chain Amino Acids Methods
5 Enzymology, 1970), then isolating the enzyme with said activity through purification, determining the protein sequence of the enzyme through techniques such as Edman degradation, design of PCR primers to the likely nucleic acid sequence, amplification of said DNA sequence through PCR, and cloning of said nucleic acid sequence. To identify
10 homologous or similar genes and/or homologous or similar enzymes, analogous genes and/or analogous enzymes or proteins, techniques also include comparison of data concerning a candidate gene or enzyme with databases such as BRENDA, KEGG, or MetaCYC. The candidate gene or enzyme may be identified within the above-mentioned databases in accordance with the teachings herein.

The terms "between... and..." and "ranging from... to..." should be understood as
15 being inclusive of the limits, unless otherwise specified. Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises", "comprising" and "possesses", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. The term "comprising" also means
20 "including" as well as "consisting" e.g. a composition "comprising" X may consist exclusively of X or may include something additional e.g. X + Y. It must be noted also that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. By way of example, a reference to "a gene" or "an enzyme" is a reference to "one or more genes" or "one or more enzymes".

25 It is to be understood that this disclosure is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present disclosure which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific
30 terms used herein have the same meanings as commonly understood by the person skilled in the art. In accordance with the present disclosure there may be conventional molecular biology, microbiology, and recombinant DNA techniques employed which are within the skill of the art.

This disclosure is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The disclosure is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. Preferably, the terms used
5 herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Kolbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Several documents are cited throughout the text of this specification. Each of the
10 documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.), whether supra or infra, is hereby incorporated by reference in its entirety.

The examples and figures which follow are presented by way of illustration and without implied limitation of the invention.

EXAMPLES

Example 1: Protocol for making a recombinant *Saccharomyces cerevisiae* strain according to the invention

5 All the hereinafter implemented recombinant *Saccharomyces cerevisiae* strains were constructed from standard strains using standard yeast molecular genetics procedure (Methods in yeast Genetics – A cold spring harbor laboratory course Manual (2000) by D. Burke, D. Dawson, T. Stearns CSHL Press).

10 Cluster of the following-mentioned genes were integrated in recombinant yeast at once using the ability of yeast to efficiently recombine free DNA ends which have sequence homology.

In addition, for a better comprehension of following genotypes:

- jlp1, lyp1, sam3, his3, leu2, trp1 and ura3 are insertion sites.

- Lowercase letters mean that the considered gene is inactive, uppercase letters reflect an active gene.

15 - “::”: following a gene name means that the gene is interrupted by what follows (if more than one gene are inserted, they are noted in brackets []). The interruption of the gene is concomitant with an entire deletion of the coding sequence but preserves the promoter. In consequence the gene followed by “::” is inactive and is noted in lowercase. If not specified the transcription of the gene inserted is controlled by the promoter of the disrupted gene.

20 - “gene.Kl” means that the gene originates from *Kluyveromyces lactis*.

More particularly, the coding sequences to be cloned were artificially synthesized. For heterologous sequences (non-yeast), the nucleic sequences were modified in order to obtain a synonymous coding sequence using the yeast codon usage. Using restriction enzyme and classical cloning technology, each synthetic sequence was cloned in between a transcription promoter and a transcription terminator. Each promoter sequence is preceded by a 50 to 200 nucleotide sequence homologous to the sequence of the terminator of the upstream gene. Similarly, the terminator of each gene (a gene comprising the promoter-coding sequence-terminator) is followed by sequences homologous to the gene immediately following. So that each of the unit to be integrated have a 50-200 nucleotide overlap with both the unit upstream and the unit downstream. For the first unit, the promoter is preceded by 50-200 nucleotides homologous to the yeast chromosome nucleotide for the locus in which it will be integrated. Similarly, for the last unit, the terminator is followed by 50-200 nucleotides homologous to the yeast chromosome nucleotide for the locus in which it will be integrated.

Each unit is then PCR amplified from the plasmids constructs, yielding X unit of linear DNA having overlapping sequences. At least one of this gene is an auxotrophic marker, in order to select for recombination event. All the linear fragments are transformed in the yeast at once, and a recombinant yeast cell is selected for the auxotrophy related to the marker used. The integrity of the sequence is then verified by PCR and sequencing.

Example 2: Comparative examples for the production of Hyaluronic acid

1. Production of Hyaluronic acid of less than 50 kDa

A. Firstly, three recombinant strains are obtained: YA5234-1, YA5235-1 and
10 YA5302-2.

Accordingly, these three strains are as follows:

YA5234-1 : MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pCCW12.sba-HYAL-3.Ts-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5235-1: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pNUP57-HYAL-3.Csa-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5302-2 : MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tIDP1, pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pCCW12.Sba-HYAL-3.Li-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-

tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.X1-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1-TRP1]_{x2}

HYAL-3 represents a nucleic acid sequence encoding a polypeptide having
5 hyaluronidase activity associated with a secretion signal but no anchoring signal.

HASA, HASA-1, HASA-A, HASA2 and XHASA2 all represent a nucleic acid encoding a polypeptide having hyaluronan synthase activity. They differ from one another in that they are different reencoded versions of a nucleic acid sequence encoding the hyaluronan synthase enzymes. HASA-1 has the sequence SEQ ID NO: 1, HASA-A has the sequence SEQ
10 ID NO: 2, HASA2 has the sequence SEQ ID NO: 7 and XHASA2 has the sequence SEQ ID NO: 6.

HASB and HASB-A represent a nucleic acid sequence encoding a polypeptide having UDP-glucose 6-dehydrogenase activity. They differ from one another in that they are different reencoded versions of a nucleic acid sequence encoding the enzyme. HASB.At has the sequence SEQ ID NO: 12, HASB.Vir has the sequence SEQ ID NO: 13 and HASB-A.Vir has the sequence SEQ ID NO: 14.
15

All these strains were incubated in 25 ml of SY medium buffered with MES 0,1 M pH 5.5 in a baffled erlenmeyer for 48 hours at 28°C under vigorous agitation.

SY medium comprises the following elements:

20 KH₂PO₄: 100 mM; MgSO₄ 7H₂O: 2,8 mM; K₂SO₄: 11,5 mM; Na₂SO₄: 1,1 mM; NaCl: 2,6 mM; CaCl₂ 2H₂O: 0,7 mM; CuSO₄ 5H₂O: 15 µM; KI: 6 µM; FeCl₃: 30 µM; ZnSO₄ 7H₂O: 61 µM; MnSO₄ H₂O: 25 µM; H₂SO₄: 110 µM; Panthotenic Acids hemicalcium salt: 42 µM; Thiamin hydrochloride: 59 µM; Pyridoxin hydrochloride: 49 µM; Myo-Inositol (C₆H₁₂O₆): 555 µM; Nicotinic acid (C₆H₅NO₂): 29 µM; D-Biotine:
25 0,82 µM; Ammonium citrate tribasic: 33 mM; and glucose or sucrose 2-30%.

Growth medium was recovered at 48h hours and assayed for hyaluronic acid content and quality. An aliquot of the medium was loaded and ran on a 0.5% agarose gel subsequently colored with "stains all" (sigma Aldrich CAS Number 7423-31-6).

The amount of hyaluronic acid present in the medium was evaluated by a
30 colorimetric determination after treatment with concentrated sulfuric acid and carbazole (Bitter and Muir (1962) analytical biochemistry 4, 330-334).

For illustrative purposes, the agarose gel obtained after running an aliquot of the supernatant of strain YA5235-1 and staining it with "stains all" (CAS Number 7423-31-6) is

provided as Figure 2A. It is classically run into the gel side-by-side with HA molecular weight (MW) standards (from left to right MW of 10 kDa, 20 kDa, 50 kDa and 300 kDa).

The amounts obtained with these different strains are respectively:

- YA5234-1: 2.4 g.L⁻¹.
- 5 - YA5235-1: 4.5 g.L⁻¹.
- YA5302-2: 3.7 g.L⁻¹.

In comparison, a native yeast strain, as for example the CC788-2D strain (Chérest et al. (2000) J Biol. Chem. 275: 14056-14063), does not produce hyaluronic acid.

10 It results from this experiment that a recombinant strain comprising the modifications according to the invention produces a greater amount of hyaluronic acid when cultured in the same conditions as compared with other recombinant strains not comprising all the genetic modifications according to the invention.

Moreover, these three strains led to the production, after 48 hours, of hyaluronic acid having a molecular weight comprised between 20kDa and 50kDa.

15 **B.** Three other recombinant strains have also been obtained: YA5110-13 and YA5260-4.

These three strains are as follows:

YA5110-13: MAT- α , can1-100, his3::[pSAM1-UGP1-tRPL3, pMET6-QRI1-tIDP1-HIS3]x5, jlp1::[LEU2.K1, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tTPI1, 20 pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pNUP57-HYAL-3.Hn-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.K1, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.XI-tRPL3, pTDH3-QRI1-tIDP1], 25 trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1-TRP1]x2

YA5260-4: MAT- α , can1-100, his3::[pSAM1-UGP1-tRPL3-pMET6-QRI1-tIDP1-HIS3]x5, jlp1::[LEU2.K1, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tIDP1, pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], 30 leu2, lyp1::[pTEF3-HYAL-31.Hn-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.K1, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-

XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1-TRP1]x2

YA5569 : MAT- α , his3::[pSAM1-UGP1- tRPL3, pMET6-QRI1-tIDP1, HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA-1.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tIDP1, 5 pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pCWP2-HYAL-31.Csa-tRPL15A, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tRPL41B, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[tRPL3-HASB.Vir-pMET6, pMET25-HASA-1.Vir-tIDP1, TRP1]x2 10

HYAL-31 represents a nucleic acid sequence encoding a polypeptide having hyaluronidase activity associated with both a secretion signal and an anchoring signal.

HASA-1, HASA-A, HASA2 and XHASA2 all represent a nucleic acid sequence 15 encoding a polypeptide having hyaluronan synthase activity. They differ from one another in that they are different reencoded versions of a nucleic acid sequence encoding the hyaluronan synthase enzyme. HASA-1 has the sequence SEQ ID NO: 1, HASA-A has the sequence SEQ ID NO: 2, HASA2 has the sequence SEQ ID NO: 7 and XHASA2 has the sequence SEQ ID NO: 6.

HASB and HASB-A represent a nucleic acid sequence encoding a polypeptide 20 having UDP-glucose 6-dehydrogenase activity. They differ from one another in that they are different reencoded versions of a nucleic acid sequence encoding the enzyme. HASB.At has the sequence SEQ ID NO: 12, HASB.Vir has the sequence SEQ ID NO: 13 and HASB-A.Vir has the sequence SEQ ID NO: 14.

25

The strains were inoculated in 2 liters of the above defined SY medium at pH 6 in 2% sucrose and 500 μ M CuSO₄ in a bioreactor. They were then grown in fed-batch adding 500 μ M CuSO₄ at 7h, 24h, 31h and between 130 and 175 g.L⁻¹ of sucrose from 10 to 48 h.

For YA5110-13, pH was maintained between 5.8 and 6.2 using 15% H₂SO₄ and 10% NH₄OH from t=31 h. 30

For YA5260-4, pH maintained between 5.8 and 6.2 using 15% H₂SO₄ and 10% NH₄OH all along the fermentation.

For YA5569, pH maintained between 5.8 and 6.2 using 15% H₂SO₄ and 10% NH₄OH all along the fermentation.

Growth medium was recovered at 48h hours and assayed for hyaluronic acid content and quality as described above.

5 The hyaluronic acid amounts obtained with these two strains are respectively:

- YA5110-13: 23 g.L⁻¹.

- YA5260-4: 16 g.L⁻¹.

- YA5569: 32 g.L⁻¹

In comparison, a native strain does not produce hyaluronic acid.

10 It results from this experiment that a recombinant strain comprising the modifications according to the invention produces a greater amount of hyaluronic acid when cultured in the same conditions as compared with other recombinant strains not comprising all the genetic modifications according to the invention.

Moreover, the hyaluronic acid produced by YA5110-13 and YA5260 strains had
15 a molecular weight of about 20 kDa while the hyaluronic acid produced by YA5569 had a molecular weight of about 20 kDa to 50 kDa.

The exact same experiment was performed using these three recombinant strains while replacing sucrose by glucose. The hyaluronic acid obtained had the same molecular weight of about 20 kDa or of about 20 kDa to about 50 kDa after 48h.

20 C. Another recombinant strain has been obtained 5672-29A, as follows:

5672-29A: MAT- α , his3::[pSAM1-UGP1-tRPL3, pMET6-QRI1-tIDP1, HIS3]x5, jlp1::[LEU2.Sba, pTEF1.Sba-HASB.Vir-tRPL3.Sm, pTDH3.Sk-HASB.Vir-tTEF1.Sba, pTDH3-1.Sba-HASA-1.Vir-tRPL3.Sba, pTDH3.Sar-HASA-1.Vir-tRPL15A.Sba, pTEF1-HYAL-31.Hn-tRPL15A], leu2, sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1.Ago-
25 GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tRPL41B, TRP1.Sba-loxP], trp1

HYAL-31 represents a hyaluronidase associated with an anchoring signal.

1 HASA-1 represents a nucleic acid sequence encoding a polypeptide having hyaluronan synthase activity. HASA-1 has the sequence SEQ ID NO: 1.

30 The strain was inoculated in 25 ml of the above defined SY medium at pH 5.5 buffered with MES 0.1 M in a baffled erlenmeyer at 28°C under vigorous agitation.

Growth medium was recovered at 48h hours and assayed for hyaluronic acid content and quality as described above.

The hyaluronic acid amount obtained with the strain was 3.7 g.L⁻¹.

2. Production of Hyaluronic acid having a molecular weight comprised between 50 kDa and 1000 kDa

5 A. Firstly, three recombinant strains are obtained: YA5233-1, YA5359-10 and YA5381-1.

Accordingly, these three strains are as follows:

YA5233-1: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tTPI1, 10 pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pTEF1-HYAL-3.Ts-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], 15 trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5359-10: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1- tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pCCW12.sba-HYAL- 31.Ts-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, 20 pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5381-1: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1- tTPI1, 25 pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pCCW12.Sar-HYAL2-31.Ts-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], 30 trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

HASA-1, HASA-A, HASA2 and XHASA2 are previously defined.

HASB and HASB-A are previously defined.

All these strains were incubated in 25 ml of the above-defined SY medium buffered with MES 0,1 M pH 5.5 baffled erlenmeyer for 48 hours at 28°C under vigorous agitation.

5 Growth medium was recovered at 48h hours and assayed for hyaluronic acid content and quality as defined above.

For illustrative purposes, the agarose gel obtained after running an aliquot of the supernatant of strain YA5359-10, after 31h and after 48h, and staining it with “stains all” (CAS Number 7423-31-6) is provided as Figure 2B. It is classically run into the gel side-by-side with HA molecular weight (MW) standards (from left to right MW of 20 kDa, 50 kDa, 10 60-120 kDa, 300 kDa and 750 kDa).

The amounts obtained with these different strains are respectively:

- YA5233-1: 3.1 g.L⁻¹.
- YA5359-10: 3.4 g.L⁻¹.
- 15 - YA5381-1: 3.0 g.L⁻¹.

In comparison, a native strain does not produce hyaluronic acid.

It results from this experiment that a recombinant strain comprising the modifications according to the invention produces a greater amount of hyaluronic acid when cultured in the same conditions as compared with other recombinant strains not comprising all 20 the genetic modifications according to the invention.

Moreover, these three strains led to the production, after 48 hours, of hyaluronic acid having a molecular weight comprised:

- for YA5233-1: between 50 kDa and 500 kDa;
- for YA5359-10: between 100 kDa and 750 kDa; and
- 25 - for YA5381-1: between 50 kDa and 250 kDa.

B. Six other recombinant strains have also been obtained: YA5262-16, YA5326-3, YA5300-15, YA5358-4, YA5264-1 and YA5331-6.

These six strains are as follows:

30 **YA5262-16**: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.K1, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tIDP1, pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pCCW10.ago-HYAL-31.Hn-tRPL15A, pCCW12.Sk-HASB-A.Vir-

tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5326-3: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pCCW10.ago-HYAL-31.Csa-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5300-15: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tIDP1, pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pTDH3-HYAL-3.Ts-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5358-4: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pCCW12.Sar-HYAL-31.Li-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5264-1: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pTDH3-HYAL-31.Ts-

tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1,
 pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba],
 sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3,
 pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-
 5 tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5331-6: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-
 tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tRPL3,
 pCUP1-QRI1-tIDP1, pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-
 tRPL15A], leu2, lyp1::[pTEF3-HYAL-31.Li-tRPL15A, pCCW12.Sk-HASB-A.Vir-
 10 tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-
 HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-
 tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-
 XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-
 HASA-1.Vir-tIDP1- TRP1]x2

15 HASA-1, HASA-A, HASA2 and XHASA2 are previously defined.

HASB and HASB-A are previously defined.

YA5262-16 was inoculated in 2 liters of the above defined SY medium at ph 4
 in 2% glucose in a bioreactor and ph was maintained at 4 all along the fermentation using
 15% H₂SO₄ and 10% NH₄OH.

20 YA5326-3 was:

- inoculated in 2 liters of the above defined SY medium at ph 6 in 2% glucose in
 a bioreactor and ph was maintained at 6 all along the fermentation using 15% H₂SO₄ and 10%
 NH₄OH; or

- inoculated in 2 liters of the above defined SY medium at ph 6 in 2% sucrose in
 25 a bioreactor and ph was maintained at 6 until 34h and at ph 4 from 34h to 48h with 15%
 H₂SO₄ and 10% NH₄OH.

YA5300-15 was inoculated in 2 liters of the above defined SY medium at ph 6
 in 2% sucrose in a bioreactor and ph was maintained at 6 all along the fermentation using
 15% H₂SO₄ and 10% NH₄OH.

30 YA5358-4 was inoculated in 2 liters of the above defined SY medium at ph 6 in
 2% glucose in a bioreactor and ph was maintained at 6 all along the fermentation using 15%
 H₂SO₄ and 10% NH₄OH.

YA5264-1 was inoculated in 2 liters of the above defined SY medium at pH 6 in 2% sucrose in a bioreactor and pH was maintained at 6 all along the fermentation using 15% H₂SO₄ and 10% NH₄OH.

YA5331-6 was inoculated in 2 liters of the above defined SY medium at pH 6 in 2% glucose in a bioreactor and pH was maintained at 6 all along the fermentation using 15% H₂SO₄ and 10% NH₄OH.

Growth medium was recovered at 48h hours and assayed for hyaluronic acid content and quality as described above.

The hyaluronic acid amounts obtained with these two strains are respectively:

- YA5262-16: 19 g.L⁻¹;
- YA5326-3: respectively 13 g.L⁻¹ and 15 g.L⁻¹;
- YA5300-15: 9 g.L⁻¹;
- YA5358-4: 14 g.L⁻¹;
- YA5264-1: 6 g.L⁻¹; and
- YA5331-6: 9 g.L⁻¹.

In comparison, a native strain does not produce hyaluronic acid.

It results from this experiment that a recombinant strain comprising the modifications according to the invention produces a greater amount of hyaluronic acid when cultured in the same conditions as compared with other recombinant strains not comprising all the genetic modifications according to the invention.

Moreover, the hyaluronic acid produced by these strains after 48 hours had a molecular weight:

- YA5262-16: of 50 kDa;
- YA5326-3: of respectively 50 kDa or comprised between 50 kDa and 250 kDa;
- YA5300-15: comprised between 100 kDa and 1000 kDa;
- YA5358-4: comprised between 100 kDa and 1000 kDa;
- YA5264-1: comprised between 100 kDa and 1000 kDa; and
- YA5331-6: comprised between 100 kDa and 1000 kDa.

Furthermore, previously described recombinant strain YA5235-1 was inoculated in a bioreactor at pH 6 in 2% sucrose and pH was maintained at 6 until 34h and at 4 from 34h to 48h with 15% H₂SO₄ and 10% NH₄OH.

After 48h the medium contains 18 g.L⁻¹ of 100-1000 kDa Hyaluronic acid.

The same experiment was performed using YA5235-1 while using glucose instead of sucrose. The obtained hyaluronic acid also has a molecular weight comprised between 100 and 1000 kDa.

These results show that, in particular, pH regulation along the fermentation process as well as the production of a hyaluronidase coupled to a secretion signal or a secretion signal and an anchoring signal influences, or even allows controlling, the size of the hyaluronic acid polymer produced.

3. Production of Hyaluronic acid having a molecular weight superior to 1000 kDa

Four recombinant strains are obtained: YA5232-1, YA5303-3, YA5374-4 and YA5382-1.

Accordingly, these four strains are as follows:

YA5232-1: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pNUP57-HYAL-3.Ts-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5303-2: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1- tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pNUP57-HYAL-3.Li-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5374-3: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1- tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pCCW12.Sar-HYAL-3.Ba-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba],

sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

YA5382-1: MAT- α , can1-100, his3::[pSAM1-UGP1- tRPL3-pMET6-QRI1-tIDP1- HIS3]x5, jlp1::[LEU2.Kl, pCUP1-HASA2.Sz-tRPL41B, pCUP1-UGP1- tTPI1, pTDH3-QRI1-tMET25, pCCW12-HASB.At-tRPL15A], leu2, lyp1::[pTEF1-HYAL2-31.Ba-tRPL15A, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tDIT1, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF3-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[pMET6-HASB.Vir-tRPL3, pMET25-HASA-1.Vir-tIDP1- TRP1]x2

HASA-1, HASA-A, HASA2 and XHASA2 are previously defined.

HASB and HASB-A are previously defined.

All these strains were incubated in 25 ml of the above-defined SY medium buffered with MES 0,1 M ph 5.5 in a baffled erlenmeyer for 48 hours at 28°C under vigorous agitation.

Growth medium was recovered at 48h hours and assayed for hyaluronic acid content and quality as defined above.

The amounts obtained with these different strains are respectively:

- YA5232-1: 4 g.L⁻¹.
- YA5303-3: 2.7 g.L⁻¹.
- YA5374-3: 2.5 g.L⁻¹.
- YA5382-1: 3.0 g.L⁻¹.

In comparison, a native strain does not produce hyaluronic acid.

It results from this experiment that a recombinant strain comprising the modifications according to the invention produces a greater amount of hyarluronic acid when cultured in the same conditions as compared with other recombinant strains not comprising all the genetic modifications according to the invention.

Moreover, these four strains led to the production, after 48 hours, of hyaluronic acid having a molecular weight:

- for YA5232-1: greater than about 1000 kDa;
- for YA5303-3: of about 1000 kDa;
- for YA5374-3: greater than about 1000 kDa; and

- for YA5382-1: greater than about 1000 kDa.

Example 3: Comparative examples for the production of Hyaluronic acid

A. The five following recombinant strains are obtained:

- 5 **YA5509**: MAT- α , can1-100, his3::[tRPL3-UGP1-pSAM1, pMET6-QRI1-tIDP1, HIS3]x5, jlp1::[LEU2.K1-RS, pCUP1-HASA.Sz-tRPL41B, pCUP1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-UGD1.At-tRPL15A], leu2, lyp1::[pCCW10.Ago-HYAL-31.Hn-tRPL15A, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tRPL41B, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.K1-RS, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tIDP1, pCCW12-XHASA2.XI-tRPL3, pTDH3-QRI1-tIDP1], trp1::[tRPL3-HASB.Vir-pMET6, pMET25-HASA.Vir-tIDP1, TRP1]x2, ura3::[tRPL3-MHPF.Ec-pPDC1, pTDH3-GDH-21.Eca-tIDP1, URA3]x2
- 15 **YA5789**: MAT- α , can1-100, his3::[tRPL3-UGP1-pSAM1, pMET6-QRI1-tIDP1, HIS3]x5, jlp1::[LEU2.K1-RS, pCUP1-HASA.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tIDP1, pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-UGD1.At-tRPL15A], leu2, lyp1::[tMET3.Sba-MET3.Sba-RS-pMET3.Sba, pCCW10.Ago-HYAL-31.Hn-tRPL15A, pTEF1.Ago-GLN1-tTDH3, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tRPL41B, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], met3::, sam3::[LEU2.K1-RS, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tIDP1, pCCW12-XHASA2.XI-tRPL3, pTDH3-QRI1-tIDP1], trp1::[tRPL3-HASB.Vir-pMET6, pMET25-HASA.Vir-tIDP1, TRP1]x2, ura3::[tRPL3-MHPF.Ec-pPDC1, pTDH3-GDH-21.Eca-tIDP1, URA3]x2
- 25 **YA5790**: MAT- α , can1-100, glt1::[MET3.Sba-RS, pCCW10.Ago-HYAL-31.Hn-tRPL15A, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tRPL41B, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba, pTEF1.Ago-GLN1-tTDH3], his3::[tRPL3-UGP1-pSAM1, pMET6-QRI1-tIDP1, HIS3]x5, jlp1::[LEU2.K1-RS, pCUP1-HASA.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tIDP1, pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-UGD1.At-tRPL15A], leu2, met3::, sam3::[LEU2.K1-RS, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tIDP1, pCCW12-XHASA2.XI-tRPL3, pTDH3-
- 30

QRI1-tIDP1], trp1::[tRPL3-HASB.Vir-pMET6, pMET25-HASA.Vir-tIDP1, TRP1]x2, ura3::[tRPL3-MHPF.Ec-pPDC1, pTDH3-GDH-21.Eca-tIDP1, URA3]x2

YA5806: MAT- α , can1-100, glt1::[MET3.Sba-RS, pCCW10.Ago-HYAL-31.Hn-tRPL15A, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tRPL41B, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], his3::[tRPL3-UGP1-pSAM1, pMET6-QRI1-tIDP1, HIS3]x5, jlp1::[LEU2.Kl-RS, pCUP1-HASA.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tIDP1, pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-UGD1.At-tRPL15A], leu2, met3::, sam3::[LEU2.Kl-RS, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[tRPL3-HASB.Vir-pMET6, pMET25-HASA.Vir-tIDP1, TRP1]x2, ura3::[tRPL3-MHPF.Ec-pPDC1, pTDH3-GDH-21.Eca-tIDP1, URA3]x2

YA5658: MAT- α , can1-100, his3::[tRPL3-UGP1-pSAM1, pMET6-QRI1-tIDP1, HIS3]x5, jlp1::[LEU2.Kl-RS, pCUP1-HASA.Sz-tRPL41B, pCUP1-UGP1-tRPL3, pCUP1-QRI1-tIDP1, pPDC1-UGP1-tTPI1, pTDH3-QRI1-tMET25, pCCW12-UGD1.At-tRPL15A], leu2, lyp1::[pCCW10.Ago-HYAL-31.Hn-tRPL15A, pTEF1.Ago-GLN1-tTDH3, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sk-HASB-A.Vir-tTEF1.Sba, pCCW12-HASA-1.Vir-tRPL41B, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], sam3::[LEU2.Kl-RS, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tIDP1, pCCW12-XHASA2.Xl-tRPL3, pTDH3-QRI1-tIDP1], trp1::[tRPL3-HASB.Vir-pMET6, pMET25-HASA.Vir-tIDP1, TRP1]x2, ura3::[tRPL3-MHPF.Ec-pPDC1, pTDH3-GDH-21.Eca-tIDP1, URA3]x2

HASA-1, HASA-A and XHASA2 are previously defined.

HASB and HASB-A are previously defined.

All these strains were incubated in 25 mL of the above-defined SY medium buffered with MES 0,1 M pH 5.5 in a baffled erlenmeyer for 48 hours at 28°C under vigorous agitation.

Growth medium was recovered at 48h hours and assayed for hyaluronic acid content and quality as defined above.

The amounts obtained with these different strains are respectively:

- YA5509: 3.5 g.L⁻¹.

- YA5789 (GLN1 overexpression): 5 g.L⁻¹.

- YA5658 (GNL1 overexpression) : 4.5 g.L⁻¹.
- YA5806 (Δ GLT1): 4.5 g.L⁻¹.
- YA5790 (GNL1 overexpression; Δ GLT1): 6.7 g.L⁻¹.

In comparison, a native strain does not produce hyaluronic acid.

5 It results from this experiment, under similar conditions, a recombinant strain according to the invention produces significantly more hyaluronic acid when either GLN1 is overexpressed or GLT1 is disrupted as compared with other recombinant strains according to the invention devoid of any of these two modifications (a respect increased yield of production by 27-32% and 27%).

10 Moreover, a recombinant strain according to the invention produces even more hyaluronic acid when both GLN1 is overexpressed and GLT1 is disrupted as compared with other recombinant strains according to the invention comprising only one of these two modifications or devoid of both (an increased yield of production by 48% compared to the one comprising none of these two modifications).

15

B. The four following recombinant strains are obtained:

YA6178: MAT- α , glt1::[MET3.Sba, pTDH3-HYAL-31.Csa-tRPL15A, pTEF1.Ago-GLN1-tTDH3, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-A.Vir-tRPL15A.Sba], his3::[pSAM1-UGP1-tRPL3, pMET6-QRI1-tIDP1, HIS3]x3, jlp1::[LEU2.Sba-loxP, pTEF1.Sba-HASB.Vir-tRPL3.Sm, pTDH3.Sk-HASB-A.Vir-tTEF1.Sba, pTDH3-1.Sba-HASA-1.Vir-tRPL3.Sba, pTDH3.Sar-HASA-A.Vir-tRPL15A.Sba], leu2, met3 Δ 1, sam3::[LEU2.K1, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tRPL41B, TRP1.Sba-loxP], trp1, ura3::[pPDC1-MHPF.Ec- tRPL3, pTDH3-GDH-21.Eca-tIDP1, URA3]x2

25 **YA6179:** MAT- α , glt1::[MET3.Sba, pTDH3-HYAL-31.Csa-tRPL15A, pTEF1.Ago-GLN1-tTDH3, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-2.Vir-tRPL15A.Sba], his3::[pSAM1-UGP1-tRPL3, pMET6-QRI1-tIDP1, HIS3]x3, jlp1::[LEU2.Sba-loxP, pTEF1.Sba-HASB.Vir-tRPL3.Sm, pTDH3.Sk-HASB-A.Vir-tTEF1.Sba, pTDH3-1.Sba-HASA-1.Vir-tRPL3.Sba, pTDH3.Sar-HASA-A.Vir-tRPL15A.Sba], leu2, met3 Δ 1, sam3::[LEU2.K1, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tRPL41B, TRP1.Sba-loxP], trp1, ura3::[pPDC1-MHPF.Ec- tRPL3, pTDH3-GDH-21.Eca-tIDP1, URA3]x2

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YA6180: MAT- α , glt1::[MET3.Sba, pTDH3-HYAL-31.Csa-tRPL15A, pTEF1.Ago-GLN1-tTDH3, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-3.Vir-tRPL15A.Sba], his3::[pSAM1-UGP1-tRPL3, pMET6-QRI1-tIDP1, HIS3] $\times$ 3, jlp1::[LEU2.Sba-loxP, pTEF1.Sba-HASB.Vir-tRPL3.Sm, pTDH3.Sk-HASB-A.Vir-tTEF1.Sba, pTDH3-1.Sba-HASA-1.Vir-tRPL3.Sba, pTDH3.Sar-HASA-A.Vir-tRPL15A.Sba], leu2, met3 Δ 1, sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tRPL41B, TRP1.Sba-loxP], trp1, ura3::[pPDC1-MHPF.Ec- tRPL3, pTDH3-GDH-21.Eca-tIDP1, URA3] $\times$ 2

YA6181: MAT- α , glt1::[MET3.Sba, pTDH3-HYAL-31.Csa-tRPL15A, pTEF1.Ago-GLN1-tTDH3, pCCW12.Sba-HASB.vir-tRPL3, pCCW12.Sm-HASA-5.Vir-tRPL15A.Sba], his3::[pSAM1-UGP1-tRPL3, pMET6-QRI1-tIDP1, HIS3] $\times$ 3, jlp1::[LEU2.Sba-loxP, pTEF1.Sba-HASB.Vir-tRPL3.Sm, pTDH3.Sk-HASB-A.Vir-tTEF1.Sba, pTDH3-1.Sba-HASA-1.Vir-tRPL3.Sba, pTDH3.Sar-HASA-A.Vir-tRPL15A.Sba], leu2, met3 Δ 1, sam3::[LEU2.Kl, pPDC1-PGM1-tIDP1, pTEF1.Ago-GFA1-tRPL15A, pENO2-UGP1-tRPL3, pCWP2-GNA1-tTPI1, pTEF1-PCM1-tRPL41B, TRP1.Sba-loxP], trp1, ura3::[pPDC1-MHPF.Ec- tRPL3, pTDH3-GDH-21.Eca-tIDP1, URA3] $\times$ 2

HASA-1 and HASA-A are previously defined.

HASB and HASB-A are previously defined.

HASA-2, HASA-3 and HASA5 all represent a nucleic acid sequence encoding a polypeptide having hyaluronan synthase activity. They differ from one another in that they are different reencoded versions of a nucleic acid sequence encoding the hyaluronan synthase enzyme. HASA-2 has the sequence SEQ ID NO: 101, HASA-3 has the sequence SEQ ID NO: 102 and HASA-5 has the sequence SEQ ID NO: 104.

All these strains were incubated in 25 mL of the above-defined SY medium buffered with MES 0,1 M ph 5.5 in a baffled erlenmeyer for 48 hours at 28°C under vigorous agitation.

Growth medium was recovered at 48h hours and assayed for hyaluronic acid content and quality as defined above.

The amounts obtained with these different strains are respectively:

- YA6178: 6.3 g.L⁻¹.
- YA6179: 5.9 g.L⁻¹.
- YA6180: 5.2 g.L⁻¹.
- YA6181: 6.4 g.L⁻¹.

In comparison, a native strain does not produce hyaluronic acid.

Moreover, these four strains led to the production, after 48 hours, of hyaluronic acid having a molecular weight of about 20 kDa.

SEQUENCES

SEQ ID NO: 1 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus PBCV-1*

5 ATGGGTAAGAACATCATTATTATGGTTTCTTGGTACACTATTATTACATCCAATCTCATCGCAGTGGGTGGCGCC
TCACTCATACTAGCCCCAGCTATTACGGGCTATGTCCTTCACTGGAACATTGCCCTTCAACAATTTGGGGAGTG
TCGGCCTACGGAATTTTCGTGTTTGGTTTCTTCTTGGCCAGGTATTATTTAGTGAACCTCAACCGGAAAAGGCTC
CGGAAGTGGATTTCCCTCCGACCCAAAGGGTGAATGATGTTAGGTTGGCTGTAATTATTGCCGGCTACCGTGAG
GACCCGTATATGTTCCAAAAGTGTCTTGAAAGTGTGCGTGACTCAGACTATGGGAATGTAGCTAGACTAATATGC
10 GTTATTGACGGCGATGAAGACGACGACATGAGGATGGCTGCAGTGTACAAGGCTATCTATAACGACAACATCAAG
AAACCTGAGTTTGTCTCTGTGAGAGTGACGATAAGGAGGGTGAGAGAATAGATAGCGATTTTACCCGTGATATC
TGCGTGCTGAACCGCATCGCGAAAGCGTGAATGTTTGTACACAGGGTTCCAATTGGCAAAGATGGACCCCTCA
GTTAATGCCGTCGTCTTAATCGACAGTGACACTGTGTTAGAAAAGGACGCGATTCTCGAaGTAGTATACCCGCTG
GCATGCGATCCAGAAATACAGGCTGTAGCAGGCGAATGCAAAATATGGAATACTGACACACTGTTGAGTTTGCTG
15 GTAGCCTGGCGATATTACAGCGCATTTTGGCTAGAGCGTAGCGCCCAATCATTCTTCAGGACAGTACAATGCGTC
GGAGGACCTCTCGGCGCCTACAAGATTGATATAATTAAGGAAATCAAGGACCCATGGATCAGCCAACGTTTCCTT
GGCCAAAAGTGCACATACGGCGACGATAGACGACTCACTAATGAAATACTAATGAGGGGTAAGAAAGTAGTTTTT
ACCCCATTCGCTGTTGGCTGGTCCGACAGCCGACGAACGCTCTCCGTTACATTGTACAGCAAACACGGTGGTCC
AAGTCGTGGTGTAGGGAGATATGGTATACACTGTTTGCAGCATGGAAGCATGGACTTTCGGGCATTTGGCTCGCA
20 TTCGAGTGCTTATACCAGATTACTTATTTCTTCCCTGGTGATTTACCTATTCTCCCGTTTGGCTGTTGAGGCTGAC
CCACGGGCGCAAACGGCCACCGTCATTGTTTCGACCACAGTTGCGCTTATTAAGTGCGGTTACTTTAGCTTCAGA
GCTAAAGACATTAGGGCCTTCTATTTCTGCTCTACACGTTTCTTACTTCTTCTGATGATTCCGGCACGAATA
ACTGCGATGATGACCTGTGGGACATAGGTTGGGGAACCAGGGGAGGAAATGAGAAGCCTTCCGTAGGCACCAGA
GTTGCTTTTGTTGGGCCAAGCAATATTTGATTGCTTACATGTGGTGGCCGCTGTCGTCGGTGCTGGCGTGTACTCC
25 ATCGTTTCATAATTGGATGTTTGACTGGAATTCACTTTCTACAGGTTGCGACTGGTAGGCATCTGTTCTGATATA
GTTTTTCATTGTAATAGTGTGTTAGTCTACTTTACAGGAAAGATCACGACCTGGAACCTTACGAAGCTACAGAAA
GAATTGATCGAGGACCGCTACTGTACGACGCAACGACCAATGCCAGTCGGTATAA

SEQ ID NO: 2 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus PBCV-1*

30 ATGGGCAAGAATATTATTATCATGGTCAGTTGGTATACCATTATTACATCAAACCTAATTGCTGTGCGTGGAGCC
TCACTAATCTTAGCTCCCGCGATTACGGGTTATGTGCTTCATTGGAATATTGCTTTATCCACTATTTGGGGTGT
AGCGCTTACGGCATCTTTGTGTTCCGTTTCTTCTTGGCTCAGGTGTTATTCTCTGAGCTAAACAGAAAGAGACTC
AGAAAGTGGATTTGCTGCGGCCGAAGGGATGGAACGATGTTGCGTTAGCGGTGATCATTGCCGGTTATCGTGAA
35 GATCCTTATATGTTTCAGAAGTGTTTGAATCTGTTTCGAGACTCAGACTACGGTAACGTTGCTAGGCTGATCTGC
GTAATCGATGGAGACGAAGATGACGATATGAGAATGGCCGCGGTTTATAAAGCCATCTATAACGATAACATTAAG
AAGCCAGAGTTTGTGTTTATGCGAATCGGATGACAAGGAGGGTGAGAGGATTGATTCTGACTTCTCGCGTGACATC
TGTGTCCTGCGACCCACAGGGGAAAGCGAGAGTGCTCTATACAGGATTCCAATTAGCTAAAATGGACCCAAGC

GTTAATGCTGTCGTCCTTATCGATAGCGACACGGTGTGGAAAAGGACGCAATATTGGAAGTAGTCTATCCACTC
GCTTGTGATCCAGAGATCCAGGCCGTGGCTGGCGAGTGCAAGATTGGAATACAGATACGCTTCTGTCACTACTG
GTGGCGTGGCGTTATTATTCAGCATTCTGTGTGGAGAGGAGCGCACAAATCGTTCTTTCGGACTGTACAATGCGTA
GGCGGTCCACTAGGAGCATATAAAATTGACATAATCAAAGAGATAAAAGATCCTTGGATTTGCGAGAGATTCCCTC
5 GGTCAAAAGTGTAACCTATGGGGACGACAGGCGGTTGACTAACGAGATTCTAATGAGAGGTAAGAAGGTGGTGTTT
ACTCCTTTTGGGTAGGTGGAGCGATTGCGCCACAAATGTATTAGATACATTGTTCAACAAACAAGGTGGTTCG
AAATCGTGGTGCCGAGAAATATGGTACACGTTGTTTCGCCGCATGGAAACACGGCTTGTTCGGGTATATGGCTCGCT
TTCGAGTGCCTTTATCAGATCACTTACTTCTTCTGTTTATTTACTTGTTCCTTCAAGACTGGCAGTGGAAGCTGAT
CCAAGGGCTCAGACTGCTACTGTATCGTTTCAACAACAGTGGCGCTAATAAAGTGTGGTTACTTCAGCTTTCGCG
10 GCGAAGGACATAAGAGCGTTTTACTTTGTTCTTTATACCTTTGTATATTTCTTCTGTATGATCCCGGCCCGGATA
ACAGCTATGATGACATTATGGGACATTGGTTGGGGCACCAGGGGTGGGAATGAGAAGCCGTCCGTAGGAACGAGA
GTAGCGCTGTGGGCCAAGCAGTATCTTATCGCATACATGTGGTGGGCAGCTGTCGTCGGGGCAGGTGTATACAGC
ATAGTACATAACTGGATGTTTACTGGAACCTCACTCTCATATCGATTGCGATTGGTCGGGATCTGCTCTTACATC
GTGTTTATTGTTATAGTCTTAGTAGTATATTTCACTGGAAAGATAACAACATGGAATTTCACTAAGCTTCAAAG
15 GAATTGATTGAAGACAGGGTGCTCTACGATGCGACAATAATGCACAAAGCGTATAA

SEQ ID NO: 3 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA)
originating from *Pasteurella multocida*

ATGAACACCCTTTCCAGGCAATTAAGGCTTATAATAGTAATGATTATCAACTAGCCCTCAAGCTGTTGAAAAG
20 TCGGCAGAAATCTACGGCAGAAAGATAGTTGA^gTTCCAAATTACGAAGTGCAAGGAGAACTTTCTGCGCACCCCA
TCCGTTAACAGTGCGCATCTCTCGGTTAAACAAAGAGAAGGTGAATGTTTGCACAGTCCATTAGACATAGCC
ACTCAATTATTGTTATCGAATGTGAAAAA^gCTCGTCCTTAGTGATAGCGAGAAAAATACACTAAAGAATAAATGG
AAGTTGTTGACTGAAAAGAAGTCTGAGAACGCAGAGGTCCGGGCCGTAGCTTTAGTTCCCAAGGACTTTCCGAAA
GATTTAGTGCTTGCGCCGTTACCAGACCATGTGAACGATTTTACGTGGTATAAGAAGCGGAAGAAACGCCTAGGA
25 ATCAAACCAGAACACCAACACGTTGGCCTCTCCATTATTGTGACAACGTTCAACCGTCCTGCCATCTCAGCATC
ACCCTGGCTTGCCCTCGTCAATCAAAAGACCCATTATCCTTTTGGGTGATCGTGACCGACGATGGTTCTCAAGAG
GATTTATCGCCTATAATCCGTCAATATGAGAACAAGCTAGACATCCGTTATGTTCCGCAAAAGGACAACGGTTTT
CAGGCATCGGCCGCACGTAATATGGGCCTACGTCTAGCCAAATATGACTTCATTGGGCTCCTTGATTGTGATATG
GCTCCCAACCCTTTGTGGGTACACTCATACGTTGCGGAACTATTAGAGGACGATGATTTGACCATAATAGGCCCC
30 AGGAAGTACATTGACACGCAACACATAGATCCCAAGGATTTCCCTAATAACGCCTCTCTGTTAGAGTCGTTGCCA
GAGGTAAAGACCAATAATTCCGTCGCGGCTAAGGGCGAGGGGACCGTATCTTTAGACTGGCGTTTGAACAATTT
GAGAAGACCGAGA^gCTTGAAGGCTATCCGATAGCCCTTTCCGATTCTTCGCAGCTGGGAATGTGGCTTTCCGCAAG
AAGTGGCTTAACAAGTCAGGATTCTTCGACGAGGAATTCAATCACTGGGGAGGTGAAGATGTAGAGTTCGGTTAT
CGTCTGTTTCCGGTACGGTTCGTTCTTCAAAACTATAGACGGCATCATGGCCTATCATCAGGAACCGCCAGGTAAA
35 GAAAACGAAACTGACAGAGAAGCGGCAAGAACATTACCCTCGATATAATGAGGGAGAAGGTGCCTTACATCTAC
CGTAAACTCCTGCCTATAGAAGACAGTCATATCAACCGAGTACCATTGGTATCAATTTACATCCCGGCCTACAAC
TGTGCTAACTATATTCAAAGATGTGTTGATTAGCTTTGAATCAAACGGTAGTTGATCTCGAAGTGTGCATTTCG
AACGACGGTAGTACTGACAACACGCTGGAAGTTATTAACAAGCTGTATGGTAATAATCCGCGTGTGCGTATAATG
TCTAAACCCAATGGCGGCATTGCGAGTGCATCCAACGCAGCGGTACGCTTCGCAAAGGGTTATTACATAGGACAG

TTGGACAGCGACGATTACTTAGAACCCGACGCGAGTGGAGTTATGTCTCAAGGAATTTCTTAAGGATAAGACCCTT
GCGTGCGTTTACACCACTAATCGTAACGTCAACCCAGATGGCTCTTTAATAGCCAATGGCTATAACTGGCCAGAG
TTCAGTCGTGAGAAGTTGACTACGGCCATGATTGCTCATCACTTCCGGATGTTTACCATTTCGTGCTTGGCATCTG
ACGGATGGGTTCAATGAGAAGATTGAGAACGCTGTTGACTACGACATGTTTCTCAAGCTCAGTGAAGTTGGTAAA
5 TTTAAGCATCTGAACAAAATATGTTATAATCGGGTGTACACGGCGATAACACCTCAATCAAGAAgCTTGGCATA
CAAAAGAAGAATCATTTCGTAGTTGTCAATCAGTCTCTAAACCGCCAAGGTATAACTTATTATAACTACGATGAA
TTTGATGATCTCGATGAGAGTCGGAATACATTTTCAACAAGACTGCAGAGTATCAAGAAGAGATAGATATTCTT
AAAGATATTAAGATCATTTCAGAACAAAGACGCCAAAATAGCTGTCTCCATTTTCTATCCGAACACTCTAAACGGG
TTGGTGAAGAAGCTAAATAATATTATAGAGTATAATAAGAATATCTTCGTTATTGTACTTCATGTGCGATAAGAAT
10 CACTTAACCCAGACATCAAGAAGGAGATATTAGCCTTCTACCATAAGCATCAGGTGAACATCTTATTGAACAA
GACATCTCCTACTATACATCAAATCGTCTGATTAAGACAGAAGCCCATTTGAGTAACATTAACAAGCTaAGTCAA
TTAAATCTTAACTGCGAATACATTATATTCGACAATCACGATTCTTATTTGTGAAGAACGATTCTATGCTACAC
ATGAAGAAGTATGATGTTGGAATGAACCTCTCTGCATTGACTCATGATTGGATTGAAAAGATAAACGCTCACCCG
CCATTTAAGAAGCTgATCAAAACTTACTTCAATGACAATGATCTTAAGTCGATGAATGTAAAGGGTGCCTCCAG
15 GGAATGTTTATGACATACGCATTAGCCACGAGTTATTGACGATCATCAAGGAGGTGATAACCTCTTGTCAATCC
ATTGACTCCGTCCCCGAATACAACACAGAAGATATTGGTTTCAGTTTGCACTTTAAATCTGGAAAAGAAGACC
GGCCACGTATTCAACAAGACAAGCACTCTCACGTATATGCCATGGGAACGTAAACTGCAGTGGACGAATGAACAA
ATAGAGTCCGCAAAGAGGGGCGAAAACATTCCGGTAAACAAGTTCATCATTAAACAGCATTACCCCTTAA

- 20 **SEQ ID NO: 4** is a reencoded nucleic acid sequence of hyaluronan synthase (HASA)
originating from *Pasteurella multocida*

ATGAACACCCTTTCCAGGCAATTAAGGCTTATAATAGTAATGATTATCAACTAGCCCTCAAGCTGTTGCGAAAAG
TCGGCAGAAATCTACGGCAGAAAGATAGTTGAgTTCCAAATTACGAAGTGCAAGGAGAACTTTCTGCGCACCCA
TCCGTTAACAGTGCGCATCTCTCGGTTAACAAGAAGAGAAGGTGAATGTTTGCACAGTCCATTAGACATAGCC
25 ACTCAATTATTGTTATCGAATGTGAAAAAATCTGTCCTTAGTGATAGCGAGAAAAATACACTAAAGAATAAATGG
AAGTTGTTGACTGAAAAGAAGTCTGAGAACGCGAGAGGTCCGGGCCGTAGCTTTAGTTCCCAAGGACTTTCCGAAA
GATTTAGTGCTTGCGCCGTTACCAGACCATGTGAACGATTTTACGTGGTATAAGAAGCGGAAGAAACGCCTAGGA
ATCAAACCAGAACACCAACACGTTGGCCTCTCCATTATTGTGACAACGTTCAACCGTCCTGCCATCTCAGCATC
ACCCTGGCTTGCTCGTCAATCAAAAGACCCATTATCCTTTTGGGTGATCGTGACCGACGATGGTTCTCAAGAG
30 GATTTATCGCCTATAATCCGTCAATATGAGAACAAGCTAGACATCCGTTATGTTCCGCAAAAGGACAACGGTTTT
CAGGCATCGGCCGACGTAATATGGGCCTACGTCTAGCCAAATATGACTTCATTGGGCTCCTTGATTGTGATATG
GCTCCCAACCCTTTGTGGGTACACTCATACGTTGCGGAACTATTAGAGGACGATGATTTGACCATAATAGGCCCC
AGGAAGTACATTGACACGCAACACATAGATCCCAAGGATTTCCCTTAATAACGCCTCTCTGTTAGAGTCGTTGCCA
GAGGTAAAGACCAATAATCCGTGCGGCTAAGGGCGAGGGGACCGTATCTTTAGACTGGCGTTTGAACAATTT
35 GAGAAGACCGAGAACTTGAGGCTATCCGATAGCCCTTTCCGATTCTTCGCAGCTGGGAATGTGGCTTTCCGCAAG
AAGTGGCTTAACAAGTCAGGATTCTTCGACGAGGAATTCAATCACTGGGGAGGTGAAGATGTAGAGTTCGGTTAT
CGTCTGTTTCGGTACGGTTCGTTCTTCAAAACTATAGACGGCATCATGGCCTATCATCAGGAACCGCCAGGTAAA
GAAAACGAACTGACAGAGAAGCGGGCAAGAACATTACCCTCGATATAATGAGGGAGAAGGTGCCTTACATCTAC
CGTAAACTCCTGCCTATAGAAGACAGTCATATCAACCGAGTACCATTGGTATCAATTTACATCCCGGCCTACAAC

TGTGCTAACTATATTCAAAGATGTGTTGATTTCAGCTTTGAATCAAACGGTAGTTGATCTCGAAGTGTGCATTTC
AACGACGGTAGTACTGACAACACGCTGGAAGTTATTAACAAGCTGTATGGTAATAATCCGCGTGTGCGTATAATG
TCTAAACCCAATGGCGGCATTGCGAGTGCATCCAACGCAGCGGTGAGCTTCGCAAAGGGTTATTACATAGGACAG
TTGGACAGCGACGATTACTTAGAACCCGACGCAGTGGAGTTATGTCTCAAGGAATTTCTTAAGGATAAGACCCCTT
5 GCGTGCCTTTACACCACTAATCGTAACGTCAACCCAGATGGCTCTTTAATAGCCAATGGCTATAACTGGCCAGAG
TTCAGTCGTGAGAAGTTGACTACGGCCATGATTGCTCATCACTTCCGGATGTTTACCATTCTGTGCTTGGCATCTG
ACGGATGGGTTCAATGAGAAGATTGAGAACGCTGTTGACTACGACATGTTTCTCAAGCTCAGTGAAGTTGGTAAA
TTTAAGCATCTGAACAAAATATGTTATAATCGGGTGTACACGGCGATAACACCTCAATCAAGAAgCTTGGCATA
CAAAAGAAGAATCATTTTCGTAGTTGTCAATCAGTCTCTAAACCGCAAGGTATAACTTATTATAACTACGATGAA
10 TTTGATGATCTCGATGAGAGTCGAAATACATTTTCAACAAGACTGCAGAGTATCAAGAAGAGATAGATATTCTT
AAAGATATTTAA

SEQ ID NO: 5 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Pasteurella multocida*

15 ATGAACACCCCTTTCCAGCAATTAAGCTTATAATAGTAATGATTATCAACTAGCCCTCAAGCTGTTTCGAAAAG
TCGGCAGAAATCTACGGCAGAAAGATAGTTGA_gTTCCAAATTACGAAGTGCAAGGAGAAACTTCTGCGCACCCA
TCCGTTAACAGTGCATCTCTCGGTTAACAAAGAAGAGAAGGTGAATGTTTGCACAGTCCATTAGACATAGCC
ACTCAATTATTGTTATCGAATGTGAAAAACTCGTCCTTAGTGATAGCGAGAAAAATACACTAAAGAATAAATGG
AAGTTGTTGACTGAAAAGAAGTCTGAGAACGCAGAGGTCCGGGCCGTAGCTTTAGTTCCCAAGGACTTTCCGAAA
20 GATTTAGTGCTTGCGCCGTTACCAGACCATGTGAACGATTTTACGTGGTATAAGAAGCGGAAGAAACGCCTAGGA
ATCAAAACGAGAACACCAACACGTTGGCCTCTCCATTATTGTGACAACGTTCAACCGTCCTGCCATCCTCAGCATC
ACCCGTGGCTTGCTCGTCAATCAAAAGACCCATTATCCTTTTGAGGTGATCGTGACCGACGATGGTTCTCAAGAG
GATTTATCGCCTATAATCCGTCAATATGAGAACAAGCTAGACATCCGTTATGTTTCGGCAAAAGGACAACGGTTTT
CAGGCATCGGCCGCACGTAATATGGGCCTACGTCTAGCCAAATATGACTTCATTGGGCTCCTTGATTGTGATATG
25 GCTCCCAACCCCTTTGTGGGTACACTCATACTGCGGAACTATTAGAGGACGATGATTTGACCATAATAGCCCCC
AGGAAGTACATTGACACGCAACACATAGATCCCAAGGATTTCCCTTAATAACGCCTCTCTGTTAGAGTCGTTGCCA
GAGGTTAAGACCAATAATTCCGTGCGCGCTAAGGGCGAGGGGACCGTATCTTTAGACTGGCGTTTGAACAATTT
GAGAAGACCGAGAACTTGAGGCTATCCGATAGCCCTTTCCGATTCTTCGCAGCTGGGAATGTGGCTTTCCGCAAG
AAGTGGCTTAACAAGTCAGGATTCTTCGACGAGGAATTCAATCACTGGGGAGGTGAAGATGTAGAGTTCCGGTTAT
30 CGTCTGTTTCGGTACGGTTCGTTCTTCAAACTATAGACGGCATCATGGCCTATCATCAGGAACCGCCAGGTAAA
GAAAACGAAACTGACAGAGAAGCGGGCAAGAACATTACCCTCGATATAATGAGGGAGAAGGTGCCTTACATCTAC
CGTAAACTCCTGCCTATAGAAGACAGTCATATCAACCGAGTACCATTGGTATCAATTTACATCCCGGCCCTACAAC
TGTGCTAACTATATTCAAAGATGTGTTGATTTCAGCTTTGAATCAAACGGTAGTTGATCTCGAAGTGTGCATTTC
AACGACGGTAGTACTGACAACACGCTGGAAGTTATTAACAAGCTGTATGGTAATAATCCGCGTGTGCGTATAATG
35 TCTAAACCCAATGGCGGCATTGCGAGTGCATCCAACGCAGCGGTGAGCTTCGCAAAGGGTTATTACATAGGACAG
TTGGACAGCGACGATTACTTAGAACCCGACGCAGTGGAGTTATGTCTCAAGGAATTTCTTAAGGATAAGACCCCTT
GCGTGCCTTTACACCACTAATCGTAACGTCAACCCAGATGGCTCTTTAATAGCCAATGGCTATAACTGGCCAGAG
TTCAGTCGTGAGAAGTTGACTACGGCCATGATTGCTCATCACTTCCGGATGTTTACCATTCTGTGCTTGGCATCTG
ACGGATGGGTTCAATGAGAAGATTGAGAACGCTGTTGACTACGACATGTTTCTCAAGCTCAGTGAAGTTGGTAAA

TTTAAGCATCTGAACAAAATATGTTATAATCGGGTGTACACGGCGATAACACCTCAATCAAGAAGCTTGGCATA
CAAAAGAAGAATCATTTCGTAGTTGTCAATCAGTCTCTAAACCGCCAAGGTATAACTTATTATAACTACGATGAA
TTTGATGATCTCGATGAGAGTCGGAATACATTTTCAACAAGACTGCAGAGTATCAAGAAGAGATAGATATTCTT
AAAGATATTGGATCCGCCATTTCTCAAATCACTGACGGTCAAATCCAAGCTACTACCACTGCTACCACCGAAGCT
5 ACCACCACTGCTGCCCATCTTCCACCGTTGAACTGTTTCTCCATCCAGCACCGAAACTATCTCTCAACAACT
GAAAATGGTGCTGCTAAGCCGCTGTCGGTATGGGTGCCGGTGTCTAGCTGCTGCTGCTATGTTGTTATAA

SEQ ID NO: 6 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA)
originating from *Xenopus laevis*

10 ATGCACTGTGAACGATTTATTTGTATATTACGAATAATTGGTACGACTTTGTTCCGAGTAAGTCTGCTACTAGGG
ATATCCGCCGCTTATATCGTGGGATACCAATTCATTGACGCGATAATTACTACTTTTCTTCGGTCTCTACGGT
GCTATTTTAGCTTTACATTTAATCATTGAGTCTCTCTTCGCATTCCTGGAGCATCGTAAGATGAAGCGGTCACTT
GAAACCCCTATAAAATTAACAAGAGCGTAGCATTGTGTATCGCTGCATACCAGGAAGACGAAGATTATCTTAGA
AAATGTTTGCTTTCTGTGAAACGGCTCACATACCCCGGTATGAAGGTAATCATGGTGATAGATGGTAAGTCTGAT
15 GACGATCTATACATGATGAACATATTGAGAGATCATGGGAAATGATAGCTGCGCCACCTATGTATGGAAGAAT
AATTTTCACATGAAGGGACCAAACGAGACCGACGAGACTCACCGGAGTCCATGCAGCATGTAACACAGATGGTG
CTTTCTAACAGAAATGCTGTATCATGCAAAAGTGAACGGGAAACGCGAGGTGATGTATACTGCATTCAAAGCG
CTGGGTGCGTGGTTGATTACGTCCAAGTGTGCGACTCAGATACAGTCTTGATCCAGCAAGCTCGGTGGAGATG
GTCAAGGTGTTGGAGGAAGACATTATGGTCGGAGGTGTAGGCGGAGACGTGCAGATACTCAATAAGTACGACTCC
20 TGGATCAGTTTCTTGAGTTCGGTTCGTTATTGGATGGCTTTCAATATAGAACGAGCATGCCAATCATACTTTGGC
TGTGTGCAGTGATATCGGGGCCTCTGGGCATGTACAGAACTCACTCTTACATGAGTTTATTGAAGACTGGTAC
AATCAAGAATTCCTCGGTTCCCAATGCTCTTTCCGGTGATGATCGGCATTTGACTAATCGAGTACTATCATTGGGC
TATGCTACTAAATACACTGCGCGCAGTAAGTGTCTGACAGAAACCCACAGAATATCTTAGATGGTTGAACCAA
CAAACCAGGTGGAGTAAGTCCTATTTCCGCGAGTGGTTGTACAACCTCTTTGTGGTTCCACAAACACCATTTATGG
25 ATGACTTATGAAGCCGTAATTACGGGTTTCTTCCCTTCTTCTTAATAGCGACGGTGATTACAGCTCTTCTATCGT
GGTCAATTTGGAATATCTTACTCTTTCTACTCACAGTTCAATTAGTTGGTCTAATAAAGTCGTCGTTTGCAAGT
GCGTTGCGTGGAACATCGTTATGGTCTTTATGAGTTTCTACAGTGTTTTATACATGAGTAGCTTGCTGCCAGCA
AAGATGTTTGCAATCGCCACGATAAACAAGGCTGGATGGGGTACTAGTGGTAGAAAGACCATAGTTGTTAATTTT
ATTGGTTTAAATCCCTATTACGGTATGGTTCACAATTTTACTAGGGGGAGTATGCTACACTATATGGCGGGAAACC
30 AAGAAGCCGTTTAGCGAGTCTGAGAAGATCGTTCTTGGGTGCGTCTATATTGTATGCTTGTATTGGGTCATG
TTGCTTACAATGTATGTCAGTCTTGTCTATGAAGTGCGGGCGCCGTAGGAAGGAACACAGCACGACCTTGTTTTA
GCATAA

35 **SEQ ID NO: 7** is a reencoded nucleic acid sequence of hyaluronan synthase (HASA)
originating from *Streptococcus zooepidemicus*

ATGAGGACGTTAAAGAATCTTATCACTGTTGTAGCGTTTCAGTATATTCTGGGTTTTGCTGATCTATGTAAATGTC
TATCTTTTCGGTGCGAAGGGTCTCTATCAATATATGGATTCTTGCTAATTGCGTATTTGCTTGTGAAGATGAGT

CTTTCATTCTTCTATAAACCATTCAAAGGTCGAGCTGGTCAGTATAAAGTCGCAGCGATTATCCCTTCATACAAC
 GAGGATGCAGAATCGCTGTTGGAAACCTTAAATCTGTTCAACAACAAACCTATCCCCTTGCTGAAATTTATGTA
 GTTGATGATGGTTCCGCAGATGAAACCGGTATCAAGCGGATCGAGGATTATGTCAGGGATACAGGCGACTTAAGT
 TCAAATGTGATTGTGCATCGATCAGAAAAGATCAAGGCAAGAGACACGCCCAAGCATGGGCATTGAAAGATCT
 5 GATGCAGATGTATTTCTAACAGTCGATAGTGATACTTACATTTATCCGGACGCCCTTGAAGAGTTATTGAAAACC
 TTCAATGATCCGACAGTATTTGCCGCAACTGGCCACTTGAACGTTGTAACAGGCAGACTAATCTATTGACCAGA
 CTCCTGATATAAGATACGACAATGCTTTTGGCGTCGAACGTGCTGCTCAAAGTGTAACGGTAATATACTGGTG
 TGTTCCGGGCCACTGTCCGTCTATCGCAGAGAAGTAGTAGTCCCGAACATTGACAGGTATATTAATCAGACTTTT
 CTGGGTATCCCCGTGTCAATTGGGGACGACCGGTGTTTAACGAACTACGCTACTGATTTGGGCAAGACTGTATAT
 10 CAGTCGACGGCTAAATGTATTACAGACGTCCAGATAAGATGTGACTTACCTTAAACAGCAGAATAGATGGAAT
 AAGTCATTCTTTAGAGAATCTATCATCAGCGTGAAGAAGATCATGAATAATCCATTCGTGGCGCTTTGGACCATT
 TTGGAGGTTTCCATGTTTATGATGCTTGTCTACTCTGTCGTTGATTTCTTTGTAGGTAACGTTTCGAGAATTGAT
 TGGCTCAGAGTTCTTGCTTTCTTAGTTATCATCTTCATCGTTGCGCTATGCAGGAATATTCATTACATGCTAAAA
 CATCCGCTTAGCTTCTTACTCAGCCCTTTCTACGGCGTTTTGCATCTCTTCGTTTTGCAGCCATTGAAGCTCTAC
 15 TCCTTATTTACCATTGAAACGCTGATTGGGGTACGCGCAAGAACTATTAtaa

SEQ ID NO: 8 is an amino acid sequence of hyaluronan synthase (HASA) originating from
Chlorella virus PBCV-1

MGKNIIIMVSWYTIITSNLIAGVGASLILAPAITGYVLHWNIALSTIWGVSAYGIFVFGFFLAQVLFSELNRKRL
 20 RKWISLRPKGWNDVRLAVIIAGYREDPYMFQKCLESVRSDSYGNVARLICVIDGDEDDMRMAAVYKAIYNDNIK
 KPEFVLCESDDKGERIDSDFSRDICVLQPHRGKRECLYTGFQLAKMDPSVNAVVLIDSDTVLEKDAILEVVYPL
 ACDPEIQAVAGECKIWNNTDLSLLVAVRYSAFCVERSAQSFFRTVQCVGGPLGAYKIDIIKEIKDPWISQRFL
 GQKCTYGDDRRRLTNEILMRGKKVFTPFVAVGWSDSPTNVFRYIVQQTRWSKSWCREIWTFLFAAWKHGLSGIWLA
 FECLYQITYFFLVIYLF SRLAVEADPRAQTATVIVSTTVALIKGYFSFRAKDIRAFYFVLYTFVYFFCMIPARI
 25 TAMMTLWDIGWTRGGNEKPSVGTRVALWAKQYLIAYMWWAAVVGAGVYSIVHNWMDWNSLSYRFALVGICSYI
 VFIVIVLVVYFTGKITTWNFTKLQKELIEDRVLYDATTNQSV

SEQ ID NO: 9 is an amino acid sequence of hyaluronan synthase (HASA) originating from
Pasteurella multocida

MNTLSQAIKAYNSNDYQLALKLFEKSAEIIYGRKIVEFQITKCKEKL SAHPSVNSAHL SVNKEEKVNVCDSPLDIA
 30 TQLLLSNVKKLVLS DSEKNTLKNKWLLTEKKSENAEVRVALVPKDFPKDLVLAPLPDHVNDFTWYKKRKRRLG
 IKPEHQHVGLSIIIVTTFNRPAIL SITLACLVNQKTHYPFEVIVTDDGSQEDLSP IIRQYENKLDIRYVRQKDNGF
 QASAARNMGLRLAKYDFIGLLDCMAPNPLWVHSYVAELLEDDDLTIIGPRKYIDTQHIDPKDFLNNASLLES LP
 EVKTNN SVAAGEGTVSLDWRLEQFEKTENLR LSDSPFRFFAAGNVAF AKKWL NKSGFFDEEFNHWGGEDEVFGY
 35 RLFYRGSFFKTIDGIMAYHQEPPGKENETDREAGKNITLDIMREKVPYIYRKLLPIEDSHINRVPLVSIYIPAYN
 CANYIQRCVDSALNQTVVDLEVCICNDGSTDNTLEVINKLYGNNPRVRIMSKPNGGIASASNAAVSFAKGYIIGQ
 LDSDDYLEPDAVELCLKEFLKDKTLACVYTTNRNVNPDGSLIANGYNWPEFSREKLTTAMIAHHFRMFTIRAWHL
 TDGFNEK IENAVDYDMFLKLSEVGKFKHLN KICYNRVLHGDNTS IKKLGIQKKNHFVVVNQSLNRQGITYYNYDE
 FDDLDESRYIFNKTA EYQEEIDILKDIKIIQNKDAKIAVSIFYPNTLNLGVKKLNNIIEYKNKIFVIVLHV DKN

HLTPDIKKEILAFYHKHQVNILLNNDISYYTSNRLIKTEAHLNINKLSQLNLNCEYIIFDNHDSL FVKND SYAY
MKKYDVGMNF SALT HDWIEKINAHPPFKKLIKTYFNDNDLKSMNVKGASQGMFMTYALAHELLTIIKEVITSCQS
IDSVPEYNTEDIWFQFALLILEKKTGHVFNKTSTLTYPWERKLQWTNEQIESAKRGENIPVNKFIINSITL

- 5 **SEQ ID NO: 10** is an amino acid sequence of hyaluronan synthase (HASA) originating from *Xenopus laevis*

MHCERFICILRIIGTTLFGVSLLLGISAAIYVG YQFIQTDNYYFSFGLYGAILALHLIIQSLFAFLEHRKMKRSL
ETPIKLNKSVALCIAAYQEDEDYLRKCLLSVKRLTYPGMKVIMVIDGNSDDDL YMMNIFREIMGNDSCATYVWKN
NFMHKGPNETDETHRESMQHVTQMVLNVRNVCIMQKWNKGREVMYTAFKALGRSVDYVQVCDSDTVLDPASSVEM
10 VKVLEEDIMVGGVGGDVQILNKYDSWISFLSSVRYWMAFNIERACQSYFGCVQCISGPLGMYRNSLLHEFIEDWY
NQEFLGSQCSFGDDRHLTNRVLSLGYATKYTARSKCLTETPTEYLRWLNQQTRWSKSYFREWLNSLWFHKKHLW
MTYEAVITGFFPFFLIATVIQLFYRGRINILLFLLTVQLVGLIKSSFASALRGNI VMVMSFYSVLYMSSLLPA
KMFAIATINKAGWGTSGRKTIVVNFIGLIPITVWFITLLGGVCYTIWRETKKPFSESEKIVLAVGAILYACYWVM
LLTMVSVLVMKCGRRRKEPQHDLVLA

15

- SEQ ID NO: 11** is an amino acid sequence of hyaluronan synthase (HASA) originating from *Streptococcus zooepidemicus*

MRTLKNLITVVAFSIFWVLLIYVNVYLF GAKGSLSIYGFLLIAYLLVKMSLSFFYKPFKGRAGQYKVAAIIPSYN
EDAESLLETLSVQQQTYPLAEIYVDDGSADETGKRIEDYVRD TGDLSSNVIVHRSEKNQKRHAQAWAFERS
20 DADVFLTVDSDTYIYPDALEELLKTFNDPTVFAATGHLNVRNRQTNLLTRLTDIRYDNAFGVERAAQSVTGNILV
CSGPLSVYRREVVVPNI DRYINQTF LGIPV SIGDDRCLTNYATDLGKTVYQSTAKCITDVPDKMSTYLKQQNRWN
KSFFRESIISVKKIMNPNFVALWTILEVSMFMMLVYSVVDFVGVNREFDWLRVLAFLVIFIIVALCRNIHYMLK
HPLSFLSPFYGVLLHFLVLQPLKLYSLFTIRNADWGTRKKLL

- 25 **SEQ ID NO: 12** is a nucleic acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Arabidopsis thaliana*

ATGGTCAAAATATGCTGTATCGGGCTGGATATGTCGGTGGGCCTACAATGGCCGTTATGGCCCTAAAGTGTCCA
GAGATTGAAGTCGTGGTAGTTGATATATCGGAGCCACGAATTAACGCATGGAACCTCAGACCGTCTACCAATTTAT
GAGCCAGGGTTAGAGGATGTCGTCAACAGTGTAGAGGGAAGAATTTGTTCTTCTCTACAGATGTAGAGAAGCAT
30 GTATTTGAGTCAGATATAGTGTGTTTTCGGTAAACACTCCTACGAAAACGCAGGGTCTGGGTGCAGGTAAAGCA
GCAGATTTGACATATTGGGAGTCCGCTGCTCGCATGATAGCTGATGTGAGCAAATCGTCGAAAATCGTCGTTGAA
AAGAGTACAGTACCAGTTCGTACAGCCGAGGCTATAGAAAAGATTTTAACGCACAATTCGAAGGGTATCGAATTC
CAGATCTTATCAAATCCAGAATTTCTTGCCGAAGGGACGGCGATTAAAGACTTATATAACCTGATAGAGTTCTA
ATCGGTGGCAGGGACACCGCTGCGGGACAAAAGGCCATTAAGGCGTTGCGTGACGTGTATGCCCACTGGGTTCCT
35 GTTGAGCAAATAATCTGTACTAATTTATGGAGTGCCGAGCTATCAAAGTTGGCTGCGAACGCATTTCTAGCTCAA
AGGATAAGTTCAGTAAACGCAATGTCAGCGCTTTGTGAGGCAACTGGTGCTGACGTAACCAAGTCGCTCACGCC
GTTGGAACCGACACTAGAAATTGGACCGAAGTTTCTTAACGCATCCGTAGGCTTCGGCGGATCTTGCTTTTCAGAAA
GACATCCTGAATCTTATTTACATCTGCGAATGCAATGGTCTTCCAGAAGCAGCCAATTATTGGAAACAGGTAGTC

AAGGTAAATGACTACCAAAAGATTAGGTTTGCTAATCGAGTCGTATCTTCTATGTTCAACACCGTCTCCGGTAAG
AAAATTGCTATTTTGGGATTTCGTTCAAGAAGGACACCGGCGACACGCGTGAAACTCCTGCCATAGATGTGTGT
AATCGCCTCGTGGCTGATAAAGCAAAGCTGTCTGATCTATGATCCGCAAGTATTAGAAGAGCAGATCCGCCGTGAT
CTGTCCATGCCCCGATTTCGATTGGGACCATCCAGTCCCACTCCAGCAGATCAAAGCTGAAGGTATCTCCGAACAG
5 GTTAACGTTGTGTCCGACGCCTACGAGGCTACGAAGGATGCTCATGGTTTATGTGTTTTAACCGAATGGGACGAA
TTCAAGTCACCTTGATTTTAAGAAGATCTTTGATAATATGCAGAAACCCGCTTTTCGTTTTTCGACGGAAGAAACGTG
GTCGACGCTGTGAAATTGAGAGAAATTGGATTTCATAGTATATTCCATAGGTAAACCTCTGGATAGTTGGCTCAAG
GATATGCCGGCTGTTGCATAA

- 10 **SEQ ID NO: 13** is a reencoded nucleic acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Chlorella virus PBCV-1*

ATGAGTAGAATTGCTGTCTGGATGCGGTTACGTGGGTACGGCCTGCGCCGTACTTTTGGCGCAGAAGAACGAA
GTTATCGTTTTGGATATCTCGGAGGACCGGGTACAACCTGATTAAGAATAAGAAGTCACCTATAGAAGATAAGGAA
ATCGAAGAATTCCTGGAGACGAAAGATTTGAATCTAACAGCGACGACGGATAAGGTGCTCGCCTATGAGAATGCT
15 GAATTCGTTATAATAGCTACACCGACCGATTACGATGTCGTCACTAGATATTTCAACACTAAGTCTGTTGAAAAT
GTGATAGGCGATGTCATTAAGAACACTCAGACACACCCTACTATAGTGATCAAGAGTACTATTCCAATCGGTTTC
GTGGATAAAGTTAGAGAACAGTTTCGATTATCAAAATATTATCTTCTCGCCGGAATTCTTGAGAGAAGGACGAGCA
TTATATGATAATCTTTACCCCTCCCGTATCATCGTCGGTGATGACTCCCAATTGCCTTAAAATTCGCGAATCTC
TTGGTCGAGGGTAGCAAACTCCACTAGCTCCCGTATTAATACTATGGGTACGCGAGAGGCCGAAGCTGTAAAATA
20 TTTTCAAATACATATTTGGCTATGAGAGTAGCATACTTCAATGAGCTAGACACATTTGCAATGTCCCATGGTATG
AACGCAAAAGAGATTATCGACGGAGTCACACTAGAACCAAGGATAGGGCAGGGTTATTTCGAATCCTTCATTTGGG
TATGGAGCCTATTGTTTCCCAAAAGATACAAAGCAATTGTTGGCTAATTTTGAAGGGGTTCCACAAGATATTATT
GGCGCAATCGTAGAGTCTAACGAACTAGAAAGGAGTTATTGTTTCTGAGGTCGAGAACAGATTTCCGACCACA
GTTGGCGTATATAAGTTGGCTGCTAAAGCTGGTTTCGGATAATTTTCAGGTCTAGTGCGATAGTCGATATCATGGAA
25 AGGTTGGCTAATAAGGGCTACCACATCAAAATATTTGAACCTACCGTTGAACAATTTGAGAATTTTGAGGTAGAT
AATAATCTCACAACCTTTTGAACCGAGTCTGATGTAATAATTGCGAACAGAGTTCAGTCGAACATCGCATTCTG
TTTGGGAAGAAGTTAATAACACGGGATGTCTATGGGGATAA

- 30 **SEQ ID NO: 14** is a reencoded nucleic acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Chlorella virus PBCV-1*

ATGTCACGAATCGCAGTAGTTGGCTGTGGGTACGTGGGAACCGCATGCGCTGTACTGCTGGCGCAAAAGAATGAG
GTAATCGTTCTTGATATTAGTGAGGATAGGGTCCAACCTAATCAAGAACAAGAAATCCCCGATCGAAGATAAGGAG
ATTGAAGAATTCCTGGAAACAAAAGACCTAACTTAACTGCAACAACCTGACAAAGTTTTAGCCTACGAAAACGCT
35 GAGTTTGTGATTATAGCGACACCCACAGATTATGACGTAGTTACCAGATACTTTAACACGAAGTCCGTAGAGAAC
GTCATTGGAGATGTTATAAAGAATACTCAGACTCATCTACGATAGTAATAAAGTCAACCATTCCCATAGGTTTC
GTAGATAAGGTTAGGGAGCAATTCGATTACCAGAACATTATATTTTCGCCAGAATTTCTGAGAGAGGGTCGCGCC
CTGTATGATAATCTATATCCATCACGGATTATAGTGGCGATGACTCTCCGATTGCACTTAAGTTTGCTAATCTT

TTAGTTGAGGGCTCCAAAACCTCCGCTCGCCCCAGTACTTACGATGGGTACACGTGAGGCTGAAGCTGTCAAGCTG
TTTTCAAACACATACCTTGCTATGCGAGTCGCATACCTTTAACGAACTAGATACCTTTGCTATGTCGCACGGTATG
AATGCTAAAGAAATCATAGATGGCGTAACGTTGGAGCCTCGGATAGGTCAAGGATATTCCAATCCATCTTTTGGC
TACGGTGCGTATTGTTTTCCCAAAGGACACGAAGCAATTATTAGCTAACTTTGAGGGTGTTCCGCAAGATATAATT
5 GGGGCGATAGTAGAAAGCAATGAAACACGGAAGGAGGTAATCGTGAGTGAAGTGAAAACCGATTCCCCACTACG
GTCGGCGTTTACAAATTAGCCGCCAAGGCTGGTTCCGACAATTTCCGATCCAGCGCAATAGTAGATATTATGGAA
AGATTAGCTAATAAGGGATACCACATTAAAATCTTTGAACCTACTGTGGAACAGTTGAGAACTTCGAGGTTGAT
AATAACTTGACGACTTTTCGCAACGGAGAGCGATGTAATTATTGCAAACCGCGTACCTGTGGAACATCGAATTTTG
TTCGGAAGAAGCTGATTACACGCGATGTATATGGCGATAACTAA

10

SEQ ID NO: 15 is a reencoded nucleic acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Streptococcus zooepidemicus*

ATGAAGATATCGGTAGCGGGTTCGGGGTACGTGGGGTTATCCTTGTCATCTTGCTTGCCCAACATAACGATGTG
ACTGTTGTAGATATAATCGACGAAAAGGTACGGCTAATTAACCAGGGCATATCTCCGATTAAGGATGCGGACATT
15 GAGGAATATCTGAAGAATGCACCGTTGAATCTTACGGCTACACTAGACGGAGCTTCGGCTTAGTAATGCTGAT
CTGATTATAATCGCAACGCCAACTAATTACGATTGAGAAGCAATTATTTGACACCAGACACGTTGAAGAAGTA
ATTGAGCAAGTATTGGATTAAATGCCTCCGCTACTATAATCATCAAGAGTACCATAACCTTGGGTTTTATTAA
CACGTAAGAGAGAAATACCAAACAGACAGAATCATCTTTTCTCCAGAGTTCTTAAGAGAGTCAAAGGCATTGTAC
GATAACTTATACCCCTCTCGTATAATAGTCAGTTATGAGAAGGATGACTCTCCAAGAGTTATACAAGCAGCTAAG
20 GCGTTCGCGGGTTTATTAAAAGAGGGGGCAAAGAGCAAGGATACCCAGTTCTGTTTATGGGCTCTCAAGAAGCT
GAAGCTGTCAAGCTGTTTGCTAATACTTTTCTCGCCATGAGGGTCAGTTACTTCAACGAGCTTGACACTTATAGC
GAATCAAAAGGACTAGACGCCCAAAGAGTTATAGAAGGCGTCTGCCATGATCAAAGGATAGGTAATCATTACAAT
AATCCATCTTCGATATGGCGTTATTGTTTACCCAAAGACTCAAAGCAACTTTTGGCTAATTATAGAGGCATA
CCTCAGTCTCTAATGTCTGCCATCGTTGAATCGAACAAGATCCGTAAGTCGTATTTAGCTGAACAAATATTAGAT
25 AGGGCTTCTTCACAAAAGCAGGCTGGTGTACCTTTAACCATAGGATTTTACCCTTTGATTATGAAGTCCAACTCC
GATAACTTTAGAGAATCAGCCATTAAAGATATTATTGACATCATTAACTGACTACGGTGTCAATATTGTCATTAT
GAACCTATGTTGGGAGAAGACATTGGTTATAGAGTCGTTAAAGATTGGAACAGTTTAAAGAACGAAAGTACAATT
ATTGTTAGTAACAGGTTTGAAGATGATTAGGTGATGTTATTGATAAAGTTTATACACGTGACGTCTTTGGTAG

30 **SEQ ID NO: 16** is an amino acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Arabidopsis thaliana*

MVKICIGAGYVGGPTMAVMALKCPEIEVVVDISEPRINAWNSDRLP IYEPGLEDVVKQCRGKNLFFSTDVEKH
VFESDIVFSVNTPTKTQGLGAGKAADLTWESAARMIADVSKSSKIVVEKSTVPVRTAEAEIEKILTHNSKGIEF
QILSNPEFLAEGTAIKDLYNPDRVLIGGRDTAAGQKAIKALRDVYAHWVPVEQIICTNLWSAELSKLAANAF LAQ
35 RISSVNAMSALCEATGADVTQVAHAVGTDTRIGPKFLNASVGGGSCFQKDILNLIYICECNGLPEAANYWKQVV
KVNDYQKIRFANRVSSMFTVSGKKIAILGFAFKKDTGDTRETPAIDVCNRLVADKAKLSIYDPQVLEEQIRRD
LSMARFDWDHPVPLQQIKAEGISEQVNVVSDAYEATKDAHGLCVLTEWDEFKSLDFKKIFDNMQKPAFVFDGRNV
VDAVKLREIGFIVYSIGKPLDSWLKDMPAVA

SEQ ID NO: 17 is an amino acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Chlorella virus PBCV1*

MSRIAVVGCYVGTACAVLLAQKNEVIVLDISEDRVQLIKNKKSPIEDKEIEEFLETKDLNLTATTDKVLAYENA
5 EFVVIATPTDYDVVTRYFNTKSVENVIGDVIKNTQTHPTIVIKSTIPIGFVDKVVREQFDYQNIIFSPEFLREGRA
LYDNLPSRIIVGDDSPIALKFANLLVEGSKTLPAPVLTMGTREAEAVKLFNTYLAMRVAYFNELDTFAMSHGM
NAKEIIDGVTLPRIGQGYSNPSFGYGAYCFPKDTKQLLANFEGVPQDIIGAIVESNETRKEVIVSEVENRFPTT
VGVIYKLAAGSDNFRSSAIVDIMERLANKGYHIKIFEPTVEQFENFEVDNLLTTFATESDVIIANRVPVEHRIL
FGKKLITRDVYGDN

10

SEQ ID NO: 18 is an amino acid sequence of UDP-Glucose dehydrogenase (HASB) originating from *Streptococcus zooepidemicus*

MKISVAGSGYVGLSLSILLAQHNDVTVDIIDEKVRLINQGISPIKDADIEEYLKNAPLNLTATLDGASAYSNA
LIIIIATPTNYDSERNYFDTRHVEEVIEQVLDLNASATIIKSTIPLGFIKHVREKYQTDRIIFSPEFLRESKALY
15 DNLPSRIIVSYEKDDSPRVIQAAKAFAGLLKEGAKSKDTPVLFMGSQAEAVKLFANTFLAMRVSYFNELDTYS
ESKGLDAQRVIEGVCHDQRIGNHYNNPSFGYGGYCLPKDSKQLLANYRGIPQSLMSAIVESNKIRKSYLAEQILD
RASSQKQAGVPLTIGFYRLIMKSNSDNFRESAIDIIIDINDYGVNIVIEPMLGEDIGYRVVKDLEQFKNESTI
IVSNRFEDDLGDVIDKVYTRDVFGRD

20 **SEQ ID NO: 19** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Bothrops atrox* with a N-terminal secretion signal

ATGCAATTTAGCACAGTCGCATCAGTAGCCTTCGTTGCCTTGGCCAACTTCGTGGCAGCACCGATGTATCCGAAC
GAACCGTTCTTAGTCTTTTGAACGCGCTACAACCTCAGTGCTAGACTTCGATATAAGGTTGACCTTGATCTGAAG
ACATTCCATATCGTGACAAATGCTAATGACTCGCTGTCAGGATCGGCTGTCACGATTTTCTATCCACGCACTTA
25 GGGGTTTACCCACATATTGATGACAGGGGCACTTCTTCAATGGCATCATACCCAAAATGAATCCCTGGTAAAG
CATTTAAACAAATCTAAATCAGATATTAATCGAATGATTCCCTTAAGAACATTCACGGGCTGGGAGTCATAGAC
TGGGAAACTGGCGGCCACAGTGGGATAGGAATTGGGGAAGTAAGAACGTTTATAGGAATAGATCAATCCAATTC
GCGCGTGATCTCCACCCAGAGCTTAGTGAGGACAAGATTAAACGCTTGGCAAAACAGGAATTCGAGAAAGCTGCA
AAGAGTTTTATGAGGGATACACTATTATTAGCCGAGGAGATGCGACCAGACGGCTACTGGGGATACTATCTGTAC
30 CCCGATTGTCAACAATTACAATTATAAGACTAAGCCAGATCAGTACACAGGAGAGTGCCCTGACATCGAGATGTCA
CGTAACAATCAACTCTTGTGGCTTTGGCGGGATAGCACTGCCCTTTTCCCCAATATCTACTTAGAGACTATACTA
AGAAGCTCTGACAATGCCCTGAAGTTTGTGCACCATAGGCTCAAGGAGGCAATGAGGATAGCCTCAATGGCTCGA
AATGACTACGCGTTGCCTTTCTTTGTTTATGCTCGACCATTTCTATGCCTATACCTTCGAACCATTTGACTCAGGAA
GACCTTGTGACTACGGTCGGAGAGACCGCGGACATGGGCGCCGCTGGGATCGTATTTTGGGGGAGTATGCAGTAC
35 GCAAGCACGTTGAATCTTGCGGCAAAGTCAAGGACTACATGAATGGCCCACTGGGGCGTTACATTGTGAATGTT
ACAACGCGGCCAAAATTTGCTCAGATTCTTGTGCAAACGTCATGGTAGGTGTGTAAGAAAGCACTCAGACTCC
AATGCATTCCCTTACCTATTTCCCGATTTCGTTTCGCATAATGGTGCACGGGAATGCAACCGAGAAGAAAGTTATA

GTAAAGGGGAAGCTGGAATTAAAGAATCTTATATTCTTACGTAATAACTTTATGTGCCAGTGTTATCAAGGCTGG
AAAGGTCTATATTGCGAGAAGCATTTCGATAAAGGAAATTCGGAATACTAA

SEQ ID NO: 20 is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating

- 5 from *Bothrops atrox* with a N-terminal secretion signal and a C-terminal anchoring signal
ATGCAATTTAGCACAGTCGCATCAGTAGCCTTCGTTGCCTTGGCCAACTTCGTGGCAGCACCGATGTATCCGAAC
GAACCGTTCTTAGTCTTTTGAACGCGCCTACAACCTCAGTGTAGACTTCGATATAAGGTTGACCTTGATCTGAAG
ACATTCCATATCGTGACAAATGCTAATGACTCGCTGTCAGGATCGGCTGTCACGATTTTCTATCCCACGCACTTA
GGGGTTTACCCACATATTGATGACAGGGGGCACTTCTTCAATGGCATCATACCCCAAATGAATCCCTGGTAAAG
10 CATTTAAACAAATCTAAATCAGATATTAATCGAATGATTCCCTTAAGAACATTCCACGGGCTGGGAGTCATAGAC
TGGGAAACTGGCGGCCACAGTGGGATAGGAATTGGGGAAGTAAGAACGTTTATAGGAATAGATCAATCCAATTC
GCGCGTGATCTCCACCCAGAGCTTAGTGAGGACAAGATTAAACGCTTGGCAAAACAGGAATTCGAGAAAGCTGCA
AAGAGTTTTATGAGGGATACACTATTATTAGCCGAGGAGATGCGACCAGACGGCTACTGGGGATACTATCTGTAC
CCCGATTGTCACAATTACAATTATAAGACTAAGCCAGATCAGTACACAGGAGAGTGCCCTGACATCGAGATGTCA
15 CGTAACAATCAACTCTTGTGGCTTTGGCGGGATAGCACTGCCCTTTTCCCAATATCTACTTAGAGACTATACTA
AGAAGCTCTGACAATGCCTGAAGTTTGTGCACCATAGGCTCAAGGAGGCAATGAGGATAGCCTCAATGGCTCGA
AATGACTACGCGTTGCCTTTCTTTGTTTATGCTCGACCATTCTATGCCTATACCTTCGAACCAATTGACTCAGGAA
GACCTTGTGACTACGGTCGGAGAGACCGCGGACATGGGCGCCGCTGGGATCGTATTTTGGGGGAGTATGCAGTAC
GCAAGCACGGTTGAATCTTGCGGCAAAGTCAAGGACTACATGAATGGCCCACTGGGGCGTTACATTGTGAATGTT
20 ACAACTGCCGCCAAAATTTGCTCAGATTCTTGTGCAAACGTCATGGTAGGTGTGTAAGAAAGCACTCAGACTCC
AATGCATTCCCTTACCTATTTCCCGATTTCGTTTCGCATAATGGTGCACGGGAATGCAACCGAGAAGAAAGTTATA
GTAAAGGGGAAGCTGGAATTAAAGAATCTTATATTCTTACGTAATAACTTTATGTGCCAGTGTTATCAAGGCTGG
AAAGGTCTATATTGCGAGAAGCATTTCGATAAAGGAAATTCGGAATACTCGATCCGCCATTTCTCAAACTACTGAC
GGTCAAATCCAAGCTACTACCACTGCTACCACCGAAGCTACCACCACTGCTGCCCCATCTTCCACCGTTGAAACT
25 GTTTCTCCATCCAGCACCGAAACTATCTCTCAACAACTGAAATGGTGCTGCTAAGGCCGCTGTCTGGTATGGGT
GCCGGTGCTCTAGCTGCTGCTGCTATGTTGTTATAA

SEQ ID NO: 21 is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating

- 30 from *Cupiennius salei* with a N-terminal secretion signal
ATGCAATTCAGCACTGTTGCATCAGTTGCATTTGTGCCCCTGGCGAATTTTGTAGCCGCGTTCAAGATTTACTGG
AACGTCCCAACTTTTTCAGTGCACGCATAACTACAAAATCGATTATGTCAAATTGTTGTCCACTTACGGGATACAG
GTCAATGATGGCGGTAAGTTTCAAGGAAACCAAGTGACTATCTTTATGAAACCCAGTTGGGTTTGTATCCACGA
ATCCTAAAATCTGGTAAAATGGAAAACGGCGGAATCCCTCAACGCGGTAACTTTGAGAAACACCTAGAAAAGGCA
35 AGCACGGACCTCCAGAAAGTGATCCCTTGGAAAGAGTTTAGCGGATTAGGTGTGATAGATTGGGAGGCTTGAGAG
CCCACATGGGAATTTAACTGGGAACCGTTGAGGATATATCAAACCGAATCAATTAAGAGAGCTAAAGAACTACAC
CCTACCGCAAACGATTCCGCAGTAAAAGAAATTGCAGAGCGGCAATGGGAAGATTAGCCAAGTTATACATGTTA
GAAACACTGCGGCTGGCAAAGAACTTCGACCTCAAGCGCCTTGGTGTTACTACTTATTTCTGATTGCTATAAT
TACGTGCGAAAGAAACCAAAAGATTTCCAATGTAGTGCCTCGATACGTAAAGGTAACGATAAGCTAAGCTGGTTG

TGGAAAGATTCTACGGCATTGTGTCCATCGATATACGTATATGAATCACAATTAGACAGGTATTCTTTTGAACAA
AGGACATGGCGCGACAATGAGAACTTCGGGAAGCGTTGCGTGTAGCCACGAGAACCTCTAAAATATACCCATAC
GTTAACTATTTTCGATAAGGAGCTTATACCGGAGCAAGAAGTATGGAGAATGCTTGCGCAGGCAGCTGCTGTCCGGT
GGCAGTGGTGCGGTAATTTGGGGCTCATCTGCTGCAGTTGCATCTGAAGAGTTATGTAAATCTTTAAACAGTAT
5 ATTATTGAAACGCTTGGGCCGGCGGCAGAGAAGGTGGCTTGGCGTAGTGACTTATGCAGCAAAGAAATTTGTAAT
AATCAGGGTCGCTGCACATTCCCGACGATGATTATGCAAACGCATGGAAATTATTTACAGATGATACTGTTAAG
TTTTATGCTGGTAATATTACATGTAGGTGCTCCGAGAATTATTCTGGTCGTTTCTGCGAAAAGAAGAATTAA

SEQ ID NO: 22 is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating
10 from *Cupiennius salei* with a N-terminal secretion signal and a C-terminal anchoring signal
ATGCAATTCAGCACTGTTGCATCAGTTGCATTTGTGCCCTGGCGAATTTTGTAGCCGCGTTCAAGATTTACTGG
AACGTCCCAACTTTTCAGTGACGCATAACTACAAAATCGATTATGTCAAATTTGTGCCACTTACGGGATACAG
GTCAATGATGGCGTAAGTTTCAAGGAAACCAAGTACTATCTTTTATGAAACCCAGTTGGGTTTGTATCCACGA
ATCCTAAAATCTGGTAAAATGGAACCGGCGAATCCCTCAACGCGGTAACCTTGAGAAACACCTAGAAAAGGCA
15 AGCACGGACCTCCAGAAAGTGATCCCTTGGAAAGAGTTTAGCGGATTAGGTGTGATAGATTGGGAGGCTTGGAGA
CCCACATGGGAATTTAACTGGGAACCGTTGAGGATATATCAAACCGAATCAATTAAGAGAGCTAAAGAACTACAC
CCTACCGCAAACGATTCCGCAGTAAAAGAAATTGCAGAGCGGCAATGGGAAGATTACGCCAAGTTATACATGTTA
GAAACACTGCGGCTGGCAAAGAACTTCGACCTCAAGCGCCTTGGTGTTACTACTTATTTCTGATTGCTATAAT
TACGTCCGAAAGAAACCAAAAGATTTCCAATGTAGTGCCTCGATACGTAAAGGTAACGATAAGCTAAGCTGGTTG
20 TGGAAAGATTCTACGGCATTGTGTCCATCGATATACGTATATGAATCACAATTAGACAGGTATTCTTTTGAACAA
AGGACATGGCGCGACAATGAGAACTTCGGGAAGCGTTGCGTGTAGCCACGAGAACCTCTAAAATATACCCATAC
GTTAACTATTTTCGATAAGGAGCTTATACCGGAGCAAGAAGTATGGAGAATGCTTGCGCAGGCAGCTGCTGTCCGGT
GGCAGTGGTGCGGTAATTTGGGGCTCATCTGCTGCAGTTGCATCTGAAGAGTTATGTAAATCTTTAAACAGTAT
ATTATTGAAACGCTTGGGCCGGCGGCAGAGAAGGTGGCTTGGCGTAGTGACTTATGCAGCAAAGAAATTTGTAAT
25 AATCAGGGTCGCTGCACATTCCCGACGATGATTATGCAAACGCATGGAAATTATTTACAGATGATACTGTTAAG
TTTTATGCTGGTAATATTACATGTAGGTGCTCCGAGAATTATTCTGGTCGTTTCTGCGAAAAGAAGAATGGATCC
GCCATTTCTCAAATCACTGACGGTCAAATCCAAGCTACTACCACTGCTACCACCGAAGCTACCACCACTGCTGCC
CCATCTTCCACCGTTGAAACTGTTTCTCCATCCAGCACCGAACTATCTCTCAACAACTGAAAATGGTGCTGCT
AAGGCCGCTGTGCGTATGGGTGCCGTGCTCTAGCTGCTGCTGCTATGTTGTTATAA
30

SEQ ID NO: 23 is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating
from *Hirudo Nipponia* with a N-terminal secretion signal
ATGCAATTCCTACTGTCGCTTCCGTTGCTTTTCGTCGCTTTGGCTAACTTTGTTGCCGCTATGAAGGAGATAGCA
GTTACCATCGATGATAAGAATGTTATAGCGTCTGTCTAGTGAAAGCTTCCATGGTGTGGCTTTTCGACGCAAGCCTA
35 TTCTCACCTAAAGGGCTATGGTCGTTTGTGACATTACTTCACCCAAGTTATTCAAGCTTCTGGAGGGACTCTCC
CCTGGTTATTTCCGTGTCGGCGGCACATTTGCGAACTGGTTATTCTTCGACCTCGATGAAAACAACAAGTGAAG
GATTACTGGGCTTTTAAGGACAAGACTCCAGAGACCGCCACGATTACCCGACGGTGGCTCTTCAGGAAGCAAAAC
AACCTCAAGAAGGAAACCTTTGATGATTTGGTAAAGCTTACAAAGGGGTCCAAGATGCGGCTGCTATTTGATCTA

AACGCAGAGGTCCGTACAGGATATGAGATAGGCAAGAAGATGACATCAACTTGGGACTCAAGCGAGGCAGAAAAG
TTGTTCAAATATTGCGTTAGCAAGGGATATGGAGATAATATCGATTGGGAGTTAGGCAACGAACCTGATCACACG
TCAGCACACAATTTGACAGAGAAGCAAGTAGGTGAAGATTTTAAGGCCCTACACAAGGTGTTGGAAAAGTATCCA
ACACTTAATAAAGGTAGCTTGGTTGGTCCAGACGTTGGGTGGATGGGAGTGTCGTACGTCAAGGGTCTGGCTGAC
5 GGGGCTGGAGATCATGTGACCGCTTTTACTCTACATCAGTATTATTTTCGATGGAAATACGAGTGATGTTAGTACC
TACTTGGATGCGACGTACTTTAAGAAGTTGCAACAGTTATTTCGATAAGGTAAAAGACGTACTCAAGAATTCTCCA
CATAAGGACAAACCTTTGTGGCTAGGGGAAACCTCTTCCGGCTACAACAGTGGGACTAAAGATGTATCCGATAGA
TACGTGTCGGGGTTCTTGACGCTGGACAAGTTGGGGCTTTTCGGCGGCAAATAACGTCAAGGTGGTCATCAGACAA
ACGATTTATAATGGTTACTATGGTTTGCTTGACAAGAATACTTTGGAGCCCAATCCGGACTACTGGCTGATGCAC
10 GTCCACAATTCCTTAGTTGGTAATACCGTTTTCAAGGTCGATGTCAGCGATCCCAAAACAAAGCCCGTGTTCAC
GCTCAGTGACGAAGACCAATAGTAAACATACCCAGTCACGTTATTATAAGGGGTCCCTAACCATTTTCGCGTTG
AATGTAGGTGATGAAGATGTTACACTCAAGATTGATCAATACAGTGGTAAGAAAATATACTCATATATTCTGACG
CCTGAAGGCGGCCAGTTAACCTCTCAAAAGGTACTGCTTAACGGTAAAGAATTGAAGTTAGTGTGATCAACTT
CCAGAACTTAACGCGGACGAAAGTAAACATCCTTCACATTGTCTCCAAAACCTTTTGATTCTTTGTGGTCTCG
15 GATGCCAACGTTGAAGCTTGCAAGAAATAA

SEQ ID NO: 24 is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Hirudo nipponia* with a N-terminal secretion signal and a C-terminal anchoring signal

ATGCAATTCTCTACTGTCGCTTCCGTTGCTTTTCGTCGCTTTGGCTAACTTTGTTGCCGCTATGAAGGAGATAGCA
20 GTTACCATCGATGATAAGAATGTTATAGCGTCTGTGTCAGTGAAAGCTTCCATGGTGTGGCTTTTCGACGCAAGCCTA
TTCTCACCTAAAGGGCTATGGTCGTTTGTGACATTACTTCACCCAAGTTATTCAAGCTTCTGGAGGGACTCTCC
CCTGGTTATTTCCGTGTGCGCGGCACATTTGCGAACTGGTTATTCTTCGACCTCGATGAAAACAACAAGTGAAG
GATTACTGGGCTTTTAAGGACAAGACTCCAGAGACCGCCACGATTACCCGACGGTGGCTCTTCAGGAAGCAAAAC
AACCTCAAGAAGGAAACCTTTGATGATTTGGTAAAGCTTACAAAGGGGTCCAAGATGCGGCTGCTATTTGATCTA
25 AACGCAGAGGTCCGTACAGGATATGAGATAGGCAAGAAGATGACATCAACTTGGGACTCAAGCGAGGCAGAAAAG
TTGTTCAAATATTGCGTTAGCAAGGGATATGGAGATAATATCGATTGGGAGTTAGGCAACGAACCTGATCACACG
TCAGCACACAATTTGACAGAGAAGCAAGTAGGTGAAGATTTTAAGGCCCTACACAAGGTGTTGGAAAAGTATCCA
ACACTTAATAAAGGTAGCTTGGTTGGTCCAGACGTTGGGTGGATGGGAGTGTCGTACGTCAAGGGTCTGGCTGAC
GGGGCTGGAGATCATGTGACCGCTTTTACTCTACATCAGTATTATTTTCGATGGAAATACGAGTGATGTTAGTACC
30 TACTTGGATGCGACGTACTTTAAGAAGTTGCAACAGTTATTTCGATAAGGTAAAAGACGTACTCAAGAATTCTCCA
CATAAGGACAAACCTTTGTGGCTAGGGGAAACCTCTTCCGGCTACAACAGTGGGACTAAAGATGTATCCGATAGA
TACGTGTCGGGGTTCTTGACGCTGGACAAGTTGGGGCTTTTCGGCGGCAAATAACGTCAAGGTGGTCATCAGACAA
ACGATTTATAATGGTTACTATGGTTTGCTTGACAAGAATACTTTGGAGCCCAATCCGGACTACTGGCTGATGCAC
GTCCACAATTCCTTAGTTGGTAATACCGTTTTCAAGGTCGATGTCAGCGATCCCAAAACAAAGCCCGTGTTCAC
35 GCTCAGTGACGAAGACCAATAGTAAACATACCCAGTCACGTTATTATAAGGGGTCCCTAACCATTTTCGCGTTG
AATGTAGGTGATGAAGATGTTACACTCAAGATTGATCAATACAGTGGTAAGAAAATATACTCATATATTCTGACG
CCTGAAGGCGGCCAGTTAACCTCTCAAAAGGTACTGCTTAACGGTAAAGAATTGAAGTTAGTGTGATCAACTT
CCAGAACTTAACGCGGACGAAAGTAAACATCCTTCACATTGTCTCCAAAACCTTTTGATTCTTTGTGGTCTCG
GATGCCAACGTTGAAGCTTGCAAGAAAGGATCCGCCATTTCTCAAATCACTGACGGTCAATCCAAGCTACTACC

ACTGCTACCACCGAAGCTACCACCACTGCTGCCCCATCTTCCACCGTTGAAACTGTTTCTCCATCCAGCACCGAA
ACTATCTCTCAACAACTGAAAATGGTGCTGCTAAGGCCGCTGTCGGTATGGGTGCCGGTGTCTAGCTGCTGCT
GCTATGTTGTTATAA

- 5 **SEQ ID NO: 25** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Loxosceles intermedia* with a N-terminal secretion signal

ATGCAGTTTTCAACCGTTGCGAGCGTCGCTTTTGTGGCTCTTGCTAATTTTCGTTGCCGCCCTTCGATGTCTTCTGG
AATGTTCCGTCGCGAGCAATGCAAGAAATATGGTATGAAATTCGTTCCGCTCTTAGAGCAATACTCTATCCTAGTC
AACAAAGAAGACAATTTCAAGGGCGACAAAATAACGATCTTTTATGAGTCACAGCTCGGGCTTTACCCACATATT
10 GGTGCAAACGACGAGTCGTTTAATGGCGGGATACCACAATTAGGTGACCTGAAAGCACACTTAGAAAAGTCAGCG
GTTGATATACGACGTGATATTTTGGATAAGTCGGCGACTGGTCTAAGAATTATAGACTGGGAAGCATGGAGACCA
ATATGGGAATTCAGTGGTCTAGCCTACGAAAGTACCAAGATAAAATGAAGAAAGTCGTCCGCCAGTTTAAACCCG
ACTGCTCATGAATCCACAGTGGCCAACTAGCACATAATGAGTGGGAAAATAGTAGTAAGTCTTGATGCTTTCT
ACATTGCAGTTGGGTAAGCAACTTCGTCCAACTCTGTATGGTGCTATTATTTATTTCCCGACTGTTATAACTAT
15 GATGGCAACTCAGTCCAAGAATTTCAAGTGTCTGAAGCTATCCGTAAGGGGAACGATAGGTTGAAATGGTTGTGG
GAAGAATCGACAGCTGTATGCCCATCTATCTACATAAAAGAAGGCCAACTGACCAATTATACCTTGCAAAAGAGA
ATCTGGTTTACCAATGGGAGATTACAGGAAGCCTTGAGAGTAGCTCAACCTAAAGCGCGTATTATCCATACATA
AATTACTCCATCAAACCCGGAATGATGGTGCCTGAAGTTGAGTTTTGGCGGTTAATCGCTCAGATAGCCTCGCTG
GGTATGGATGGAGCAGTGATTTGGGGATCAAGTGCAGTGTAGGCAGTAAGAATCATTGTGCGCAATTAATGAAG
20 TACATTGCAGACGTATTGGGTCCTGCAACTTTGCGCATAAAAGAAAATGTAGCACGGTGCAGTAAACAGGCGTGC
TCTGGTAGGGGTAGATGTACCTGGCCTAAAGATACCTCTGTTATTGCTTGGAAGTTCCTCGTTGAAAAGGAAGAC
TATGACTTTTATCTGGGTGATATAGAGTGTAATGTGTTGAAGGCTACGAAGGTAGGTACTGTGAACAAAAGACT
AAGTAA

- 25 **SEQ ID NO: 26** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Loxosceles intermedia* with a N-terminal secretion signal and a C-terminal anchoring signal

ATGCAGTTTTCAACCGTTGCGAGCGTCGCTTTTGTGGCTCTTGCTAATTTTCGTTGCCGCCCTTCGATGTCTTCTGG
AATGTTCCGTCGCGAGCAATGCAAGAAATATGGTATGAAATTCGTTCCGCTCTTAGAGCAATACTCTATCCTAGTC
30 AACAAAGAAGACAATTTCAAGGGCGACAAAATAACGATCTTTTATGAGTCACAGCTCGGGCTTTACCCACATATT
GGTGCAAACGACGAGTCGTTTAATGGCGGGATACCACAATTAGGTGACCTGAAAGCACACTTAGAAAAGTCAGCG
GTTGATATACGACGTGATATTTTGGATAAGTCGGCGACTGGTCTAAGAATTATAGACTGGGAAGCATGGAGACCA
ATATGGGAATTCAGTGGTCTAGCCTACGAAAGTACCAAGATAAAATGAAGAAAGTCGTCCGCCAGTTTAAACCCG
ACTGCTCATGAATCCACAGTGGCCAACTAGCACATAATGAGTGGGAAAATAGTAGTAAGTCTTGATGCTTTCT
35 ACATTGCAGTTGGGTAAGCAACTTCGTCCAACTCTGTATGGTGCTATTATTTATTTCCCGACTGTTATAACTAT
GATGGCAACTCAGTCCAAGAATTTCAAGTGTCTGAAGCTATCCGTAAGGGGAACGATAGGTTGAAATGGTTGTGG
GAAGAATCGACAGCTGTATGCCCATCTATCTACATAAAAGAAGGCCAACTGACCAATTATACCTTGCAAAAGAGA
ATCTGGTTTACCAATGGGAGATTACAGGAAGCCTTGAGAGTAGCTCAACCTAAAGCGCGTATTATCCATACATA

AATTACTCCATCAAACCCGGAATGATGGTGCCTGAAGTTGAGTTTGGCGGTTAATCGCTCAGATAGCCTCGCTG
GGTATGGATGGAGCAGTGATTGGGGATCAAGTGCAGTGTAGGCAGTAAGAATCATTGTGCCAATTAATGAAG
TACATTGCAGACGTATTGGGTCTGCAACTTTGCGCATAAAAGAAAATGTAGCACGGTGCAGTAAACAGGCGTGC
TCTGGTAGGGTAGATGTACCTGGCCTAAAGATACTCTGTTATTGCTTGGAAGTTCCTCGTTGAAAAGGAAGAC
5 TATGACTTTTATCTGGGTGATATAGAGTGTAATGTGTTGAAGGTACGAAGGTAGGTACTGTGAACAAAAGACT
AAGGGATCCGCCATTTCTCAAATCACTGACGGTCAAATCCAAGCTACTACCCTGCTACCACCGAAGCTACCACC
ACTGCTGCCCATCTTCCACCGTTGAACTGTTTCTCCATCCAGCACCAGAACTATCTCTCAACAACTGAAAAAT
GGTGCTGCTAAGGCCGCTGTCGGTATGGGTGCCGTGCTCTAGCTGCTGCTGCTATGTTGTTATAA

- 10 **SEQ ID NO: 27** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Tityus serrulatus* with a N-terminal secretion signal

ATGCAATTCTCTACTGTCGCTTCCGTTGCTTTTCGTCGCTTTGGCTAACTTTGTTGCCGCTGCTGATTTTAAAGTT
TACTGGGAAGTGCCTTCCTTCTTTGTTCTAAACGTTTTAAATTAATGTAACGGAAGTTTTAACAAGTCACGAG
ATTCTTGTCATCAGGGTGAGAGTTTCAACGGTGACAAGATAGTAATCTTTTACGAAAACCAATTGGGGAAGTAC
15 CCGCATATTGACTCAAACAATGTGGAGATCAATGGAGGAATACTTCAAGTAGCCGATTTGGCGAAGCATTTGAAA
GTAGCCAAGGATAATATCACTAAATTCGTCCCGAATCCTAATTTCAACGGTGTGCGAGTGATCGACTGGGAAGCT
TGGCGGCCATCATGGGAATTTAACTGGGGTAAGTTAAAAGTATATAAAGAAAAGAGCATTGACTTGGTCAAGTCG
AAACATCCGGAGTGGCCCTCCGACAGGGTTGAAAAGGTTGCTAAAGAGGAGTGGGAGGAGAGTGCCAAAGAATGG
ATGGTGAAGACCCTGAAGTTAGCACAGGAAATGCGACCGAACGCAGTTTGGTGCTATTATCTATTCCCTGACTGC
20 TACAATTATTTGCGTAAGGATCAACCTCTCAATTCAGCTGCTCGTCTCGAATTCAGAAGGAAAATTCTCGTCTT
TCTTGGCTCTGGAATCAATCAACAGCCATTTGCCTAAGCATTTATATCCAGGAATCCCATGTTACCAAATATAAT
ATGTCCCAGCGGACATGGTGATCGATGCGAGATTAAGAGAAGCAATTCGAGTCAGCGAACACAGACCAACATA
CCCATCTACCCTTACATTAATTATATTCTACCTGGAACATACTAACTGTACCAGCAATGGACTTTAAAAGGACA
CTGGGTCAAATAGCTAGCCTCGGCCTAGATGGTGCTTTGTTATGGGGATCTAGCTATCATGTTTAAACAGAATCT
25 CAATGCAAAATCACTTCTGATTATGTGAAATCAGTGATTGCTCCTACCGTGGCTACTGTCGTTCTCAATACAAAC
AGATGCTCACAGATAATTTGTAAGGGTCGCGGCAACTGTGTTTGGCCTGAAGAACCATTTAGTTCTTGGAAATAC
TTAGTTGACCCCAAAATGCCAGTGTTCAAGCCAACCAACATCCACTGTAAATGTAAAGGTTACCTAGGTAGATAC
TGTGAGATCCCAAAGTAA

- 30 **SEQ ID NO: 28** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Tityus serrulatus* with a N-terminal secretion signal and a C-terminal anchoring signal

ATGCAATTCTCTACTGTCGCTTCCGTTGCTTTTCGTCGCTTTGGCTAACTTTGTTGCCGCTGCTGATTTTAAAGTT
TACTGGGAAGTGCCTTCCTTCTTTGTTCTAAACGTTTTAAATTAATGTAACGGAAGTTTTAACAAGTCACGAG
ATTCTTGTCATCAGGGTGAGAGTTTCAACGGTGACAAGATAGTAATCTTTTACGAAAACCAATTGGGGAAGTAC
35 CCGCATATTGACTCAAACAATGTGGAGATCAATGGAGGAATACTTCAAGTAGCCGATTTGGCGAAGCATTTGAAA
GTAGCCAAGGATAATATCACTAAATTCGTCCCGAATCCTAATTTCAACGGTGTGCGAGTGATCGACTGGGAAGCT
TGGCGGCCATCATGGGAATTTAACTGGGGTAAGTTAAAAGTATATAAAGAAAAGAGCATTGACTTGGTCAAGTCG
AAACATCCGGAGTGGCCCTCCGACAGGGTTGAAAAGGTTGCTAAAGAGGAGTGGGAGGAGAGTGCCAAAGAATGG

ATGGTGAAGACCCTGAAGTTAGCACAGGAAATGCGACCGAACGCAGTTTGGTGCTATTATCTATTCCCTGACTGC
TACAATTATTTTCGGTAAGGATCAACCCTCTCAATTCAGCTGCTCGTCTCGAATTCAGAAGGAAAATTCTCGTCTT
TCTTGGCTCTGGAATCAATCAACAGCCATTTGCCTAAGCATTTATATCCAGGAATCCCATGTTACCAAATATAAT
ATGTCCCAGCGGACATGGTGGATCGATGCGAGATTAAGAGAAGCAATTCAGTCAGCGAACACAGACCAAACATA
5 CCCATCTACCCCTTACATTAATTATATTCTACCTGGAACATACTCAAACTGTACCAGCAATGGACTTTAAAAGGACA
CTGGGTCAAATAGCTAGCCTCGGCCTAGATGGTGCTTTGTTATGGGGATCTAGCTATCATGTTTTAACAGAACTCT
CAATGCAAAATCACTTCTGATTATGTGAAATCAGTGATTGCTCCTACCGTGGCTACTGTCTGTTCTCAATACAAAC
AGATGCTCAGATAAATTTGTAAGGGTCGCGGCAACTGTGTTTGGCCTGAAGAACCATTTAGTTCTTGGAATAAC
TTAGTTGACCCCAAAATGCCAGTGTTCAAGCCAACCAACATCCACTGTAAATGTAAAGGTTACCTAGGTAGATAC
10 TGTGAGATCCCAAAGGGATCCGCCATTTCTCAAATCACTGACGGTCAAATCCAAGCTACTACCCTGCTACCACC
GAAGCTACCACCACTGCTGCCCCATCTCCACCGTTGAACTGTTTCTCCATCCAGCACCGAACTATCTCTCAA
CAAACGTAAATGGTGCTGCTAAGGCCGTGTCGGTATGGGTGCCGGTGCTCTAGCTGCTGCTGCTATGTTGTTA
TAA

15 **SEQ ID NO: 29** is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Vespa magnifica* with a N-terminal secretion signal

ATGCAATTTTCTACAGTGGCAAGTGTTCGATTCGTTGCACTAGCCAACCTTTGTGGCGGCAGATAGCTGTGGGTCA
AACTGCGAAAAGAGTGAGAGACCGAAAAGGGTCTTCAACATTTACTGGAACGTACCTACATTCATGTGTACCAG
TACGGACTATACTTTGACGAGGTACGAATTTTAATATAAAGCACAACAGCAAAGACAATTTCAAGGGGACAAG
20 ATCGCGATCTTTTATGACCCCGGGAGTTTCCCGCTCTGCTGCCACTAACTATGGTAAGTACAAGATCAGGAAT
GGTGGTGTTCACAAAGAGGGTAACATCACCATCCATCTGCAGAGATTCATAGAGTACCTAGATAAGACCTATCCG
AACCCTAACTTTTCAGGCATCGGTGTGATCGATTTTCGAGAGGTGGAGACCAATTTTCAGACAGAATTGGGGTAAT
ATGAAGATTTACAAGAACTTCTCCATCGATCTTGTGCGTAAAGAGCATCCTTTCTGGAATAAGAAAATGATCGAG
TTGGAAGCTTCTAAAAGATTCGAGAAATACGCCCGTCTGTTTCATGGAAGAAACATTAAAGTTGGCTAAGAAAAC
25 AGAAAACAGCCGATTGGGGCTACTACGGTTACCCCTATTGCTTCAACATGTCTCCTACTAATTTTCGTTCTTGAC
TGCGATGTCACAGCTAGGGATGAGAACAACGAGATGTCTTGGTTGTTTAAACAACCAGAATGTCTATTACCAAGT
GTATACATTAGGAGAGAGCTAACTCCTGACCAGAGGATTGGGCTTGTAAGGGGAGAGTGAAGGAAGCTGTGAGA
ATTTCAAATAAACTGAAGCACTCACCTAAAGTCTTCAGCTATTGGTGGTATGTTTACCAAGACGAGACCAACACC
TTCTTAACGGAGACCGACGTCAAGAAGACGTTTCAGGAGATTGTGATCAACGGTGGAGATGGGATTATAATCTGG
30 GGTTCTGCTCTGATGTAAACAGCTTGTCCAAGTGTACGAGGTTAAGGGAGTACCTATTGACAGTCTTGGGACCA
ATTGCTGTTAACGTGACTGAAGCAGTAAACTAA

SEQ ID NO: 30 is a reencoded nucleic acid sequence of hyaluronidase (HYAL) originating from *Vespa magnifica* with a N-terminal secretion signal and a C-terminal anchoring signal

35 ATGCAATTTTCTACAGTGGCAAGTGTTCGATTCGTTGCACTAGCCAACCTTTGTGGCGGCAGATAGCTGTGGGTCA
AACTGCGAAAAGAGTGAGAGACCGAAAAGGGTCTTCAACATTTACTGGAACGTACCTACATTCATGTGTACCAG
TACGGACTATACTTTGACGAGGTACGAATTTTAATATAAAGCACAACAGCAAAGACAATTTCAAGGGGACAAG
ATCGCGATCTTTTATGACCCCGGGAGTTTCCCGCTCTGCTGCCACTAACTATGGTAAGTACAAGATCAGGAAT

GGTGGTGTTCACAAGAGGGTAACATCACCATCCATCTGCAGAGATTCATAGAGTACCTAGATAAGACCTATCCG
 AACCGTAACTTTTAGGCATCGGTGTGATCGATTTTCGAGAGGTGGAGACCAATTTTCAGACAGAATTGGGGTAAT
 ATGAAGATTTACAAGAACTTCTCCATCGATCTTGTGCGTAAAGAGCATCCTTTCTGGAATAAGAAAATGATCGAG
 TTGGAAGCTTCTAAAAGATTTCGAGAAAATACGCCCCGTCTGTTTCATGGAAGAAACATTAAAGTTGGCTAAGAAAAT
 5 AGAAAACAGGCCGATTGGGGCTACTACGGTTACCCCTATTGCTTCAACATGTCTCCTACTAATTTCTGTTCTGAC
 TGCATGTACAGCTAGGGATGAGAACACGAGATGTCTTGGTTGTTTAAACAACCAGAATGTCTTATTACCAAGT
 GTATACATTAGGAGAGAGCTAACTCCTGACCAGAGGATTGGGCTTGTACAGGGGAGAGTGAAGGAAGCTGTGAGA
 ATTTCAAATAAACTGAAGCACTCACCTAAAGTCTTCAGCTATTGGTGGTATGTTTACCAAGACGAGACCAACACC
 TTCTTAACGGAGACCGACGTCAAGAAGACGTTTCAGGAGATTGTGATCAACGGTGGAGATGGGATTATAATCTGG
 10 GGTTCTGCTCTGATGTAAACAGCTTGTCCAAGTGTACGAGGTTAAGGGAGTACCTATTGACAGTCTTGGGACCA
 ATTGCTGTTAACGTGACTGAAGCAGTAAACGGATCCGCCATTTCTCAAATCACTGACGGTCAAATCCAAGCTACT
 ACCACTGCTACCAACGAAGCTACCACCACTGCTGCCCCATCTTCCACCGTTGAACTGTTTCTCCATCCAGCACC
 GAAACTATCTCTCAACAACTGAAAATGGTGCTGCTAAGGCCGCTGTCGGTATGGGTGCCGGTGTCTAGCTGCT
 GCTGCTATGTTGTTATAA

15

SEQ ID NO: 31 is an amino acid sequence of hyaluronidase (HYAL) originating from *Bothrops atrox* with a N-terminal secretion signal

MQFSTVASVAFVALANFVAAPMYPNEPFLVFWNAPTQCRLRYKVDLDLKTFFHIVTNANDSLSGSAVTIFYPHTL
 GVPYHIDDRGHFFNGIIPQNESLVKHLNKSXSDINRMIPLRTFHGLGVIDWENWRPQWDRNWGSKNVYRNRSIQF
 20 ARDLHPELSEDKIKRLAKQEFKAAKSFMRDTLLLAEMRPDGYWGYLYPDCHNYNYKTKPDQYTGECPDIEMS
 RNNQLLWLWRDSTALFPNIYLETILRSSDNALKFVHRLKEAMRIASMARNDYALPFFVYARPFYAYTFEPLTQE
 DLVTTVGETADMGAAGIVFWGSMQYASTVESCGKVKDYMNGLGRYIVNVTTAAKICSRFLCKRHGRVCRKHSDS
 NAFLHLFPDSFRIMVHGNAATEKKVIVKGKLELKNLIFLRNNFMCQCYQGWKGLYCEKHSIKEIRKI

25 **SEQ ID NO: 32** is an amino acid sequence of hyaluronidase (HYAL) originating from *Bothrops atrox* with a N-terminal secretion signal and a C-terminal anchoring signal

MQFSTVASVAFVALANFVAAPMYPNEPFLVFWNAPTQCRLRYKVDLDLKTFFHIVTNANDSLSGSAVTIFYPHTL
 GVPYHIDDRGHFFNGIIPQNESLVKHLNKSXSDINRMIPLRTFHGLGVIDWENWRPQWDRNWGSKNVYRNRSIQF
 ARDLHPELSEDKIKRLAKQEFKAAKSFMRDTLLLAEMRPDGYWGYLYPDCHNYNYKTKPDQYTGECPDIEMS
 30 RNNQLLWLWRDSTALFPNIYLETILRSSDNALKFVHRLKEAMRIASMARNDYALPFFVYARPFYAYTFEPLTQE
 DLVTTVGETADMGAAGIVFWGSMQYASTVESCGKVKDYMNGLGRYIVNVTTAAKICSRFLCKRHGRVCRKHSDS
 NAFLHLFPDSFRIMVHGNAATEKKVIVKGKLELKNLIFLRNNFMCQCYQGWKGLYCEKHSIKEIRKIGSAISQITD
 GQIQATTTATTEATTTAAPSSTVETVSPSSTETISQQTENGAAKAAVGMGAGALAAAAMLL

35 **SEQ ID NO: 33** is an amino acid sequence of hyaluronidase (HYAL) originating from *Cupiennius salei* with a N-terminal secretion signal

MQFSTVASVAFVALANFVAAFKIYWNVPTFQCTHNYKIDYVKLLSTYGIQVNDGGKFQGNQVTIFYETQLGLYPR
 ILKSGKMENGIPQRGNFKEHLEKASTDLQKVIPWKEFSGLGVIDWEAWRPTWEFNWEPLRIYQTESIKRAKELH

PTANDSAVKEIAERQWEDSAKLYMLETLR LAKKLRPQAPWCYYLFPDCYNVVGKKPKDFQCSASIRKGNDKLSWL
WKDSTALCPSIYVYESQLDRYSFEQRTWRDNEKLREALRVATRTSKIYPYVNYFDKELIPEQEVWRMLAQAAAVG
GSGAVIWGSSAAVASEELCKSLKQYIIETLGPAAEKVAWRSD LCSKEICNNQGRCTFPDDDYANAWKLFTDDTVK
FYAGNITCRCSENYSGRFCEKKN

5

SEQ ID NO: 34 is an amino acid sequence of hyaluronidase (HYAL) originating from *Cupiennius salei* with a N-terminal secretion signal and a C-terminal anchoring signal

MQFSTVASVAFVALANFVAAFKIYWNVPTFQCTHNYKIDYVKLLSTYGIQVNDGGKFQGNQVTIFYETQLGLYPR
ILKSGKMENG GIPQRGNF EKHLEKASTDLQKVIPWKEFSGLGVIDWEAWRPTWEFNWEPLRIYQTESIKRAKELH
PTANDSAVKEIAERQWEDSAKLYMLETLR LAKKLRPQAPWCYYLFPDCYNVVGKKPKDFQCSASIRKGNDKLSWL
WKDSTALCPSIYVYESQLDRYSFEQRTWRDNEKLREALRVATRTSKIYPYVNYFDKELIPEQEVWRMLAQAAAVG
GSGAVIWGSSAAVASEELCKSLKQYIIETLGPAAEKVAWRSD LCSKEICNNQGRCTFPDDDYANAWKLFTDDTVK
FYAGNITCRCSENYSGRFCEKKN GSAISQITDGQIQATTTATTEATTTAAPSSTVETVSPSSTETISQQTENGAA
KAAVGMGAGALAAAAMLL

10

15

SEQ ID NO: 35 is an amino acid sequence of hyaluronidase (HYAL) originating from *Hirudo Nipponia* with a N-terminal secretion signal

MQFSTVASVAFVALANFVAA~MKEIAVTIDDKNVIASVSESFHGVAFDASLFS PKGLWSFVDITSPKLFKLEGL
SPGYFRVGGTFANWLF FDLDENNKWKDYWAFKDKTPETATITRRWLF RKQNNLKKETFDDLVLKLTGSKMRLLFD
LNAEVRTGYEIGKMTSTWDSSEAELFKYCVSKGYGDNIDWELGNEPDHTSAHNLTEKQVGEDFKALHKVLEKY
PTLNKGS L VGPDV GWMGVS YVKGLADGAGDHVTAFTLHQYYFDGNTSDVSTYLDATYFKKLQQLFDKVKDVLKNS
PHKDKPLWLGETSSGYNSG TKDVS DRYVSGFLTLDKLGLSAANNVKVIRQTIYNGYYGLLDKNTLEPNPDYWLM
HVHNSLVGNTVFKVDVSDPTNKARVYAQCTKTNSKHTQSRYYKGS LTIFALNVGDEDVTLKIDQYSGKKIYSYIL
TPEGGLTSQKVLLNGKELKLVSDQLPELNADESKTSFTLSPKTFGFFVVS DANVEACKK GSAISQITDGQIQAT
TTATTEATTTAAPSSTVETVSPSSTETISQQTENGAAKAAVGMGAGALAAAAMLL

25

SEQ ID NO: 36 is an amino acid sequence of hyaluronidase (HYAL) originating from *Hirudo Nipponia* with a N-terminal secretion signal and a C-terminal anchoring signal

MQFSTVASVAFVALANFVAA~MKEIAVTIDDKNVIASVSESFHGVAFDASLFS PKGLWSFVDITSPKLFKLEGL
SPGYFRVGGTFANWLF FDLDENNKWKDYWAFKDKTPETATITRRWLF RKQNNLKKETFDDLVLKLTGSKMRLLFD
LNAEVRTGYEIGKMTSTWDSSEAELFKYCVSKGYGDNIDWELGNEPDHTSAHNLTEKQVGEDFKALHKVLEKY
PTLNKGS L VGPDV GWMGVS YVKGLADGAGDHVTAFTLHQYYFDGNTSDVSTYLDATYFKKLQQLFDKVKDVLKNS
PHKDKPLWLGETSSGYNSG TKDVS DRYVSGFLTLDKLGLSAANNVKVIRQTIYNGYYGLLDKNTLEPNPDYWLM
HVHNSLVGNTVFKVDVSDPTNKARVYAQCTKTNSKHTQSRYYKGS LTIFALNVGDEDVTLKIDQYSGKKIYSYIL
TPEGGLTSQKVLLNGKELKLVSDQLPELNADESKTSFTLSPKTFGFFVVS DANVEACKK

35

SEQ ID NO: 37 is an amino acid sequence of hyaluronidase (HYAL) originating from *Loxosceles intermedia* with a N-terminal secretion signal

MQFSTVASVAFVALANFVAAFDVFWNVPSQQCKKYGKMFVPLLEQYSILVNKEDNFKGDKITIFYESQLGLYPHI
 GANDESFNNGGIPQLGDLKAHLEKSAVDIRRDILDKSATGLRIIDWEAWRP IWEFNWSSLRKYQDKMKKVVRQFNP
 5 TAHESTVAKLAHNEWENSSKSWMLSTLQLGKQLRPNSVWCYYLFPDCYNYDGNQSVQEFQCSEAIRKGNDRKWLW
 EESTAVCPSTIYIKEGQLTNYTLQKRIWFTNGRLQEALRVAQPKARIYPYINYSIKPGMMVPEVEFWRLIAQIASL
 GMDGAVIWGSSASVSGSKNHCAQLMKYIADVLGPATLRIKENVARCSKQACSGRGRCTWPKDTSVIAWKFLVEKED
 YDFYLGDIIECKCVEGYEGRYCEQKTK

10 **SEQ ID NO: 38** is an amino acid sequence of hyaluronidase (HYAL) originating from *Loxosceles intermedia* with a N-terminal secretion signal and a C-terminal anchoring signal

MQFSTVASVAFVALANFVAAFDVFWNVPSQQCKKYGKMFVPLLEQYSILVNKEDNFKGDKITIFYESQLGLYPHI
 GANDESFNNGGIPQLGDLKAHLEKSAVDIRRDILDKSATGLRIIDWEAWRP IWEFNWSSLRKYQDKMKKVVRQFNP
 TAHESTVAKLAHNEWENSSKSWMLSTLQLGKQLRPNSVWCYYLFPDCYNYDGNQSVQEFQCSEAIRKGNDRKWLW
 15 EESTAVCPSTIYIKEGQLTNYTLQKRIWFTNGRLQEALRVAQPKARIYPYINYSIKPGMMVPEVEFWRLIAQIASL
 GMDGAVIWGSSASVSGSKNHCAQLMKYIADVLGPATLRIKENVARCSKQACSGRGRCTWPKDTSVIAWKFLVEKED
 YDFYLGDIIECKCVEGYEGRYCEQKTKGSAISQITDGGIQATTATTEATTTAAPSSTVETVSPSSTETISQQTEN
 GAAKAAVGMGAGALAAAAMLL

20

SEQ ID NO: 39 is an amino acid sequence of hyaluronidase (HYAL) originating from *Tityus serrulatus* with a N-terminal secretion signal

MQFSTVASVAFVALANFVAAADFKVYWEVPSFLCSKRFKINVTEVLTSHEILVNQGESFNGDKIVIFYENQLGKY
 25 PHIDSNNVEINGGILQVADLAKHLKVAKDNIKFVPNPNFNGVGVIDWEAWRPSWEFNWGKLVYKEKSIDLVKS
 KHPEWPSDRVEKVAKEEWEESAKEWMVKTLKLAQEMRPNNAVWCYYLFPDCYNYFGKDQPSQFSCSSRIQKENSRL
 SWLWNQSTAICLSIIYIQESHVTKYNMSQRTWWIDARLREAIRVSEHRPNIPYIPYINYLPGTNQTPAMDFKRT
 LGQIASLGLDGALLWGSSYHVLTESQCKITS DYVKS VIAPTVATVVLNTNRCSQIICKGRGNCVWPEEPFSSWKY
 LVDPKMPVFKPTNIHCKCKGYLGRYCEIPK

30

SEQ ID NO: 40 is an amino acid sequence of hyaluronidase (HYAL) originating from *Tityus serrulatus* with a N-terminal secretion signal and a C-terminal anchoring signal

MQFSTVASVAFVALANFVAAADFKVYWEVPSFLCSKRFKINVTEVLTSHEILVNQGESFNGDKIVIFYENQLGKY
 PHIDSNNVEINGGILQVADLAKHLKVAKDNIKFVPNPNFNGVGVIDWEAWRPSWEFNWGKLVYKEKSIDLVKS
 35 KHPEWPSDRVEKVAKEEWEESAKEWMVKTLKLAQEMRPNNAVWCYYLFPDCYNYFGKDQPSQFSCSSRIQKENSRL
 SWLWNQSTAICLSIIYIQESHVTKYNMSQRTWWIDARLREAIRVSEHRPNIPYIPYINYLPGTNQTPAMDFKRT
 LGQIASLGLDGALLWGSSYHVLTESQCKITS DYVKS VIAPTVATVVLNTNRCSQIICKGRGNCVWPEEPFSSWKY

LVDPKMPVFKPTNIHCKCKGYLGRYCEIPKGS AISQITDGQIQATTTATTEATTTAAPSSTVETVSPSSTETISQ
QTENGA AKA AVGMGAGALAAA AMLL

SEQ ID NO: 41 is an amino acid sequence of hyaluronidase (HYAL) originating from *Vespa*

5 *magnifica* with a N-terminal secretion signal

MQFSTVASVAFVALANFVAADSCGSNCEKSERPKRVFN IYWNVPTFMCHQYGLYFDEVTFNFIKHNSKDNFQGDK
IAIFYDPGEFPALLPLNYGKYKIRNGGVPQEGNIT IHLQRFIEYLDKTYPNRNFSGIGVIDFERWRP IFRQNWGN
MKIYKNFSIDLVRKEHPFWNKKMIELEASKRFEKYARLFMEETLKLAKKTRKQADWGYGYGYPCFNMSPTNFVPD
CDVTARDENNEMSWLFNNQNVLLPSVYIRRELTDPQRI GLVQGRVKEAVRISNKLKHSPKVS YWWYVYQDETNT
10 FLTETDVKKTFQEIVINGGDGII IWGSSSDVNSLSKCTRLREYLLTVLGPIAVNVTEAVN

SEQ ID NO: 42 is an amino acid sequence of hyaluronidase (HYAL) originating from *Vespa*
magnifica with a N-terminal secretion signal and a C-terminal anchoring signal

MQFSTVASVAFVALANFVAADSCGSNCEKSERPKRVFN IYWNVPTFMCHQYGLYFDEVTFNFIKHNSKDNFQGDK
15 IAIFYDPGEFPALLPLNYGKYKIRNGGVPQEGNIT IHLQRFIEYLDKTYPNRNFSGIGVIDFERWRP IFRQNWGN
MKIYKNFSIDLVRKEHPFWNKKMIELEASKRFEKYARLFMEETLKLAKKTRKQADWGYGYGYPCFNMSPTNFVPD
CDVTARDENNEMSWLFNNQNVLLPSVYIRRELTDPQRI GLVQGRVKEAVRISNKLKHSPKVS YWWYVYQDETNT
FLTETDVKKTFQEIVINGGDGII IWGSSSDVNSLSKCTRLREYLLTVLGPIAVNVTEAVNAISQITDGQIQATTT
ATTEATTTAAPSSTVETVSPSSTETISQQTENGA AKA AVGMGAGALAAA AMLL

20

SEQ ID NO: 43 is a nucleic acid sequence of the secretion sequence added in 5'

ATGCAATTTAGCACAGTCGCATCAGTAGCCTTCGTTGCCTTGCCAACTTCGTGGCAGCA

SEQ ID NO: 44 is an amino acid sequence of the secretion sequence added in N-ter

25 MQFSTVASVAFVALANFVAA

SEQ ID NO: 45 is a nucleic acid sequence of the anchoring sequence added in 3'

GGATCCGCCATTTCTCAAATCACTGACGGTCAAATCCAAGCTACTACCACTGCTACCACCGAAGCTACCACCACT
GCTGCCCATCTTCCACCGTTGAACTGTTTCTCCATCCAGCACCGAACTATCTCTCAACAACTGAAAATGGT
30 GCTGCTAAGCCGCTGTCGGTATGGGTGCCGGTGCTCTAGCTGCTGCTGCTATGTTGTTATAA

SEQ ID NO: 46 is an amino acid sequence of the anchoring sequence added in C-ter

AISQITDGQIQATTTATTEATTTAAPSSTVETVSPSSTETISQQTENGA AKA AVGMGAGALAAA AMLL

35 **SEQ ID NO: 47** is a nucleic acid sequence of glutamine-fructose-6-phosphate
amidotransferase (GFA1) originating from *Saccharomyces cerevisiae*

ATGTGTGGTATCTTTGGTTACTGCAATTATCTAGTGGAAAGATCCAGAGGAGAAATTATCGACACCTTAGTGGAT
GGTTTACAAAGATTAGAATATAGAGGCTATGATTCCACCGGTATTGCTATCGATGGTGACGAAGCTGATTCTACT
TTCATCTATAAGCAAATCGGTAAAGTGAGTGCTTTGAAAGAGGAGATTACTAAGCAAAATCCGAACAGAGACGTT
ACTTTTGTCTCTCATTGTGGTATTGCGCATACTAGATGGGCTACTCACGGTCGACCAGAACAAGTTAACTGTCAC
5 CCTCAAAGATCTGACCCAGAAGACCAATTTGTGGTCGTTTCATAATGGTATCATCACAATTTTAGAGAACTGAAG
ACTCTTTTAATTAACAAAGGTTATAAATTCGAAAGTGATACCGATACCGAGTGTATTGCTAACTATATTTGCAT
TTATACAATACAAATTTACAAAATGGGCATGACTTAGATTTCCACGAATTAACCAAGCTAGTTCCTTTTAGAACTA
GAAGGTTTCATACGGGTTATTATGTAAATCTTGTCACATATCCTAATGAGGTTATCGCCACTAGAAAAGGTTCCCTT
TTACTGATTGGTGTCAAATCTGAAAAAACTAAAAGTCGACTTCGTGGATGTGGAATTTCCCGAAGAAAACGCT
10 GGTCACCGGAAATTCATTGAAATCTAACAACAAATCATTGTGGCTTGGGCCCAAAGAAAGCTCGTGAATTTGAA
GCTGGTTCCCAAATGCCAATTTACTACCAATTGCCGCCAATGAATTTAACTTGAGACATTCTCAATCCAGGGCT
TTCCTATCAGAAGATGGATCTCCAACACCGGTGGAATTTTTTGTTCCTTCGGATGCGGCATCTGTTGTTAAACAT
ACCAAGAAGGTGCTATTTTTAGAAGATGACGATTTGGCTCATATTTACGATGGTGAGTTACATATTCATAGATCT
AGAAGAGAAGTAGGCGCATCAATGACAAGGTCCATTCAAACCTTTAGAGATGGAGTTAGCTCAGATCATGAAGGGC
15 CCTTACGACCATTTTATGCAAAAGGAAATCTATGAGCAACCAGAATCTACTTTCAATACTATGAGAGGTAGAATC
GACTATGAAAAATAATAAGTGATATTGGGTGGTTTTAAAGGCATGGTTACCAGTTGTCAGAAGAGCACGGAGACTG
ATCATGATCGCATGCGGTACTTCTTATCATTATGTTTGGCTACTCGTGCTATCTTCGAAGAATTATCAGATATC
CCAGTTAGTGTGGAATTAGCGTCTGACTTTCTGGACAGAAAATGCCCTGTCTTCAGAGACGATGTATGCGTGTTT
GTTTCACAAAGTGGTGAAGTGCAGTACCATGCTGGCTCTAAATTATTGTTTAGAAAGAGGAGCCTTAACTGTC
20 GGAATTGTTAACAGTGTGGTTCCTTCTATCTCTCGTGTCACCCACTGTGGTGTTCATATTAACGCTGGTCTGAA
ATTGGTGTGCTCTACAAAAGCTTATACTTCCAGTATATTGCCCTTAGTGATGTTTGCTCTATCGCTGTCAGAT
GACCGTGTATCGAAAATAGACAGAAGAATTGAAATCATTCAAGGCTTGAAGTTAATCCCGGCCAAATTAAGCAG
GTATTAAAGCTGGAACCAAGAATAAAAAAGCTCTGTGCGACTGAATTAAAGGATCAAAAATCTCTATTGTTATTG
GGTAGAGGTTACCAATTTGCTGCTGCTCTGGAAGGTGCTTTGAAGATCAAAGAAATTTCTTATATGCATTCTGAA
25 GGTGTTTTGGCAGGTGAGTTGAAGCACGGTGTCTTGGCCTTGGTGGACGAAAACCTGCCAATCATTGCTTTTGGT
ACCAGAGACTCTCTATTCCCTAAAGTAGTTTCTCTATTGAGCAAGTTACTGTAAGAAAGGGCCATCCAATTATT
ATTTGTAACGAAAATGATGAAGTGTGGGCGCAAAAATCTAAATCAATCGACCTGCAAACCTTAGAAGTTCCACAA
ACTGTTGATTGTTTACAAGGTCTAATTAATATTATCCATTACAACTAATGTATATTGGTTGGCTGTTAATAAA
GGGATTGATGTTGATTTTCCAAGAACTTGGCTAAATCTGTTACCGTCGAATAA
30

SEQ ID NO: 48 is a reencoded nucleic acid sequence of glutamine-fructose-6-phosphate
amidotransferase (GFA1) originating from *Chlorella virus 1* (PBCV-1)

ATGTGCGGGATCTTCGGTGTGTGTCGAACAATAATAGCATAGAAGTTTCCATCAAGGGTATACAGAAGCTAGAG
TACCGCGGGTACGATTCTGTGGAATAGCCTATACAGACGGAGGCCATTGAACGGATCAGGTCAATAGATGGG
35 ATCGACGACTTAAGGAAGAAAACAATAACAGAGTCTTCCCGGTAGCTATCGCTCATTCAAGGTGGAGTACCACT
GGGATTCCAAGTGTGTGAACGCCCACCGCACATCTCTCGGGGCACGTCTGGATGCGAGTCGCGCATTGCAGTC
GTACACAATGGCATTATTGAAAATTACCAGCAGATCCGCAAGTACTTGATAAATCTTGGGTATACTTTTCGATTCA
CAGACCGACACGGAAGTCATTGCTCATCTAATAGACTCGCAGTATAACGGGAACATCTGCACACCGTCCAAATG
GCAGTTAAGCACCTCAAAGGAAGCTACGCCATAGCAGTCATGTGCCATAAGGAGTCCGGGAAGATCGTGGTGGCA

AAACAGAAGAGTCCGTTGGTATTGGGTATAGGAAGCGACGGAGCTTATTATATCGCATCGGACGTATTGGCTCTT
CCCCTAACAAGTGGTCTACATAAGTGACGGATTCTCTGCTGAGTTAAGTCCTGGATCGATGACTATCTATGAT
CTGGATGGCAATAAGGTAGAATACGAGGTGGAAGACGTTGAAATGGAGCAGACGTGATGTCTTTAGATAACTTT
GATCACTACATGATCAAAGAGATCAATGAACAGCCGATATCAATTCTCAATACTATTAAGAACAAGGATTCTAT
5 GCAGAGATATTTGGTGATCTAGCACATGAAATATTTGAGAAGATCGACAATATATTGATTCTAGCCTGCGGAACT
TCGTATCATGCAGGTCTCGTCGGGAAGCAATGGATAGAGACCATCTCACGCATCCCGGTGGATGTTTCATATTGCA
AGCGAGTATGAGCCGACAATTCCACGAGCCAATACACTTGTGATAACGATTAGTCAAAGCGGCGAGACTGCGGAT
ACAATCGCAGCCCTTCAACGAGCGCAAAACGCAGGAATGATATACACTTTGTGTATCTGCAACAGCCCCAAGTCA
ACTCTAGTGCAGGAGTCGATAATGAAATACATAACCAAATGCGGTTGAGAAGTTCTGTTGCCAGTACTAAAGCA
10 TTTACGTCGCAACTTGTGGTGTGTATATGCTTGCGAACGTTCTTGCGAACAAAAGTATGATGATCTGCTAGGGGAT
CTTCCTCAAGCTATCGAGAGAGTAATCTGTCTTACTAATGACGAAATGAAGCGTTGGGCGGATGAAATTTGTACT
GCCAAAAGTGCCATCTTCTTAGGGAGAGGACTGAACGCACCTGTAGCATTTGAGGGCGCGCTAAAGTTGAAGGAA
ATCTCATATATCCATGCCGAGGGTTTCTCGGTGGAGAGTTGAAGCATGGGCCGCTGGCTTTGTTGGACGATAAG
ATCCCTGTAATCGTGACTGTTGCTGACCACGCTTATTTGGATCACATTAAGGCTAACATCGACGAGGTATTGGCA
15 CGAAACGTTACCGTATACGCGATCGTCGATCAGTATGTTAATATTGAACCACAGGAGCGACTGCATGTAGTAAAA
GTGCCGTTTGTGAGTAAAGAGTTTTCTCCCATCATCCACACAATTCCGATGCAATTGTTATCGTATTATGTCGCG
ATCAAGCTGGGCAAGAACGTTGACAAGCCACGTAACCTGGCGAAAAGTGTTACAACATTCTaa

20 **SEQ ID NO: 49** is a reencoded nucleic acid sequence of glutamine-fructose-6-phosphate
amidotransferase (GFA1) originating from *Chlorella virus 1* (PBCV-1)

ATGTGTGGCATCTTTGGAGCAGTGTCAAACAACACTCTATCGAGGTGTCAATCAAGGGTATTGAGAAGCTAGAA
TATCGTGGGTATGATTCTGCGGTATTGCGTATACAGATGGGGGTGCGATTGAGCGTATACGTTCTATTGACGGC
ATTGACGATCTGCGTAAGAAAACAATCACAGAATCATCACCAGTGGCCATTGCTCACTCGCGGTGGAGCACCCT
25 GGAATTCATCAGTGGTGAACGCACATCCTCATATTTCTCGCGGAACAGTGGGTGTGAGTCTCGTATCGCGGTA
GTCCACAACGGTATCATTGAAAACATCAGCAGATCCGAAAATATCTCATCAATCTTGTTTATACGTTTGTAGT
CAAACGGACACAGAGGTCATTGCGCATTGATTGATTCTCAGTACAATGGGAATATCTTGACACCGTCCAAATG
GCTGTCAAGCACCTGAAGGGCTCTTATGCCATTGCAGTTATGTGTATATAAGAGTCTGGTAAAAATAGTCGTGGCG
AAACAGAAGTCACCCCTCGTACTTGGAAATCGGCTCAGATGGTGCTTACTACATCGCTTCGGACGTGCTGGCGCTG
30 CCGACAAATAAAGTTGTTTATATTTGAGACGTTTTCTCCGAGAACTATCTCCAGGGAGTATGACATTTACGAT
CTTGATGGAATAAAGTAGAATATGAAGTAGAGGACGTTGAAATGGAACAACTAGTATGTCTCTCGATAACTTT
GATCATTACATGATTAAGGAAATTAATGAGCAACCAATCAGTATTCTAAACACTATAAAAAATAAAGGGTTCTAT
GCAGAAATATTCGGTGATTGGCTCATGAAATCTTCAAAAAATAGACAACATCCTGATACTGGCTTGTGGTACA
AGTTATCACGCCGGTCTTGTAGGAAAACAGTGGATAGAGACCATCTCTAGAATCCCCGTGGATGTTACATCGCG
35 AGTGAATACGAACCTACTATTCCGAGAGCGAACACATTGGTAATCACTATTTACAGTCGGGTGAAACTGCGGAC
ACGATAGCGGCTTTGCAACGGGCCCCAAACGCCGGGATGATTTATACATTGTGTATTGCAATTCACCAAAGAGC
ACTCTTGTTCGTGAGAGCATTATGAAGTACATCAGGAAATGTGGTTCTGAGGTGTCAGTGGCATCAACGAAGGCG
TTTACTTCTCAGCTCGTAGTACTGTACATGCTGGCAAACGATTGGCAAATAAAACCGATGATTTGCTGGGAGAC
CTCCACAGGCAATAGAACGGGTAATTTGTTTGACAAATGACGAAATGAAACGATGGGCCGACGAAATTTGCACT

GCGAAATCCGCGATCTTCTCGGGAAGAGGACTAAACGCACCAGTTGCCTTTGAGGGAGCGTTGAAGCTCAAAGAA
ATCTCTTACATTTCATGCAGAGGGCTTCTCGGAGGTGAGTTGAAACACGCCCCCTCGCACTCCTTGATGACAAG
ATTCTGTTATCGTAACCGTAGCAGATCATGCTTATTTGGACCATATCAAAGCAAATATCGACGAAGTGCTTGCG
AGGAACGTTACGGTATACGCCATAGTAGACCAGTATGTGAACATCGAGCCCCAGGAACGCCTTCACGTCGTCAAG
5 GTTCCGTTTGTATCCAAAGAATTTCTCCGATAATTCACACTATCCCGATGCAACTGCTTTCTGATTACGTGGCA
ATTAAGCTTGGAAGAAGCGTTGACAAACCAAGGAATCTTGCAAAATCCGTGACTACCTTTTAA

SEQ ID NO: 50 is an amino acid sequence of glutamine-fructose-6-phosphate
amidotransferase (GFA1) originating from *Saccharomyces cerevisiae*

10 MCGIFGYCNLYVERSRGEIIDTLVDGLQRLEYRGYDSTGIAIDGDEADSTFIYKQIGKVSALKEEITKQNPNRDV
TFVSHCGIAHTRWATHGRPEQVNCHPQRSDPEDQFVVVHNGIITNFRELKTLINKGYKFESDTECIAKLYLH
LYNTNLQNGHDLDFHELTKLVLLELEGSYGLLCKSCHYPNEVIATRKGSPLLIGVKSEKKLVDFVDVEFPEENA
GQPEIPLKSNKSFGLGPKKAREFEAGSQNANLLPIAANFNLRHSQSRAFLSEDGSPTPVEFFVSSDAASVVKH
TKKVLFLDDDLAHIYDGLHIIHRSRREVGASMTRSIQTLEMELAQIMKGPYDHFQKEIYEQPESTFNTMRGRI
15 DYENNKVILGGLKAWLPVRRARRLIMACGTSYHSCLATRAIFEELSDIPVSVELASDFLDRKCPVFRDDVCVF
VSQSGETADTMLALNYCLERGALTGVIVNSVGSSISRVTHCGVHINAGPEIGVASTKAYTSQYIALVMFALSLS
DRVSKIDRRIEIIQGLKLIPGQIKQVLKLEPRIKKLCATELKDQKSLLLLGGRGYQFAAALLEGALIKIKEISYMHSE
GVLGELKHGVLALVDENLPIIAFGTRDSLFPKVVSSIEQVTARKGHP I I ICNENDEVWAQSKSIDLQTLVPQ
TVDCLQGLINIPLQLMSYWLAVNKGIDVDFPRNLAKSVTVE

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SEQ ID NO: 51 is an amino acid sequence of glutamine-fructose-6-phosphate
amidotransferase (GFA1) originating from *Chlorella virus 1* (PBCV-1)

10 MCGIFGAVSNNSIEVSIKGIQKLEYRGYDSCGIAYTDGGAIERIRSIDGIDDLRKKITITESSPVAIAHSRWSTT
GIPSVVNAHPHISRGTSGCESRIAVVHNGI I ENYQQIRKYLINLGYTFDSQTDTEVIAHLIDSQYNGNIIHTVQM
25 AVKHLKGSYAIAMVCHKESGKIVVAKQKSPVLVLIGSDGAYYIASDVLALPTNKVVYISDGFSAELSPGSMITID
LDGNKVEYEVEDVEMEQTSMSLDNFDHYMIKEINEQPI S I LNT I K N K G F Y A E I F G D L A H E I F Q K I D N I L I A C G T
SYHAGLVGQWIETISRIPVDVHIASEYEPTIPRANTLVITISQSGETADTIAALQRAQNAGMIYTLICNSPKS
TLVRESIMKYITKCGSEVSVASTKAFTSQLVVLYMLANVLANKTDDLGLDLPQAIERVICLTNDEMKRWADEICT
AKSAIFLGRGLNAPVAFEGALKLKEISYIHAEGFLGGELKHGPLALLDDKIPVIVTVADHAYLDHIKANIDEVLA
30 RNVTVYAIVDQYVNIEPQERLHVVKVPFVSKEFSPI I HTIPMQLLSYYVAIKLGKNVDKPRNLAKSVTTF

SEQ ID NO: 52 is a nucleic acid sequence of UDP-N-acetylglucosamine pyrophosphorylase
(QR11) originating from *Saccharomyces cerevisiae*

10 ATGACTGACACAAAACAGCTATTTCATTGAAGCCGGACAAAGTCAACTTTTCCACAATTGGGAAAGCTTGCTCGC
35 AAAGACCAAGAAGAATTGCTTTCAAACCTGGAGCAAATATCTTCCAAGAGGTCCCCTGCAAACTACTGGAAGAC
TGTCAAAATGCTATTAAATTCTCACTAGCTAACTCTTCTAAGGATACTGGCGTCGAAATTTACCAATTGCCCCCT
ACTTCGTACGAGTCGCTTATTGGCAACAGTAAGAAAGAAAATGAATACTGGCGTTTAGGCCTTGAAGCTATTGGC
AAGGGTGAAGTCGCAGTGATTTTAATGGCTGGCGGACAAGGTACGCGTTAGGATCCTCTCAACCAAAGGGCTGT

TACGACATTGGATTGCCTTCTAAGAAATCTCTTTTTCAAATTCAAGCTGAAAAGTTGATCAGGTTGCAAGATATG
GTAAAGGACAAAAAGGTAGAAATTCCTTGGTATATTATGACATCAGGCCCCACTAGAGCGGCTACTGAGGCATAC
TTTCAAGAACACAATTATTTGGCTTGAATAAAGAACAAATTACGTTCTTCAACCAGGGAACCCTGCCTGCCTTT
GATTTAACCGGGAAGCATTTCCTAATGAAAGACCCAGTAAACCTATCTCAATCACCAGATGGAAATGGTGGACTC
5 TACCGTGCCATCAAGGAAAAACAAGTTGAACGAAGACTTTGATAGGAGAGGAATCAAGCATGTTTACATGTACTGT
GTCGATAATGTCTATCTAAAAATCGCAGACCCTGTATTTATTGGTTTTGCCATCAAGCATGGCTTCGAACCTGGCC
ACCAAAGCCGTTAGAAAGAGAGATGCGCATGAATCAGTTGGGTTAATTGCTACTAAAAACGAGAAACCATGTGTC
ATAGAATATCTGAAATTTCCAATGAATTGGCTGAAGCAAAGGATAAAGATGGCTTATTAATACTACGCGCAGGC
AACATTGTAAATCATTATTACCTAGTGGATTTACTAAAACGTGATTGGATCAGTGGTGTGAGAATATGCCATAT
10 CACATTGCGAAGAAGAAAAATTCAGCTTATGATAGTGTTACCGGCAAGTACACTAAGCCTACCGAACCACCGGT
ATAAAATTAGAGCAATTCATATTTGATGTCTTTGACACTGTACCACTGAACAAGTTTGGGTGCTTAGAAGTAGAT
AGATGCAAAGAATTTTACCTTTAAAAACGGTCTCGTTCTAAGAACGATAATCCTGAGACCAGCAGACTAGCA
TATTTGAACTAGGAACCTCGTGGTTGGAAGATGCAGGCGCTATTGTAAAAGATGGGGTACTAGTCGAAGTTTCC
AGCAAATTGAGTTATGCAGGTGAAAATCTATCCAGTTCAAAGGTAAAGTCTTTGACAGAAGTGGTATAGTATTA
15 GAAAAATAA

SEQ ID NO: 53 is an amino acid sequence of UDP-N-acetylglucosamine pyrophosphorylase (QRI1) originating from *Saccharomyces cerevisiae*

MTDTKQLFIEAGSQLFHNWESLSRKDQEELLSNLEQISSKRSPAKLLEDQCNAIKFSLANSSKDTGVEISPLPP
20 TSYESLIGNSKKENEYWRGLGLEAIGKGEVAVILMAGGQGTRLGSSQPKGCYDIGLPSKKSLEFQIQAEKLIRLQDM
VKDKKVEIPWYIMTSGPTRAAATEAYFQEHNYFGLNKEQITFFNQGLPAFDLTGKHFLMKDPVNLSSQSPDNGGL
YRAIKENKLNEDFDRRGIKHVYMYCVDNVLSKIADPVFIGFAIKHGFELATKAVRKRDAHESVGLIATKNEKPCV
IEYSEISNELAEAKDKDGLLKLKLRAGNIVNHYYLVDLLKRDLDQWCENMPYHIAKKKIPAYDSVTGKYTKPTPENG
IKLEQFIFDVFDTPVLPNKFGLCEVDRCKEFSPLKNGPGSKNDNPETSRLAYLKLGTSWLEDAGAIKVDGVLVEVS
25 SKLSYAGENLSQFKGKVFDRSGIVLEK

SEQ ID NO: 54 is a nucleic acid sequence of Phosphoglucomutase-1 (PGM1) originating from *Saccharomyces cerevisiae*

ATGTCACCTTCTAATAGATTCTGTACCAACAGTTGCTTATAAGGACCAAAAACCGGGTACTTCAGGTTTACGTAAG
30 AAGACCAAGGTTTTTCATGGATGAGCCTCATTATACTGAGAACTTCATTCAAGCAACAATGCAATCTATCCCTAAT
GGCTCAGAGGGAACCACTTTAGTTGTTGGAGGAGATGGTCGTTTCTACAACGATGTTATCATGAACAAGATTGCC
GCAGTAGGTGCTGCAACGGTGTGAGAAAGTTAGTCATTGGTCAAGGCGGTTTACTTTCAACACCAGCTGCTTCT
CATATAATTAGAACATACGAGGAAAAGTGTAACCGTGGTGGTATCATATTAAGTGCCTCACACAACCCAGGCGGT
CCAGAGAATGATTTAGGTATCAAGTATAATTTACCTAATGGTGGGCCAGCTCCAGAGAGTGTCACTAACGCTATC
35 TGGGAAGCGTCTAAAAAATTAACCTACTATAAAATTATAAAGAACTTCCCAAGTTGAATTTGAACAAGCTTGGT
AAAAACCAAAAATATGGCCCATTTGTTAGTGGACATAATTGATCCTGCCAAAGCATACGTTCAATTTCTGAAGGAA
ATTTTTGATTTGACTTAATTAAGCTTCTTAGCGAAACAGCGCAAAGACAAAGGGTGAAGTTGTTGTTTGAC
TCCTTAAATGGTATTACAGGACCATATGGTAAGGCTATATTTGTTGATGAATTTGGTTTACCGGCAGAGGAAGTT
CTTCAAATTTGGCACCTTTACCTGATTTGCGCGGTTTACATCCCGATCCGAATCTAACCTATGCACGAACCTCTT

5 GTTGACAGGGTTGACCGCGAAAAAATTGCCTTTGGAGCAGCCTCCGATGGTGATGGTGATAGGAATATGATTTAC
GGTTATGGCCCTGCTTTTCGTTTCGCCAGGTGATTCTGTTGCCATTATTGCCGAATATGCACCCGAAATTCATAC
TTCGCCAAACAAGGTATTTATGGCTTGGCAGGTTCAATTCCTACATCCTCAGCCATTGATCGTGTGCAGCAAAA
AAGGGATTAAGATGTTACGAAGTTCCAACCGGCTGAAAATTCTTCTGTGCCTTATTTGATGCTAAAAAGCTATCA
10 ATCTGTGGTGAAGAATCCTTCGGTACAGGTTCCAATCATATCAGAGAAAAGGACGGTCTATGGGCCATTATTGCT
TGGTTAAATATCTTGGCTATCTACCATAGGCGTAACCTGAAAAGGAAGCTTCGATCAAACTATTTCAGGACGAA
TTTTTGAACGAGTATGGCCGTACTTTCTTCACAAGATACGATTACGAACATATCGAATGCGAGCAGGCCGAAAAA
GTTGTAGCTCTTTTGAGTGAATTTGTATCAAGGCCAAACGTTTGTGGCTCCCACTTCCCAGCTGATGAGTCTTTA
ACCGTTATCGATTGTGGTGATTTTTCTGTATAGAGATCTAGATGGCTCCATCTCTGAAAATCAAGGCCTTTTCGTA
15 AAGTTTTCGAATGGGACTAAATTTGTTTTGAGGTTATCCGGCACAGGCAGTTCTGGTGCAACAATAAGATTATAC
GTAGAAAAGTATACTGATAAAAAGGAGAACTATGGCCAAACAGCTGACGTCTTCTTGAAACCCGTCATCAACTCC
ATTGTAAAATTCCTTAAGATTTAAAGAAATTTTAGGAACAGACGAACCAACAGTCCGCACATAG

SEQ ID NO: 55 is an amino acid sequence Phosphoglucomutase-1 (PGM1) originating from
15 *Saccharomyces cerevisiae*

MSLLIDSVPTVAYKDQKPGTSGLRKKTVMDEPHYTENFIQATMQSIPNGSEGTTLVVGGDGRFYNDVIMNKIA
AVGAANGVRKLVIGQGGLLSTPAASHIIRTVEEKTGGGIILTASHNPGGPENDLGIKYNLNPNGGPAPESVINA
WEASKKLTHYKIIKNFPKLNLNKLGNQKYGPLLVDIIDPAKAYVQFLKEIFDFDLIKSFLAKQRKDKGWKLLFD
SLNGITGPYGAIFVDEFGLPAAEVLQNWHLPLPDFGGLHDPNLTARTLVDRVDREKIAFGAASDGDGDRNMIY
20 GYGPAFVSPGDSVAIIAEYAPEIPYFAKQGIYGLARSFPTSSAIDRVAARKGLRCYEVPTGWKFFCALFDAKKLS
ICGEESFGTGSNHIREKDGLWAI IAWLNILAIYHRRNPEKEASIKTIQDEFWNEYGRFTFFTRYDYEHIECEQAEK
VVALLSEFVSRPNVCGSHFPADESLTVIDCGDFSYPDLDGSIENQGLFVKFSNGTKFVLRLSGTGSSGATIRLY
VEKYTDKKENYQTADVFLKPVINSIVKFLRFKEILGTDEPTVRT

25 **SEQ ID NO: 56** is a nucleic acid sequence of UTP--glucose-1-phosphate uridylyltransferase
(UGP1) originating from *Saccharomyces cerevisiae*

ATGTCCACTAAGAAGCACACCAAAACACATTCCACTTATGCATTGAGAGCAACACAAACAGCGTTGCTGCCTCA
CAAATGAGAAACGCCTTAAACAAGTTGGCGGACTCTAGTAACTTGACGATGCTGCTCGCGCTAAGTTTGAGAAC
GAACTGGATTTCGTTTTTTCACGCTTTTTCAGGAGATATTTGGTAGAGAAGTCTTCTAGAACCACCTTGGAATGGGAC
30 AAGATCAAGTCTCCCAACCCGGATGAAGTGGTTAAGTATGAAATTATTTCTCAGCAGCCCAGAAATGTCTCAAAC
CTTTCCAAATTGGCTGTTTTTGAAGTTGAACGGTGGGCTGGGTACCTCCATGGGCTGCGTTGGCCCTAAATCTGTT
ATTGAAGTGAGAGAGGGAAACACCTTTTTGGATTTGCTGTTCTGTCAAATTGAATACTTGAACAGACAGTACGAT
AGCGACGTGCCATTGTTATTGATGAATTCTTTCAACACTGACAAGGATACGGAACACTTGATTAAGAAGTATTCC
GCTAACAGAATCAGAATCAGATCTTTCAATCAATCCAGGTTCCCAAGAGTCTACAAGGATTCTTTATTGCCTGTC
35 CCCACCGAATACGATTCTCCACTGGATGCTTGGTATCCACCAGGTCACGGTGATTGTTTTGAATCTTTACACGTA
TCTGGTGAACCTGGATGCCTTAATTGCCAAGGAAGAGAAATATTATTTGTTTCTAACGGTGACAACCTTGGGTGCT
ACCGTCGACTTAAAAATTTAAACCACATGATCGAGACTGGTGCCGAATATATAATGGAATTGACTGATAAGACC
AGAGCCGATGTTAAAGGTGGTACTTTGATTTCTTACGATGGTCAAGTCCGTTTATTGGAAGTCGCCCCAAGTTCCA
AAAGAACACATTGACGAATTCAAAAATATCAGAAAGTTTACCAACTTCAACACGAATAACTTATGGATCAATCTG

AAAGCAGTAAAGAGGTTGATCGAATCGAGCAATTTGGAGATGGAAATCATTCCAAACCAAAAACTATAACAAGA
GACGGTCATGAAATTAATGTCTTACAATTAGAAACCGCTTGTGGTGCTGCTATCAGGCATTTTGATGGTGCTCAC
GGTGTGTGCGTTCCAAGATCAAGATTCTTGCCTGTCAAGACCTGTTCCGATTTGTTGCTGGTTAAATCAGATCTA
TTCCGCTCTGGAACACGGTTCTTTGAAGTTAGACCCATCCCGTTTTGGTCCAAACCCATTAATCAAGTTGGGCTCG
5 CATTTCAAAAAGGTTTCTGGTTTTAACGCAAGAATCCCTCACATCCCAAAATCGTCGAGCTAGATCATTGACC
ATCACTGGTAACGTCTTTTAGGTAAAGATGTCACCTTGAGGGGTACTGTCATCATCGTTTGCTCCGACGGTCAT
AAAATCGATATTCCAAACGGCTCCATATTGGAAAATGTTGTCGTTACTGGTAATTTGCAAATCTTGGAACATTGA

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SEQ ID NO: 57 is an amino acid sequence of UTP--glucose-1-phosphate uridylyltransferase (UGP1) originating from *Saccharomyces cerevisiae*

MSTKKHTKTHSTYAFESNTNSVAASQMRNALNKLADSSKLDDAARAKFENELDSFFTLFRRYLVEKSSRTTLEWD
KIKSPNPDEVVKYEIIISQQPENVSNLKSLAVLKLNGGLGTSMGCVGPKSVIEVREGNTFLDLSVRQIEYLNRYD
15 SDVPLLLMNSFNTDKDTEHLIKKYSANRIRIRSFNQSRFPRVYKDSLLPVPTEYDSPLDAWYPPGHGDLFESLHV
SGELDALIAQGREILFVSNGLGATVDLKILNHMIETGAEYIMELTDKTRADVKGGLTISYDGQVRLLEVAQVP
KEHIDEFKNIRKFTNFNTNNLWINLKAVKRLIESSNLEMEIIPNQKTITRDGHEINVLQLETACGAAIRHFDGAH
GVVVP RSRLP VKTCS D L L L V K S D L F R L E H G S L K L D P S R F G P N P L I K L G S H F K K V S G F N A R I P H I P K I V E L D H L T
ITGNVFLGKDVTLRGTVIIVCSDGHKIDIPNGSILENVVVTGNLQILEH

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SEQ ID NO: 58 is a nucleic acid sequence of Glucosamine 6-phosphate N-acetyltransferase (GNA1) originating from *Saccharomyces cerevisiae*

ATGAGCTTACCCGATGGATTTTATATAAGGCGAATGGAAGAGGGGGATTTGGAACAGGTCCTGAGACGCTAAAG
GTTTTGACCACCGTGGGCACTATTACCCCGAATCCTTCAGCAAACCTATAAAATACTGGAATGAAGCCACAGTA
25 TGGAATGATAACGAAGATAAAAAAATAATGCAATATAACCCCATGGTGATTGTGGACAAGCGACCGAGACGGTT
GCCGCTACGGGAATATCATCATCGAAAGAAAGATCATTCATGAACTGGGGCTATGTGGCCACATCGAGGACATT
GCAGTAACTCCAAGTATCAGGGCCAAGGTTTGGGCAAGCTCTTGATTGATCAATTGGTAACTATCGGCTTTGAC
TACGGTTGTTATAAGATTATTTTAGATTGCGATGAGAAAAATGTCAAATTCATGAAAAATGTGGGTTTAGCAAC
GCAGGCGTGGAATGCAAATTAGAAAATAG

30

SEQ ID NO: 59 is an amino acid sequence of Glucosamine 6-phosphate N-acetyltransferase (GNA1) originating from *Saccharomyces cerevisiae*

MSLPDGFYIRRMEEGDLEQVTETLKVLTTVGTITPESFSKLIKWNEATVWNDNEDKKIMQYNPMVIVDKRTETV
AATGNIIIERKIIHELGLCGHIEDIAVNSKYQGGLGKLLIDQLVTIGFDYGCYKIIILDCDEKNVKFYEKCGFSN
35 AGVEMQIRK

SEQ ID NO: 60 is a nucleic acid sequence of phosphoacetylglucosamine mutase (PCM1) originating from *Saccharomyces cerevisiae*

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ATGAAGGTTGATTACGAGCAATTGTGCAAACTCTACGATGACACGTGCCGCACAAAGAATGTGCAGTTCAGTTAC
GGTACGGCCGATTTCAGAACGCTGGCCAAGAATTTGGATACGGTGATGTTTCACTACTGGTATACTGGCGGTTCTC
5 AGGTCGCTGAAGCTTCAGGGTCAGTATGTGGGGTGATGATCACGGCGTCGCACAACCCATACCAGGACAACGGG
GTCAAGATCGTGGAACGAGACGATCGATGCTTTTGCCACATGGGAGCCATATGCCATGCAGTTGGCCAATGCG
GCCTCTTTTGCCACTAATTTTGAAGAATTTTCGTGTTGAGTTGGCCAAGCTGATTGAACACGAAAAGATTGATTTG
AATAACAACCGTCGTGCCTCACATCGTGGTTGGGAGAGACTCTAGGAAAAGTAGTCCATACTTGTCTGCGCTGCTTG
ACTTCTCCATGGCCAGCGTCTTCCACGCGCAAGTTTGGACCTAGGCTGTGTCACTACGCCTCAATTGCATTAC
10 ATTACTGATTTGTCCAACAGGCGGAACTGGAAGGAGACACAGCGCCAGTTGCCACAGAACAGGACTACTATTTCG
TTCTTTTATAGGAGCCTTCAACGAGCTCTTCGCCACGTATCAGCTGGAGAAGAGGCTGTCTGTCCCAAATTTGTTT
ATAGACACAGCCAATGGTATCGGTGGTCCACAGTTGAAAAAACTACTGGCCTCCGAAGATTGGGACGTGCCAGCG
GAGCAAGTTGAGGTAATCAACGACAGGTCCGATGTTCCAGAAGTGTGAATTTTGAATGCGGTGCGGATTATGTG
AAGACTAACCAGAGATTACCCAAGGTCCTTCTCCATCCTCGTTTGATTGCTATATTGCTCCTTTGATGGTGAC
15 GCAGACAGGGTTGTGTTCTACTATGTCGACTCAGGATCAAAATTTTCAATTTGTTGGATGGTGACAAAATTTCCACT
TTGTTTGCAAAGTTCTTGTCTAAACAAGTAGAATTGGCACACCTAGAACATTCTTTGAAGATTGGTGTGTTGCAA
ACTGCCTATGCAAACGGCAGTTCCACCGCTTACATAAAAAAATACGTTGCACTGTCCCGTGTCTTGCACTAAGACA
GGTGTTAAACACTTGCAATCATGAAGCTGCCACTCAGTACGATATTGGCATTATTTTGAAGCAAATGGACATGGT
ACGATTATATTAGCGAAAAATTTTATCGAACTATCAAATCTGAATTATCCAAGTCCAAGTTAAATGGTGATACG
20 TTAGCTTTGAGAACTTTGAAGTGTCTCTGAATTGATTAATCAGACCGTGGGAGATGCTATTTCAGACATGCTT
GCTGTCTTTGCTACTTTGGCGATTTTGAAAATGTCGCCAATGGATTGGGATGAAGAGTATACTGATTTGCCCAAC
AAGCTGGTTAAGTGCATCGTTTCTGATAGGTCAATTTTCCAAACCACGGACCAGGAAAGAAAATTGCTCAATCCA
GTGGGGTTGCAAGACAAGATAGATCTTGTGGTAGCCAAGTATCCCATGGGAAGAAGCTTTGTCAGAGCCAGTGGT
ACGGAGGATGCGGTGAGGTTTATGCGGAATGTAAGGACTCCTCTAAGTTAGGTCAATTTTGTGACGAAGTGGTG
25 GAGCACGTTAAGGCATCTGCTTGA
```

SEQ ID NO: 61 is an amino acid sequence of phosphoacetylglucosamine mutase (PCM1) originating from *Saccharomyces cerevisiae*

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MKVDYEQCLKLYDDTCRTKNVQFSYGTAGFRTLAKNLDIVMFSTGILAVLRSLKLQGGYVGVMITASHNPYQDNG
30 VKIVEPDGSMMLLATWEPYAMQLANAASFATNFEEFRVELAKLIEHEKIDLNNTTVPHIVVGRDSRESSPYLLRCL
TSSMASVFHAQVLDLGCVTTPQLHYITDLSNRRKLEGDTAPVATEQDYYSFFIGAFNELFATYQLEKRLSVPKLF
IDTANGIGGPQLKKLLASEDWDVPAEQVEVINDRSDVPELLNFECGADYVKTNRQLPKGLSPSSFDSL YCSFDGD
ADRVVFYYVDSGSKFHLLDGDKISTLFAKFLSKQLELAHLEHSLKIGVVQTAYANGSSTAYIKNTLHCPVSTCKT
GVKHLHHEAATQYDIGIYFEANGHGTIIFSEKFHRTIKSELSKSKLNGLTLALRTLKCFSELINQTVGDAISDML
35 AVLATLAILKMSPMDWDEEYTDLPNKLVKCIVPDRSIFQTTDQERKLLNPVGLQDKIDLVVAKYPMGRSFVRASG
TEDAVRVYAECKDSSKLGQFCDEVVEHVKASA
```

SEQ ID NO: 62 is the nucleic acid sequence of promotor pTDH3

CTGCTGTAACCCGTACATGCCCAAAATAGGGGGCGGGTTACACAGAATATATAACATCGTAGGTGTCTGGGTGAA
CAGTTTATTCTTGGCATCCACTAAATATAATGGAGCCCGCTTTTTAAGCTGGCATCCAGAAAAAAAAAGAATCCC
AGCACCAAAATATTGTTTTCTTCACCAACCATCAGTTCATAGGTCCATTCTCTTAGCGCAACTACAGAGAACAGG
GGCACAACAGGCAAAAAACGGGCACAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAATGATGACACAAGG
5 CAATTGACCCACGCATGTATCTATCTCATTTTCTTACACCTTCTATTACCTTCTGCTCTCTCTGATTTGGAAAAA
GCTGAAAAAAAAAGGTTGAAACCAGTTCCTGAAATTATTCCCCTACTTGACTAATAAGTATATAAAGACGGTAGG
TATTGATTGTAATTCTGTAAATCTATTCTTAAACTTCTTAAATTCTACTTTTATAGTTAGTCTTTTTTTTAGTT
TTAAACACCAAGAACTTAGTTTCGAATAAACACACATAAACAAACAAA

10

SEQ ID NO:63 is the nucleic acid sequence of promotor pTDH3.Sk

CAATTTCATCGGCCCTTTTAGCGGCTACCCGCGCCATCTAAATGATAGGGCGGGTGACACTATGGTAAATCCCATA
ATTAGGTGTCTGGGTGAGTGGTTCTGATGCCGGCATCCACTAAATATATTGGAGCCCATTTTTTACGCGGGCTTC
CAGAAAAAAAAAGAGAATCCCAGCACCAAAAGGTGGTTCTCTTCACCAACCATCAGATCATAGGTCCACAACCACAC
15 ATAACAGGGGCACAAAAAGGCAAAAAACGGACATAACCTCAATGGAGTGATGCAAAATTGACTGGAGCAAAAGCTG
ACACAAGGCATTGATTGACCTACGCATGTATCTGTATTCTTTTCTTACACCTTCTATTACCTTCTAACTCTTTGG
GTTGGAAAAAACTGAAAAAAAGGTTGGGACCTGGTTCCCCCAAGTTGTCCCCCTACTTGGTTATTAAATATATA
AAGACAGCAAGTGTTGATTATAATCTTGTAATCTATAGTTCTTAATCTATACTTCTATTTTATAATTTAAATTAG
TCTTTTTATTTCGAAGTCCCCAAGAACTTAGTTTCGAATAAACACACACAAATAAACACA

20

SEQ ID NO: 64 is the nucleic acid sequence of promotor pTDH3-1.sba

GCAGCGCTTCTTCGCTCTAGTTTTTATAGTTATTATTACTACCACCTTAAAAATACGTAAATACTCAAAATAGT
AGTGATATTCCCAACCTTATTCATCCAAGGCACATCATCATCAGCCATTCTTTTACCTGCCATTAGTAA
CCCGTCTTCTCATTGAGCGGGTTACGGCAGCCACAGGCCACATTCGAATGTCTGGGTGAGCGGTCCCTTTTCCA
25 GCATCCACTAAATATCTCCGATCCCGCTTTTTAATCTGGCTTCCTGAAAAAAGAGAATCCCAGCACCAAAAAAT
GGCTCTCTTTCACCAACCATCAGATCATAGGTCCCATTCTCTTACCGAACCGTACAGAACAGGGGAAAACGGGTA
CAACCTCAATGGAGTGATGCAAACTGACTGGAGCAAAAAGCTGACACAAGGCAATCGACCTACGTGTCTGTCTAT
TTTCTCACACCTTCTATTACCTTCTAACTCTCTGGGTTGGAAAAAACTGAAAAAAGGTTGAGACCAGTTTCCAC
AAATCATCCCCCTGTTTGATTAATAAATATATAAAGACGACAACCTATCGATCATAACTCATAAACTATAACTC
30 CTTTACACTTCTTATTTTATAGTTATTCTATTTTAAATTCTTATTGATTTTAAACCCCAAGAACTTAGTTTCGAA
AACACACACACACAAACAATTAATA

SEQ ID NO: 65 is the nucleic acid sequence of promotor pTDH3.Sar

GAGCTAAATATCAGCCCTTCGGGTCCTGCCTGCTACCCGGTCCTGTTTCGAATAAAAAACGCGGGTAACACGACCCA
35 GTAACACCTGTCTGTTGGGTGCTGAGTTCGATACCGGCTTCCACTAAATAGATTGGGTTCCGCTCTT
TACGCTGGCTTCTGAAAAAAGAGATTCCGGGCACCAAAAAATTGGTCTCTTTGCCAACCATCAGATCATAGGTCT
CATTCTCTTACCATAACCACACAGGATAGGGGCACCACAGGCGAAAAATGGGCACAAAATCTCAATGGAGTGATGC
AAATTAGCTGGAACAAAAGCTGACACAAGGCAATTAACCTGCGCATGTATCCATCTCTTTTCTTACACCTTCTC

TTACGTTCTAACTGTTTGGGTTGGAAAAATTAAAAAAAAGGTTGAGACCAGTTTCCCCAAATCGTCCCCCTAC
TTGATTCTATAAATATATAAAGACGACAACCTATTGATTATAATCTTGTAATCTATAACTCTTTACTTTCTCCTAT
TTATAATTTAACTTAATCTTTTTAGATTAAAACCCCAAGAACTTAGTTTCGAACAAACACACAAAATAAACAA
AA

5

SEQ ID NO: 66 is the nucleic acid sequence of promotor pENO2

CGCTCAGCATCTGCTTCTTCCCAAAGATGAACGCGCGTATGTCACTAACGACGTGCACCAACTTGCAGAAAGT
GGAATCCCGTTCCAAACTGGCATCCACTAATTGATACATCTACACACGCGACGCTTTTTTCTGAAGCCCACTT
TCGTGGACTTTGCCATATGCAAAATTCATGAAGTGTGATACCAAGTCAGCATACACCTCACTAGGGTAGTTTCTT
10 TGGTTGTATTGATCATTGGTTTCATCGTGGTTTCTAATTTTTTCTCCATTGCTTTCTGGCTTTGATCTTACT
ATCATTGGATTTTTTGTGGAAGTTGTAGAATTGTATGTGACAAGTGGCACCAAGCATATATAAAAAAAAAGC
ATTATCTTCTACCAGAGTTGATTGTTAAAAACGTATTTATAGCAAACGCAATTGTAATTAATTCTTATTTTGTA
TCTTTTCTTCCCTTGTCTCAATCTTTTATTTTATTTTATTTTCTTTTCTTAGTTTCTTTCATAACACCAAGCA
ACTAATACTATAACATACAATAATA

15

SEQ ID NO: 67 is the nucleic acid sequence of promotor pTEF3

GGCTGATAATAGCGTATAAACAATGCATACTTTGTACGTTCAAAATACAATGCAGTAGATATATTTATGCATATT
ACATATAATACATATCACATAGGAAGCAACAGGCGCGTTGGACTTTAATTTTCGAGGACCGCGAATCCTTACAT
CACACCCAATCCCCCACAAGTGATCCCCCACACCATAGCTTCAAAATGTTTCTACTCCTTTTTTACTCTTCCA
20 GATTTTCTCGGACTCCGCGCATCGCCGTACCACTTCAAAACACCCAAGCACAGCATACTAAATTTCCCTCTTTC
TTCTCTAGGGTGTGCTTAATTACCCGTACTAAAGTTTGGAAAAGAAAAAGAGACCGCCTCGTTTCTTTTCT
TCGTGAAAAAGGCAATAAAAAATTTTATCACGTTTCTTTTCTGAAAATTTTTTTTTTTGATTTTTTCTCTT
TCGATGACCTCCCATTTGATATTTAAGTTAATAAACGGTCTTCAATTTCTCAAGTTTCAGTTTCATTTTTCTTGTT
CTATTACAACTTTTTTTACTTCTTGCTCATTAGAAAGAAAGCATAGCAATCTAATCTAAGTTTTAATTACAAA

25

SEQ ID NO: 68 is the nucleic acid sequence of promotor pTEF1

GTTTAGCTTGCCCTCGTCCCCGCCGGGTCAACCGGCCAGCGACATGGAGGCCCAAGAAATACCCTCCTTGACAGTCTT
GACGTGCGCAGCTCAGGGGCATGATGTGACTGTGCGCCGTACATTTAGCCCATACATCCCCATGTATAATCATTT
GCATCCATACATTTTGATGGCCGCACGGCGCAAGCAAAATACGGCTCCTCGCTGCAGACCTGCGAGCAGGGA
30 AACGCTCCCTCAGACGCGTTGAATTGTCCACGCGCGCCCTGTAGAGAAATATAAAAGGTTAGGATTTG
CCACTGAGGTTCTTCTTTTCATATACTTCTTTTAAATCTTGCTACGATACAGTTCTCACATCACATCCGAACAT
AAACAACC

SEQ ID NO: 69 is the nucleic acid sequence of promotor pTEF1.ago

GTTTAGCTTGCCCTCGTCCCCGCCGGGTCAACCGGCCAGCGACATGGAGGCCCAAGAAATACCCTCCTTGACAGTCTT
35 GACGTGCGCAGCTCAGGGGCATGATGTGACTGTGCGCCGTACATTTAGCCCATACATCCCCATGTATAATCATTT
GCATCCATACATTTTGATGGCCGCACGGCGCAAGCAAAATACGGCTCCTCGCTGCAGACCTGCGAGCAGGGA
AACGCTCCCTCAGACGCGTTGAATTGTCCACGCGCGCCCTGTAGAGAAATATAAAAGGTTAGGATTTG

CCACTGAGGTTCTTCTTTTCATATACTTCCTTTTAAATCTTGCTACGATACAGTTCTCACATCACATCCGAACAT
AAACAACC

SEQ ID NO: 70 is the nucleic acid sequence of promotor pTEF1.Sba

- 5 CGCCAACAAACCTTCGAACACTTTAATTTTCGAGGACCGCAGATCCTCACATCACACCCACACCCAAGACTGCTT
CCCCCACACACCTTGCATCTGTACACTTTCTTCTGCTCTGTTTTTCTCTCCGGCGTTCTCTCGGGTCGCCGCAT
CGCCGCGCCGGCTGGAACCGCCACGCACCGCATATTGCAAATCGCCTGCCCTCTTGCTCCTTTTTGAGGGCG
CGCCGTTACCCGCGCCAGGGTCCGAAAAAGAAACAAGGCTCTACCGCGTTTCTTTTCTTGTCGAAAAAGGC
AAAAATGAAAAATTTTATCACGTTTCTTTTTTTTGAAAAATTTTTTTTTTGTTTTTTCTTCGATGGCCTC
10 CCATTGATATTTAAGTTAATAAATGGTTTTTCAGTTTTCAAGTTTCAGTTTGTGTTCTTCTTTGCTAACTTTCCT
TACACCTCGAAAGAAAGTATAGCAATCTAATCTTAGTTTTAATTACAA

SEQ ID NO: 71 is the nucleic acid sequence of promotor pPDC1

- TTATTTACCTATCTCTAACTTCAACACCTTATATCATAACTAATATTTCTTGAGATAAGCACACTGCACCCATA
15 CCTTCCTTAAAAACGTAGCTTCCAGTTTTTGGTGGTTCCGGCTTCCTTCCCGATTCCGCCCCGCTAAACGCATATT
TTTGTTGCCCTGGTGGCATTTGCAAAATGCATAACCTATGCATTTAAAAGATTATGTATGCTCTTCTGACTTTTTCG
TGTGATGAGGCTCGTGAAAAAATGAATAATTTATGAATTTGAGAACAATTTTGTGTTGTACGGTATTTTACTA
TGGAATAATCAATCAATTGAGGATTTTATGCAAATATCGTTTGAATATTTTTCCGACCTTTGAGTACTTTTCTT
CATAATTGCATAATATTGTCCGCTGCCCTTTTTCTGTTAGACGGTGTCTTGATCTACTTGCTATCGTTCAACAC
20 CACCTTATTTTCTAACTATTTTTTTTTTAGCTCATTGAAATCAGCTTATGGTGTATGGCACATTTTGCATAAACC
TAGCTGTCTCGTTGAACATAGGAAAAAATATATAACAAGGCTCTTTCACTCTCCTTGCAATCAGATTGCG
GTTTGTTCCCTTTATTTTCATATTTCTTGTCATATTCCTTTCTCAATTATTATTTCTACTCATAACCTCACGCA
AAATAACACAGTCAAATCAATCAAA

- 25 **SEQ ID NO: 72** is the nucleic acid sequence of promotor pCCW12

- aaccagggcaaagcaaaataaaagAAACTTAATACGTTATGCCGTAATGAAGGGCTACCAAAAACGATAATCTCA
ACTGTAAACAGGTACAATGCGGACCTTTTGCCACAAAACATACATCATTTCATTGCCGAAAAAGAAAGAGTGA
AGACAGCAGTGCAGCCAGCCATGTTGCGCCAATCTAATTATAGATGCTGGTGCCTGAGGATGTATCTGGAGCCA
GCCATGGCATCATGCGCTACCGCCGGATGTAAAATCCGACACGCAAAAAGAAAACCTTCGAGGTTGCGCACTTCGC
30 CCACCCATGAACCACACGGTTAGTCCAAAAGGGGCAGTTTCAGATTCCAGATGCGGGAATTAGCTTGCTGCCACCC
TCACCTCACTAACGCTGCGGTGTGCGGATACTTCATGCTATTTATAGACGCGGTGTGGAATCAGCACGCGCAA
GAACCAAATGGGAAAATCGGAATGGGTCCAGAACTGCTTTGAGTGCTGGCTATTGGCGTCTGATTTCGTTTTGG
GAATCCTTTGCGCGCGCCCTCTCAAACTCCGCACAAGTCCCAGAAAGCGGAAAGAAATAAAACGCCACCAA
AAAAAAAATAAAAGCCAATCCTCGAAGCGTGGGTGGTAGGCCCTGGATTATCCCGTACAAGTATTTCTCAGGAG
35 TAAAAAACCCTTTGTTTTGGAATTCCTTCGCGGCCACCTACGCCGCTATCTTTGCAACAACCTATCTGCGA
TAACTCAGCAAATTTTGCAATTCGTGTTGCAGTATTGCGATAATGGGAGTCTTACTTCCAACATAACGGCAGAA
AGAAATGTGAGAAAATTTGCATCCTTTGCCTCCGTTCAAGTATATAAAGTCGGCATGCTTGATAATCTTTCTTT

CCATCCTACATTGTTCTAATTATTCTTATTCTCCTTTATTCTTTCTTAACATACCAAGAAATTAATCTTctgtca
ttcgcttaaacactatatcaata

SEQ ID NO: 73 is the nucleic acid sequence of promotor pCCW12.Sm

5 CGTTGTGCTGTAGTGAAGGAAGACTAAAAAGGATAATCACAGTTGTAAAGAGGTATAATGCGGACCCTTTTGCCA
CAAAACACACATCTTTTCGTTTCCAAAATAAGAAAGAAGAAAGCAAAAAGATTAGCAGCCACGTTGCTGCGATCTA
ATTATAGACGGTGGCGTCATCATTCTACCCAAGATTGTGTCTTGAACCTGCCACGGGTCTGCGTTATCGCCGG
ATGTAAACTAGACATGCAAAAAAGGACCTTCCAGGTAGCGTGTCCACACCACCCATGACCACCACAGTTAGT
10 CCAAAAGAGGCAGCACCACCTTCCCGATGGGGGAATTAGATTGCTGCCACCCTCACCTACTAATGCTGCGGTGTG
CGGATATGCCCTGCTATATATAGCTCCGCGTTTTTGAACCAGCACAGCGCGAGCACCAAAAAGGAAAATCGCATA
GGCCCAGAACTGATTTTCAGCACGGGCTATTGGCGTTGGGTTTTCCGTTCTGGGAAACCTTCGCCGCGTCCCCCTCA
CAAACCTCCGCACAAGTTCGAGCAAGCGGGAAAAAACGAAAAACGCCATTAATACTAAATAAAGCAAATCCTCGA
AGCGTGGGTGGCAAGCCCTGGATTTTTCCGCACAAGTACTCTTCTCAGGAGTAAAAAACCCGTTTGTTTTGGA
ATTCCCCATTTTCGCGGCCATCTACGCCGGTATCTTCGCAATATCTATCAGCGATAACTCAGCAATTTTAATATTC
15 GTGTTGCAGTGCTGCGATAGCGGGAGTCTTGTTTGAACATAACGGCAGAAAGGAATGAGAGAAAATTTTCCATT
CTTTGGCCTCCGTTCAAGTATATAAAGCCGGCATGCTTGGTAATCTTTCTCTCTCTTCTGTATTGTTTCTATAAT
ACTTTTATCTTCTAATTATTTTCTGAAAAACCAAGAAATTAATCTTCTGTCTTCGCTTAAACACTATATCAAT
A

20 **SEQ ID NO: 74** is the nucleic acid sequence of promotor pCCW12.Sk

TGTAATCCTACACGCAAAAAACCTCTTGCGTTGCGCGTTTTCCACCACCCACGACCCGCACAATCAATCCAAA
AGGGGCAACGCCAGTTCCCAATGCGGGAATTAGCTCCTCACCCCTCACCCGCTAACGCTGCGGTGTGCGGACA
CGCAGCAGTATTTATAGATCCTCGTGTGGAACCAGCCGCGTGAGCACCAATTGGAAGTGCGAATGGCCCCA
GAACCGCTTTCAGTACTGGGCCATTGGCGTCTAGTTTCCGTTTTGAGCGTCCTTCGCCGCGTCTCTCTGTGAAAT
25 CCCCCACAAAGTCTGAGCAGGCAAAAAAAGCCACCAAAATAAATAAAGCCAATCCTCGAAGGATGAGT
AGGAAAGGAAGCCCTGGTTTTTCCCGCACGAATATTTTTCAGGAGTGAAAAATCCGTTTGTGTTTGGAAATTC
CCATTTTCGCGCTACCTACGCCGGTATCTTTGGAACAATATCAGCGATAACTCAGCAAAATTGCAATATCCGTG
CTGCAATAGTGCATAGTGGGATTGGGAGTCTTGTTGCATCATAACGGCAGAAAGGAATGAATAAAAAATTTCCG
TTCTTTGTCTCCGTTCAAGTATATAAAGTCGGCATGCTTGATTATCTTTTCTCTCTTCTACTACATTTCTATTT
30 CACTTTCTACTCTATTCTTCCCTGAAAAACCAAGAAAATAATCTTCTGTCTTCGCTTAAACAATATATCAAAA

SEQ ID NO: 75 is the nucleic acid sequence of promotor pCCW12.Sba

ACCATGCCACGGTGTGCCCCACTTCCACCCACGACCCGCACGGTTGGCCCCGAAAGGGGCAACACCAGTCCCCA
ATACGGAAATTAGTCCGCCACCACCTCACCTCGCTAGAGCTGCGGTGTGCGGGCGTGATCGCTATTTATAGAC
35 ATGCCTGTGCGGTACCGCGCGAGCACCAACAGGAAATCGCACTGGGCCAGAACCCAGCTATGCGCTGG
GCCGATGGCGTCCGTTTCCCTTTGGGAGCCCCCTGCCAGTTTCGCTAACAAATCCCCGCACCGGCTTGAGAAA
AAAGCGAAAAGCGAAAAAATCAACGCCACCAAAATTAAGAGGAGCCATCCTCGAAGGGTGAATAGT
AGCCCCTGACTTTTCCCGCACAGACAGACACCTTTCAGGAGTGAACAAAAAGCAGTTGTTTGGAAATTTCCCC
ATTTGCGGTGGCCTGCGCAGGTATCTCTGCGTCACTATCAGCGATAACTCAGCAAAATTTGCAATATTCGTGTT

GCGATACTACGATAATGGGAGTCTGTGCGCTAATAACGGCAACAAGGAATGAGAGAGAAAAATTTTCTTCATTCT
CCAGCTCCCGTTCAAGTATATAAGGTCGGCATGGTCGATTGTCTTTCCTTCTCTCAGTTACGTCTCTCTATTTA
CATTATTCTTATTTTTATTATAAAAAACCCCAAGAAATTAATCTTCTGTCAATTCGCTTAAACACTATATCAAAA

5 **SEQ ID NO: 76** is the nucleic acid sequence of promotor pCCW12.Sar

AAAAACAACCTTCTGCCAACCTGCGTGCTTCTCACCACCCATGACCCACACAATTGACCCGAATGGGGCAACTC
CAGTTCCCAATACGGGAATTAAGTCGCCACCATATTTACCGCGTTGAAGCTGTGGTGTGCGGACACTCCGTACTA
TTTATAGACCCACGCGGTGGAACCAGCACGCGCGCGCACTAAACAGGAAAATCGCATTGAGTCCAGAACCGCCAC
CAGCACTTGGCCATTGGCGTCTAATTTCCGTTTTTCGGCGCCCCCTACCGCGTCTTCTAACAAAGCGCGACAAG
10 CTTGAGCAAGTGAAGAAAATTAATAAAAAACCGCCACCAAAACAAATAAAGCAATCTTCGAAGTGTGGGT
TGGTGGGAAGCCCCTGGCTTTTCCCGCACCAGTCGTTTTTCAGGAGTAAAAAATACCGTTTTGTTTTGGAATTCC
CCATTTTCGCGGCGACCTGCGCCGGTATCTTTGCAACAACCTATTTGCGATAACTCAGCAAAATTTGCATATTCGTG
TTGGGATATTGCGATAGTGGGAGTCTTGTTCATAATAACGGTAAAAAGAAGTGAAGGAAAAAATTTGCATCCT
TTAGTCTCAGTTCAAGTATATAAAGTCGGGATATTCAATTATCTTTCTTTCTCTTGCTCAAAGGTTTCTATATTT
15 TTTTTATAGTATTTCTTTTGTATAAAATACCAAGAAATTAATCTTCTGTCAATTCGCTTAAATACTACATCAATA

SEQ ID NO: 77 is the nucleic acid sequence of promotor pNUP57

TCATCTGCGCAATGACTATCAAGACCTTCTGCAAGAATTTCAAATCTCACTGAAAATCTTGACCGAAAAGTGTCT
TGAAAACCCATCAAGCCTGCAAAACCTATCTTTGACATTAGTCTCCATTATAAAAAACGGCATAGTTGGGAGAAAA
20 CTTTCCATACTTCAATTGTGGACTGATATAAGTATTTTAGTTTTGCCCGCATGATCATCCACATGGCTACAGCA
GTTCTCTCATAGGAAAATAGTACAATAGCTACGTGATATAATCTAAATAATTGTTGCCAATGTGTAATTATATCA
TTTTGAACGTACGCGAAATGGATTATTTTCAAAAATTTGTTTCTTGAAATGAGTAAAAGCAAAAGTCCAACCTCT
CCAAGTCGATGTAAACAACCTTTTGGCAAAGGGACTGAAAGACTAAATCGAGGATTATCCCGTTCAAACCTATTC
AGAAACGCTCGTTAGTAACAAAAGACATACCTTGTGTGACCAATTGATCAC
25

SEQ ID NO: 78 is the nucleic acid sequence of promotor pCCW10.ago

GGTACCACGGCAACCTCGTTCGCTGTTTCATCCCCCTTCGTACACAGGACGTTGGATGCCGTAAGCAGCGTTGCTT
TTGATCCTCagGATCGGCCGGGTAACCCGCGGCtGCTTCTATTTTAGTATTTCATATCTCAAGCACATCCATTCCG
GCCGTTTGGGGGCGCGCCGCACTCGTGTCCATTCTACCGTGGCACTTAGGGCTATCCTGTGCGGAGCGCCCCGC
30 CGACCGCCTTATCGGCACCAAAAGTAGAAGCCCCGCGCCCGCGTGGCTCAGACTCACCATCGGTGCTATTTACTT
TTCGATCAGATCGCGGCGCGCGGTGGCCGGCATTTCGGAAGCGGCCACGGAGCAGAGGTGGCGCATTTCGAATCG
CATACGTCTTCGCCACGCGGAAAAAATTTTCGGCTATATAAGGAGAGGCGCCGTCTTGTGTCAGGCAGTTT
CACTTTCTCTAAAACCAAGAACATCGATTTCTTTAGTCACTCGCTTCCTTACACCGAACTCGAGGCGGCCGC

35 **SEQ ID NO: 79** is the nucleic acid sequence of promotor pCWP2

CTAGCCTCCCTTTTTTATTTGTGCGGTACCGCAAGGGACAAAGCTTTTCTTAGAAAACCGTCTGAGAAGCATA
ACGTACGCCATCCCTAGACATATTAATAATGCTACAGATACTATGCTGCTCGTCTTTTTTTGACGACCTTTTA
TTGCAATGTGCAACTAATGGCAAACAACCACATAGTATCACAGTATTACATTGCCTCCACCGATGCGGATGTTAG

GGCGCCAAGTCTGTCATGAAGCATGTTCTCTGTCATAATCTTGTATGCAAAATACCGGTTCTGCGCCACTGATAT
GCTAGGCAGCAGCAACCTATGCAGAAGATTGCTTTTCCACGCCTGTTTTACGTCTCCAGGGCACTTGAAACAAT
GCAGCGATCGCCGCCACAACACGCCAAAAGAGAAGCGAAAGTGGGCCTGGGCGGCCTCAGTTTCGGCAGAGGTAAA
CAACACGAACTGAAGTGCCTTAGCTCCGAAGGGCAATTCCACAGGCACTCCGCGGGGCCCGCCAAGGCCAAAA
5 GGCGTGGAATATGCGCGTTTTTGGGGCCATAACACCCAGTACCACGGCCGGAACGGGCCATATAATAAGTTTTTCA
CTCTCAAGAATGGTAAACGTAAATAGGAACATCCCACTACCCTAGAAATTGCGGAAATTTGCGCGTTATCATTAG
AAAACTGGAAACCGTCCTTTTCTCTTTCTTGCATTTCCCTTTCCGTATTATTGCCATTTCTTAAGTGCATTTG
GGGAACCGTAGACCAAAAGCCAAACAGAGAAATGTAAACGTTCTAAAAAACAACGAAAAAATTGAAAAATAA
GATACAATAATCGTATATAAATCAGGCTTCTTGTTCATCATTTTCAATTCTCTTCTTGCCATCCCTTTTCTATC
10 TTTGTTCTTTCTTCTCATAATCAAGAATAAATAACTTCATCA

SEQ ID NO: 80 is the nucleic acid sequence of promotor pRPLA1

TCAAGTTGGATACTGATCTGATCTCTCCGCCCTACTACCAGGGACCCTCATGATTACCGCTCGAATGCGACGTTT
CCTGCCTCATAAACTGGCTTGAAATATTTATTGCTGAACAGTAGCCTAGCTTATAAAAAATTTCAATTAATTA
15 ATGTAATATGAAACTCACATGCCTTCTGTTTCTAAATTTGTACAGCAAGAAATAACATTACCATACGTGATCT
TATTAACTCTAGTATCTTGTCTAATACTTCATTTAAAAGAAGCCTTAACCCTGTAGCCTCATCTATGCTGCTA
CATATCGTGAGGTACGAATATCGTAAGATGATACCACGCAACTTTGTAATGATTTTTTTTTTTCATTTTTTAA
GAATGCCTTTACATGGTATTTGAAAAAATATCTTTATAAAGTTTGCATCTCTTCTGTTCTGAATAATTTTAG
TAAAAGAAATCAAAGAATAAAGAAATAGTCCGCTTGTCCAATACAACAGCTTAAACCGATTATCTCTAAATA
20 ACAAGAAGAA

SEQ ID NO: 81 is the nucleic acid sequence of promotor pCUP1

CGGCAAACTTCAACGATTTCTATGATGCATTTTATAATTAGTAAGCCGATCCCATTACCGACATTTGGGCGCTAT
ACGTGCATATGTTTCATGTATGTATCTGTATTTAAACACTTTTGTATTATTTTCTCATATATGTGTATAGGTT
25 TATACGGATGATTTAATTATTACTTACCACCCTTTATTTAGGCTGATATCTTAGCCTTGTTACTAGTTAGAAA
AAGACATTTTGTCTGTCAGTCACTGTCAAGAGATTCTTTGTCTGCATTTCTTCTAGAAGCAAAAAGAGCGATGC
GTCTTTTCCGCTGAACCGTTCCAGCAAAAAGACTACCAACGCAATATGGATTGTCAGAATCATATAAAGAGAA
GCAAATAACTCCTTGTCTTGTATCAATTGCATTATAATATCTTCTTGTTAGTGCAATATCATATAGAAGTCATCG
AAATAGATATTAAGAAAAACAACTGTACAATCAATCAATCAATCATCACATAAA
30

SEQ ID NO: 82 is the nucleic acid sequence of promotor pMET6

CCACAGGAAATATTTACGTGACTTACAAACAGAGTCGTACGTACGGACCGAGTCAGGTGAAAAATGTGGGCC
GGTAAAGGGAAAAACCAGAAACGGGACTACTATCGAACTCGTTTAGTCGCGAACGTGCAAAAGGCCAATATTTT
TCGCTAGAGTCATCGCAGTCATGGCAGCTCTTTGCTCTATCTCCCGGTCGCAAACTGTGGTAGTCATAGCTCG
35 TTCTGCTCAATTGAGAACTGTGAATGTGAATATGGAACAAATGCGATAGATGCACTAATTTAAGGGAAGCTAGCT
AGTTTTCCCAACTGCGAAAGAAAAAAGGAAAGAAAAAATTTCTATATAAGTGATAGATATTTCCATCTTTAC
TAGCATTAGTTTCTTTTACGTATTCAATATTTTTGTAACTCTTCTTTATCATAAAAAAGCAAGCATCTAA
GAGCATTGACAACACTCTAAGAAACAAAAATCCAATATAATTTCAAAGTACATATCAAAA

SEQ ID NO: 83 is the nucleic acid sequence of promotor pMET25

TTACATTATCAATCCTTGCGTTTCAGCTTCCACTAATTTAGATGACTATTTCTCATCATTTGCGTCATCTTCTAA
CACCGTATATGATAATATACTAGTAACGTAAATACTAGTTAGTAGATGATAGTTGATTTTTATTCCAACACTAAG
5 AAATAATTTGCGCCATTTCTTGAATGTATTTAAAGATATTTAATGCTATAATAGACATTTAAATCCAATTCTTCCA
ACATACAATGGGAGTTTGGCCGAGTGGTTTAAGGCGTCAGATTTAGGTGGATTAAACCTCTAAAATCTCTGATAT
CTTCGGATGCAAGGGTTCGAATCCCTTAGCTCTCATTATTTTTGCTTTTTCTCTTGAGGTCACATGATCGCAA
ATGGCAAATGGCACGTGAAGCTGTCGATATTGGGGAAGTGTGGTGGTTGGCAAATGACTAATTAAGTTAGTCAAG
GCGCCATCCTCATGAAAAGTGTGTAACATAATAACCGAAGTGTGAAAAGGTGGCACCTTGTTCCAATTGAACACG
10 CTCGATGAAAAAATAAGATATATATAAGGTTAAGTAAAGCGTCTGTTAGAAAGGAAGTTTTCTTTTTCTTGC
TCTCTTGTCTTTTCATCTACTATTTCTTCGTGTAATACAGGGTCGTGAGATACATAGATACAATTCTATTACCC
CCATCCATACA

SEQ ID NO: 84 is the nucleic acid sequence of promotor pSAM1

15 GAAACGGACGTAAGACGGAAATAGAATTTGAAGATAAAGTTATATATCACTACACACGAATACTTTCTTTTTTTT
TTTTACAGGAAAAGTGTGGTGGCGCCCTTGCTACTAGTGCATTCTTTTTTCGGGTTCTTGCTCGAaGAAAT
TTTAGCCTCATCGTAGTTTTCTACTCTGGTATCGATGAAAAGGGAAGAGTAAAAAGTTTTCCGTTTAGTACTTA
ATGGGATTGGTTTGGGACGTATATATCGACTGGTGTGTCTGTTATTCATCGTTGTTTTTCGGTTAGCTTCGAAA
AAAAATAGAGTAAAAACCAGGAATTTACCCTAAAAACAAGAAAAAATAAGATAAACGAAAAAT

20

SEQ ID NO: 85 is the nucleic acid sequence of terminator tTPI1

GATTAATATAATTATATAAAAAATATTATCTTCTTTCTTTATATCTAGTGTTATGTAAATAAATTGATGACTAC
GGAAAGCTTTTTATATTGTTTCTTTTCATTCTGAGCCACTTAAATTTCTGTAATGTTCTTGTAAGGGACGGTA
GATTTACAAGTGATACAACAAAAAGCAAGGCGCTTTTCTAATAAAAAAGAAGAAAAGCATTAAACAATTGAACAC
25 CTCTATATCAACGAAGAATATTACTTTGTCTCTAAATCCTTGTAATGTGTACGATCTCTATATGGGTTACTCA

SEQ ID NO: 86 is the nucleic acid sequence of terminator tMET25

GTGTGCGTAATGAGTTGTAAAATTATGTATAAACCTACTTTCTCTCACAAGTACTATACTTTTATAAAACGAACT
TTATTGAAATGAATATCCTTTTTTCCCTTGTTACATGTCGTGACTCGTACTTTGAACCTAAATTGTTCTAACAT
30 CAAAGAACAGTGTTAATTCGCAGTCGAGAAGAAAAATATGGTGAACAAGACTCATCTACTTCATGAGACTACTTT
ACGCCTCTATAAAGCTGTCACACTGGATAAATTTATTGTAGGACCAAGTTACAAAAGAGGATGATGGAGGTTT

SEQ ID NO: 87 is the nucleic acid sequence of terminator tDIT1

TAAAGTAAGAGCGCTACATTGGTCTACCTTTTTGTTCTTTTACTTAAACATTAGTTAGTTCGTTTTCTTTTTCTC
35 ATTTTTTTATGTTTCCCCCCTAAGTTCTGATTTTATAATTTTTATTTACACAATTCCATTTAACAGAGGGGG
AATAGATTCTTTAGCTTAGAAAATTAGTGATCAATATATATTTGCCTTTCTTTTCATCTTTTCAGTGATATTAAT
GGTTTCGAGACACTGCAATGGCCCTAGTTGTCTAAGAGGATAGATGTTACTGTCAAAGATGATATTTGAATTC

SEQ ID NO: 88 is the nucleic acid sequence of terminator tRPL3

GAAGTTTTGTTAGAAAATAAATCATTTTTTAATTGAGCATTCTTATTCCTATTTTATTTAAATAGTTTTATGTAT
TGTTAGCTACATACAACAGTTTAAATCAAATTTTCTTTTCCCAAGTCCAAAATGGAGGTTTATTTTGATGACCC
5 GCATGCGATTATGTTTTGAAAGTATAAGACTACATACATGTACATATATTTAAACATGTAAACCCGTCCATTATA
TTGCTTACTTTCTTCTTTTGGCGTTTGGACTTGGACCTCTGGTTTGCTATTTCTTACAATCTTTGCTACAAT

SEQ ID NO: 89 is the nucleic acid sequence of terminator tRPL3.sm

GAAGTTTTTAAAGCATTTTTTAGACACTTCTCATTTTTCTAAGTTTTTTTAAATAGTTTTATGTATTTACTACG
10 TATCACAATTTGAAATAATTCATCTTCCCAAAAACTAAGATTTTATCCTTGTCACGATCCGTAACCAGTTTAT
AATATTTTAGAGCTTATACACGTACGTATACACACGTGTCGGTACATGAGAATTACGTTCAAAATTATTCACTTT
TTTTTCTCTGCCGTTTACTTTTGAACCTCTGTCTCGCTATTTCTTACAATCTTCGCTACAATACCACTTGCCC
TTGG

15 **SEQ ID NO: 90** is the nucleic acid sequence of terminator tRPL3.sba

GAAGTTTTCTGACAAAAACATAACGTTTTTCCAATCATTTCTTATTTTTCCGTTTATTTAAATAGTTTTATG
TACTATTATACGTATGACTATTTAACTTAAATTCCTCCTCCCAAGAAATCTCCCAAGTTTTTCATTATCATGGCA
TACACCACTATCAGTTACAAAATGGTAGCTCAACCATATATATCTCTATATACACATATAAATGCAACACGGT
CCAAGTCACCGCTCACTGCAGTTTCTTTTGCCGTTTGGACTTCGATCTCTGCTTGGCTATTTTCTCACAATCCT

20

SEQ ID NO: 91 is the nucleic acid sequence of terminator tRPL41B

GCGGATTGAGAGCAAATCGTTAAGTTCAGGTCAAGTAAAAATTGATTTGAAAACTAATTTCTCTTATACAATCC
TTTGATTGGACCGTCATCCTTTTGAATATAAGATTTTGTTAAGAATATTTTAGACAGAGATCTACTTTATATTTA
ATATCTAGATATTACATAATTTCTCTCTAATAAAATATCATTAAATAAAATAAAATGAAGCGATTTGATTTTGT
25 GTTGTCACCTTAGTTTGGCGCTATGCCCTTTGGGTAATGCTATTATTGAATCGAAGGGCTTTATTATATTACCTT

SEQ ID NO: 92 is the nucleic acid sequence of terminator tRPL15A

GCTGGTTGATGGAAAATATAATTTTATTGGGCAAACTTTGTATTATCTGATGTGTTTTATACTATTATCTTTTAA
ATTAATGATTCTATATACAAACCTGTATATTTTTCTTTAACCAATTTTTTTTTTTATAGACCTAGAGCTGTACT
30 TTTATTCTGCTATCAAGCAAACCCCTACCCCTCTTCTCAATCCTCCCCTCAGGCAGAACTTATCTACCTGTATC
AAGGAGCGGACGAGGGAGTCCTAATTGTTCTACGTATACCAATGCTAGCAGCTTACATAGGTGGTGCACTACCA

SEQ ID NO: 93 is the nucleic acid sequence of terminator tRPL15A.sba

GCTGGCTGATGAAAATATAGTTCTGTTGGGCAAGCATTTGTTTACCTAGCATCTCTTTTATACTATTATTATCT
35 TTATATTTGATGATTTTATATACAAGTTGTATACCTTTTCTTTAACCAATTTTTTTTTTTCTAATGGTGACCTA
GAAGTACATTTTTTCTCACCAATAGATAGTCAAGATACTCCAGCCTCTATGGCGTTACCAGGAGCCGACAAGG
GAAAGCTGCTTATCTTACATATGCAGATGCCAAGGCCCGTTACAGGCCGCTTATTGATGTTTAGAAATAGCTTC

SEQ ID NO: 94 is the nucleic acid sequence of terminator tIDP1

TCGAATTTACGTAGCCCAATCTACCACCTTTTTTTTTTTCATTTTTTAAAGTGTATACCTTAGTTATGCTCTAGGAT
AATGAACACTTTTTTTTTTTTTTTTTTACTGTTATCATAAATATATATACCTTATTGTTGTTTGCAACCGTCGG
5 TTAATTCCTTATCAAGGTTCCTCAAGTTCGGATCATTACCATCAATTTCCAACATTTTCATGAGTTCTTCTTCTT
CATTACCGTGTTTTAGGGGGCTGTTTCGCACTTCTAATAGGGCTATCACCAAGCTGTTCTAATTCGTCCAAAAGTT

SEQ ID NO: 95 is the nucleic acid sequence of terminator tTEF1.sba

GGAGATTGATAGGACTTTTCTAGTTGCATATCTTTATTTTTTAAATCTTATCTATTAGTTAATTTTTTGTAATTT
10 ATCCTTATATATATAGTTTGGTTATTCTAAAACATCATTTTCAGTATCTAAAACCTCTCTTATTCATTACCTTTTT
ATTTAATGGTTTTTGCTACAGGCAAAAATTTAATGGTTTTTGCTACAGGCAAAAATCCGCCGTGGACTTATTCC
ACGTTAACTCGGTTACAGGGTCATGAACCATTTTGTCAATTATCGAAATAACTTCTTCAAAAGTCCCTCTTACTT

SEQ ID NO: 96 is a nucleic acid sequence of Glutamine synthetase (GLN1) originating from

15 *Saccharomyces cerevisiae*

ATGGCTGAAGCAAGCATCGAAAAGACTCAAATTTTACAAAAATATCTAGAACTGGACCAAAGAGGTAGAATAATT
GCCGAATACGTTTGGATCGATGGTACTGGTAACTTACGTTCCAAAGGTAGAACTTTGAAGAAGAGAATCACATCC
ATTGACCAATTGCCAGAAATGGAACCTCGACGGTTCTTCTACCAACCAAGCGCCAGGCCACGACTCTGACATCTAT
TTGAAACCCGTTGCTTACTACCCAGATCCCTTCAGGAGAGGTGACAACATTGTTGTCTTGGCCGCATGTTACAAC
20 AATGACGGTACTCCAAACAAGTTCAACCACAGACACGAAGCTGCCAAGCTATTTGCTGCTCATAAGGATGAAGAA
ATCTGGTTTTGGTCTAGAACAAGAATACACTCTATTTGACATGTATGACGATGTTTACGGATGGCCAAAGGGTGGG
TACCCAGCTCCACAAGGTCTTACTACTGTGGTGTGGTGCCGGTAAGGTTTTATGCCAGAGACATGATCGAAGCT
CACTACAGAGCTTGTGTTGTATGCCGGATTAGAAATTTCTGGTATTAACGCTGAAGTCATGCCATCTCAATGGGAA
TTCCAAGTCCGGTCCATGTACCGGTATTGACATGGGTGACCAATTATGGATGGCCAGATACTTTTTGCACAGAGTG
25 GCAGAAGAGTTTGGTATCAAGATCTCATTCCATCCAAAGCCATTGAAGGGTGACTGGAACGGTGCCGGTTGTCAC
ACTAACGTTTCCACCAAGGAAATGAGACAACCAGGTGGTATGAAATACATCGAACAAGCCATCGAGAAGTTATCC
AAGAGACACGCTGAACACATTAAGTTGTACGGTAGCGATAACGACATGAGATTAAGTGGTAGACATGAAACCGCT
TCCATGACTGCCTTTTCTTCTGGTGTGCGCAACAGAGGTAGCTCAATTAGAATCCCAAGATCCGTCGCCAAGGAA
GGTTACGGTTACTTTGAAGACCGTAGACCAGCTTCCAACATCGACCCATACTTGGTTACAGGTATCATGTGTGAA
30 ACTGTTTGGGTGCTATTGACAATGCTGACATGACGAAGGAATTTGAAAGAGAATCTTCATAA

SEQ ID NO: 97 is an amino acid sequence of Glutamine synthetase (GLN1) originating from

Saccharomyces cerevisiae

MAEASIEKTQILQKYLELDQGRGRIIAEYVWIDGTGNLRSKGRITLKKRITSIDQLPEWNFDGSSTNQAPGHSDIY
35 LKPVAYYPDFFRRGDNIVVLAACYNNDGTPNKFNRHREAALFAAHKDEE IWFGLEQEYTLFDMYDDVYGWPKGG
YPAPQGPYYCGVGAGKVYARDMIEAHYRACLYAGLEISGINAEVMP SQWEFQVGPCTGIDMGDQLWMARYFLHRV
AEEFGIKISFHPKPLKGDWNGAGCHTNVSTKEMRQPGGMKYIEQAIEKLSKRHAHEHIKLYGSDNDMRLTGRHETA
SMTAFSSGVANRGSSIRIPRSVAKEGYGYFEDRRPASNIDPYLVTGIMCETVCGAIDNADMTKEFERESS

SEQ ID NO: 98 is a nucleic acid sequence of Glutamate synthase (GLT1) originating from *Saccharomyces cerevisiae*

ATGCCAGTGTGAAATCAGACAATTCGATCCATTGGAAGAAGCTTACGAAGGTGGGACAATTCAAACTATAAC
5 GATGAACACCATCTTCATAAATCTTGGGCAAATGTGATTCCGGACAAACGAGGACTTTACGACCCTGATTATGAA
CATGACGCTTGTGGTGTCCGGTTTCGTAGCAAATAAGCATGGTGAACAGTCTCACAAGATTGTTACTGACGCTAGA
TATCTTTTAGTGAATATGACACATCGTGGTGCCGTCTCATCTGATGGGAACGGTGACGGTGCCGGTATTCTGCTA
GGTATTCCCTCACGAATTTATGAAAAGAGAATTCAAGTTAGATCTTGATCTAGACATACCTGAGATGGGCAAATAC
GCCGTAGGTAACGTCTTCTTCAAGAAGAACGAAAAAATAACAAGAAAAATTTAATTAAGTGTGAGAAGATTTTC
10 GAGGATTTAGCTGCATCCTTCAACTTATCCGTATTAGGTTGGAGAAACGTCCCCGTAGATTCTACTATTTTAGGA
GACGTTGCATTATCTCGTGAACCTACTATTCTACAGCCATTATTGGTTCCATTGTATGATGAAAAACAACCGGAG
TTTAATGAAACTAAATTTAGAACTCAATTGTATCTTTTAAGGAAGGAGGCCTCTCTTCAAATAGGACTGGAAAAAC
TGGTTCTATGTTTGTTCCTAAACAATACCACCATTTGTTTACAAGGGTCAATTGACGCCAGCTCAAGTGATAAC
TACTATCCCGACTTGACTAATGCGCATTTCAAATCCACATGGCGTTGGTCCATTCAAGATTTTCCACTAATACT
15 TTCCCTCTTGGGATAGAGCTCAACCTTTACGTTGGCTAGCTCATAATGGTGAAATTAACACCTTAAGAGGTAAC
AAGAATTGGATGCGCTCCAGAGAAGGTGTGATGAATTCAGCAACTTTCAAAGATGAGTTAGACAACTATACCCA
ATTATCGAAGAAGGTGGTTCTGATTACGCTGCATTGGATAACGTTTTAGAACTATTGACTATTAATGGCACATTA
TCTCTACCTGAAGCTGTTATGATGATGGTTCTGAAGCGTATCATAAGGATATGGATTCTGACCTAAAAGCATGG
TACGACTGGGCTGCATGTCTGATGGAACCTTGGGATGGTCCAGCTTTGTTAACTTTCACTGATGGACGTTACTGT
20 GGTGCTATATTGGATAGAAATGGTTTAAAGACCTTGTCTGTTATTACATCACTAGTGATGACAGAGTTATCTGTGCT
TCAGAGGTAGGTGTCAATTCCTATCGAAAATTCATTGGTTGTTCAAAAAGGTAACTGAAGCCAGGTGATTATTTC
CTAGTGGATACTCAATTGGGTGAAATGGTCGATACTAAAAAGTTAAATCTCAAATCTCAAAAAGACAAGATTTT
AAGTCTTGTTATCCAAAGTCATCAAGTTAGACGACTTGTTATCAAAAACCGCTAATTTGGTTCTCAAAGAATTT
ATATCACAGGATTCATTGTCTTTGAAAGTTCAAAGTGACCCACGCTCTATTGGCCAATGGTTATACCTTCGAACAA
25 GTCACATTTCTGTTAACTCCAATGGCTTTAACAGGTAAAGAAGCTTTAGGTTTCGATGGGTAACGATGCGCCACTG
GCTTGTTTAAATGAAAATCCTGTCTTACTTTATGATTATTTTACAGACAATTGTTTGCTCAAGTGACCAATCCTCCA
ATTGACCCAATTCGTGAAGCAAATGTTATGTCTGTTAGAATGTTATGTGCGACCTCAAGGCAACCTTTTGAAATG
CATTCACTCAATGTGATCGTTTATTATTGAAATCTCCTATTTTGCAATTGGAATGAGTTCCAAGCTTTGAAAAAC
ATTGAAGCTGCTTACCCATCATGGTCTGTAGCAGAAATTGATATCACATTTCGACAAGAGTGAGGGTCTATTGGGC
30 TATACCGACACAATTGATAAAATCACTAAGTTAGCGAGCGAAGCAATTGATGATGGTAAAAAGATCTTAATAATT
ACTGACAGGAAAAATGGGTGCCAACC GTTTCCATCTCCTCTTTGATTGCAATTTTCATGTATTTCATCATCACCTA
ATCAGAAACAAGCAGCGTTCCCAAGTTGCTTTGATTTTGAAACAGGTGAAGCCAGAGAAATTCACCATTTCTGT
GTCCTACTAGGTTATGGTTGTGATGGTGTATCCATACTTAGCCATGGAACTTTGGTCAGAATGAATAGAGAA
GGTCTACTTCGTAATGTCAACAATGACAATGATACTTGAGGAAGGGCAAATACTAGAAAATTACAAGCACGCT
35 ATTGATGCAGGTATCTTGAAGGTTATGTCTAAAATGGGTATCTCCACTCTAGCATCCTACAAAGGTGCTCAAATT
TTTGAAGCCCTAGGTTTAGATAACTCTATTGTTGATTGTGTTTACAGGTACTTCTTCCAGAATTAGAGGTGTA
ACTTTTCGAGTATTTGGCTCAAGATGCCTTTTCTTTACATGAGCGTGGTTATCCATCCAGACAAACCATTAGTAAA
TCTGTAACTTACCAGAAAGTGGTGAATACCCTTTAGGGATGGTGGTTACAAACACGTCAACGAACCAACCGCA
ATTGCTTCGTTACAAGATACTGTCAGAAACAAAAATGATGTCTCTTGGAATTATATGTAAAGAAGGAAATGGAA

GCAATTAGAGACTGTACACTAAGAGGACTGTTAGAATTAGATTTTGAAAATTCTGTCAGTATCCCTCTAGAACAA
GTTGAACCATGGACTGAAATTGCCAGAAGATTTGCGTCAGGTGCAATGTCTTATGGTTCTATTCTATGGAAGCT
CACTCTACATTGGCTATTGCCATGAATCGTTTAGGGGCCAAATCCAATTGTGGTGAAGGTGGTGAAGACGCAGAA
CGTTCTGCTGTTCAAGAAAACGGTGATACTATGAGATCTGCTATCAAACAAGTTGCTTCCGCTAGATTTCGGTGTA
5 ACTTCATACTACTTGTGAGATGCTGATGAAATCCAAATTAAGATTGCTCAGGGTGCTAAGCCGGGTGAAGGTGGT
GAACTACCAGCCCACAAAGTGTCTAAGGATATCGCAAAAACCAGGCACTCCACCCTAATGTTGGGTTAATCTCT
CCTCCTCCTCATCACGATATTTATTCCATTGAAGATTTGAAACAACCTGATTTATGATTTGAAATGTGCTAATCCA
AGAGCGGGAATTTCTGTAAAGTTGGTTTCCGAAGTTGGTGTGGTATTGTTGCCCTCTGGTGTAGCTAAGGCTAAA
GCCGATCATATCTTAGTTTCTGGTCATGATGGTGGTACAGGTGCTGCAAGATGGACGAGTGTCAAATATGCGGGT
10 TTGCCATGGGAATTAGGTCTAGCTGAAACTCACCAGACTTTAGTCTTGAATGATTTAAGACGTAATGTTGTGTGTC
CAAACCGATGGTCAATTGAGAACTGGGTTTGATATTGCTGTTGCAGTTTTATTAGGGGCAGAATCTTTTACCTTG
GCAACAGTTCATTAAATGCTATGGGTTGTGTTATGTTAAGAAGATGTCACCTTGAACCTCTTGTGCTGTTGGTATT
GCCACACAAGATCCATATTTGAGAAGTAAGTTTAAAGGGTCAGCCCGAACATGTTATCAACTTCTTCTATTACTTG
ATCCAAGATTTAAGACAAATCATGGCCAAGTTAGGATTCCGTACCATTGACGAAATGGTGGGTCATTCTGAAAAA
15 TTAAAGAAAAGGGACGACGTAAATGCCAAAGCCATAAATATCGATTTATCTCCTATTTTGACCCAGCACATGTT
ATTCTGCCAGGTGTTCCAACCAAGTTCACAAAGAACACAACTCCACACCCGTCTAGATAATAAGTTA
ATCGATGAGGCTGAAGTTACTTTGGATCGTGGCTTACCAGTGAATATTGACGCCCTCTATAATCAATACTGATCGT
GCACTCGGTTCTACTTTTCTTACAGAGTCTCGAAGAAATTTGGTGAAGATGGTTTGCCAAAGGACACCGTTGTC
GTTAACATAGAAGGTTTCCGCGGTCAATCTTTTGGTGCTTTCCCTAGCTTCTGGTATCACTTTTATCTTGAATGGT
20 GATGCTAATGATTATGTTGGTAAAGGTTTATCCGGTGGTATTATTGTCATTAAACCACCAAAGGATTCTAAATTC
AAGAGTGATGAAAATGTAATTGTTGGTAACACTTGTCTTATGGTGCTACTTCTGGTACTGCATTCATTTACAGT
AGTGCCGGTGAGCGTTTTCGGTGTGAGAACTCTGGTGCCACCATCGTTGTTGAGAGAATTAAGGGTAACAATGCC
TTTGAGTATATGACTGGTGGTCTGCCATTGTCTTATCACAAATGGAATCCCTAAACGCCTTCTCTGGTGCTACT
GGTGGTATTGCATACTGTTTAACTTCCGATTACGACGATTTTGTGGAAAGATTAAACAAAGATACTGTTGAGTTA
25 GAATCATTATGTGACCCGGTCGAGATTGCGTTTGTAAAGAATTTGATCCAGGAGCATTGGAACACACACAATCT
GATCTAGCAGCCAGGATTCTCGGTAATTTCAACCATTATTTGAAAGATTTGTTAAAGTCATTCCAACCTGATTAT
AAGAAAGTTTTGTTGAAGGAGAAAGCAGAAGCTGCCAAGGCAAAGGCTAAGGCAACTTCAGAATACTTAAAGAAG
TTTAGATCGAACCAAGAAGTTGATGACGAAGTCAATACTCTATTGATTGCTAATCAAAAAGCTAAAGAGCAAGAA
AAAAAGAAGAGTATTACTATTTCAAATAAGGCCACTTTGAAGGAGCCTAAGGTTGTTGATTAGAAGATGCAGTT
30 CCAGATTCCAAACAGCTAGAGAAGAATAGCGAAAGGATTGAAAAAACACGTGGTTTTATGATCCACAAACGTCGT
CATGAGACACACAGAGATCCAAGAACCAGAGTTAATGACTGGAAAGAATTTACTAACCCTATTACCAAGAAGGAT
GCCAAATATCAAACCTGCGAGATGTATGGATTGTGGTACACCATTCTGTTTATCTGATACCGGTTGTCCCCTATCT
AACATTATCCCAAGTTTAAATGAATTGTTATTCAAGAACCAATGGAAGTTGGCACTGGACAAATTGCTAGAGACA
AACAATTTCCAGAAATCACTGGAAGAGTATGTCCAGCACCTGTGAGGGAGCTGTACACTAGGTATTATTGAA
35 GACCCAGTCGGCATAAAATCGGTTGAAAGAATTATCATTGACAATGCTTTCAAGGAAGGATGGATTAAGCCTTGT
CCACCAAGTACACGCACTGGCTTTACAGTGGGTGTCATTGGTTCTGGTCCAGCAGGTTTAGCGTGTGCTGATATG
TTGAACCGTGCCGGACATACGGTCACTGTTTATGAAAGATCCGACCGTTGTGGTGGGTTATTGATGTATGGTATT
CCAAACATGAAGTTGGATAAGGCTATAGTGCAACGTCGTATTGATCTATTGAGTGCCGAAGGTATTGACTTTGTT
ACCAACACCGAAATTGGTAAAACCATAAGCATGGATGAGCTAAAGAACAAGCACAATGCAGTAGTGTATGCTATC
40 GGTTCTACCATTCCACGTGACTTACCTATTAAGGGTCGTGAATTGAAGAATATTGATTTTGCCATGCAGTTGTTG

GAATCTAACACAAAAGCTTTATTGAACAAAGATCTGGAAATCATTCTGTGAAAAGATCCAAGGTAAGAAAGTAATT
 GTTGTTCGGTGGTGGTGACACAGGTAACGATTGTTTAGGTACATCTGTAAGACACGGTGCAGCATCAGTTTTGAAT
 TTCGAATTGTTGCCTGAGCCACCAGTGGAACTGCCAAAGACAATCCATGGCCTCAATGGCCGCGTGTATGAGA
 GTGGACTACGGTCATGCTGAAGTGAAGAGCATTATGGTAGAGACCCTCGTGAATACTGCATCTTGTCCAAGGAA
 5 TTTATCGGTAACGATGAGGGTGAAGTCACTGCCATCAGAACTGTGCGCGTAGAATGGAAGAAGTCACAAAGTGGC
 GTATGGCAAATGGTAGAAATTCCTAACAGTGAAGAGATCTTTGAAGCCGATATCATTTTGTGTCTATGGGTTTC
 GTGGGTCTGAATTGATCAATGGCAACGATAACGAAGTTAAGAAGACAAGACGTGGTACGATTGCCACACTCGAC
 GACTCCTCATACTCTATTGATGGAGGAAAGACTTTTGCATGTGGTGACTGTAGAAGAGGGCAATCTTTGATTGTCT
 TGGGCCATCCAAGAAGGTAGAAAATGTGCTGCCTCTGTGATAAGTTCTAATGGACGGCACTACGTATCTACCA
 10 AGTAATGGTGGTATCGTTCAACGTGATTACAACTATTGAAAGAATTAGCTAGTCAAGTCTAA

SEQ ID NO: 99 is an amino acid sequence of Glutamate synthase (GLT1) originating from *Saccharomyces cerevisiae*

MPVLKSDNFDPLEEAYEGGTIQNYNDEHHLHKSANVIPDKRGLYDPDYEHDACGVGFVANKHGEQSHKIVTDAR
 15 YLLVNMTHRGAVSSDGNNGDAGILLGIPHEFMKREFKLDLDDIPEMGKYAVGNVFFKKNEKNKKNLIKQKIF
 EDLAASFNLVGLWRNPVDSTILGDVALSREPTILQPLLPLYDEKQPEFNETKFRTOQLYLLRKEASLQIGLEN
 WFYVCSLNNNTTIVYKQLTPAQVYNYYPDLTNAHFKSHMALVHSRFSNTNTPSWDRAQPLRWLAHNGEINTLRGN
 KNWMRSREGVMNSATFKDELDKLYPIIEEGGSDSAAALDNVLELLTINGTSLPEAVMMMVPEAYHKDMDSDLKAW
 YDWAACLMEPWDGPALLTFTDGRYCGAILDRNGLRPCRYYITSDDRVICASEVGVPIENSLVVQKGLKPGDLF
 20 LVDTQLGEMVDTKKLSQISKRQDFKSWLSKVIKLDLLSKTANLVPKEFISQDSLKLKQSDPRLLANGYTFEQ
 VTFLLTPLMALTGKEALGSMGNDAPLACLNNENPVLLYDYFRQLFAQVTNPPIDPIREANVMSLECYVGPQGNLLEM
 HSSQCDRLLLKSPILHWNFQALKNIEAAYPSWSVAEIDITFDKSEGLLGYTDITDKITKLASEAIDDGKKILII
 TDRKMGANRVSISSLIAISCIHHHLIRNKQRSQVALIILETGEAREIHHFCVLLGYGCDGVYPYLA METLVRMNR
 GLLRNVNNDNTLEEGQILENYKHAI DAGILKVM SKMGISTLAS YKGAQIFEALGLD NSIVDLCTGTSSRIRGV
 25 TFEYLAQDAFSLHERGYPSRQTISKSVNLPESGEYHFRDGGYKHVNEPTAIASLQD TVRNKNDVSWQLYVKKEME
 AIRDCTLRGLLELDFENSVSIPLEQVEPWTEIARRFASGAMSYSISMEAHSTLAIAMNRLGAKSNCEGEGGDAE
 RSAVQENGDTMRSAIKQVASARFGVTSYYLSDADEIQIKIAQGA KPGE GELPAHKVSKDIAKTRHSTPNVGLIS
 PPPHHDIIYSIEDLKQLIYDLKCANPRAGISVKLVSEVGVIVASGVAKAKADHILVSGHDGGTGAARWTSVKYAG
 LPWELGLAETHQTLVLNDLRRNVVQTDGQLRTGFDIAVAVLLGAESFTLATVPLIAMGCVMLRRCHLNSCAVGI
 30 ATQDPYLRSKFKGQPEHVINFYYLIQDLRQIMAKLGFRTIDEMVGHSEKLKKRDDVNAKAINIDLSPILTPAHV
 IRPGVPTKFTKKQDHLHTRLNKLIDEAEVTLDRGLPVNIDASIINTDRALGSTLSYRVSKKFGEDGLPKD TVV
 VNIEGSAGQSFGAFLASGITFILNGDANDYVGKGLSGGIIVIKPPKDSKFKSDENVIVGNTCFYGATSGTAFISG
 SAGERFGVRNSGATIVVERIKGNNAFEYMTGGRAIVLSQMESLNAFSGATGGIAYCLTSDYDDFVGKINKDTVEL
 ESLCDPVEIAFVKNLIQEHWN YTSQDLAARILGNFNHYLKDFVKVIPTDYKKVLLKEKAEAAKAKAKATSEYLLK
 35 FRSNQEVDDEVNTLLIANQKAKEQEKKSITISNKATLKEPKVVDLEDAVPDSKQLEKN SERIEKTRGFMHKRR
 HETHRDPRTVRNDWKEFTNPITKKDAKYQTARCMDCGTPFCLSDTGCPLSNII PKFNELLFKNQWKLALDKLLET
 NNFPEFTGRVCPAPCEGACTLGIIEDPVGIKSVERIIIDNAFKEGWIKPCPPSTRGTGTVGVIGSGPAGLACADM
 LNRAGHTVTYERSDRCGLLMYGIPNMKLDKAI VQRRIDL LLSAEGIDFVTNTEIGKTI SMDELKNKHNAVYIAI
 GSTIPRDLPIKGRELKNIDFAMQLLESNTKALLNKDLEIIREKIQGKKVIVVGGGDTGNDCLGTSVRHGAASVLN

FELLPEPPVERAKDNPWPQWPRVMRDYGHAEVKEHYGRDPREYCILSKEFIGNDEGEVTAIRTVRVIEWKKSQSG
VWQMVEIPNSEEIFEADIILLSMGFVGPILINGNDNEVKKTRRGTIATLDDSSYSIDGGKTFACGDCRRGQSLIV
WAIQEGRKCAASVDKFLMDGTTYLPSNGGIVQRDYKLLKELASQV

- 5 **SEQ ID NO: 100** is the nucleic acid sequence of terminator tTDH3

GTGAATTTACTTTAAATCTGCATTTAAATAAATTTCTTTTTATAGCTTTATGACTTAGTTTCAATTTATATAC
TATTTTAATGACATTTTCGATTCATTGATTGAAAGCTTTGTGTTTTCTTGATGCGCTATTGCATTGTTCTTGT
CTTTTCGCCACATGTAATATCTGTAGTAGATACCTGATACATTGTGGATGCTGAGTGAAATTTAGTTAATAAT
GGAGGCGCTCTTAATAATTTGGGGATATTGGCTTTTTTTTTTAAAGTTTACAAATGAATTTTTCCGCCAGGAT

10

- SEQ ID NO: 101** is a reencoded nucleic acid sequence of hyaluronan synthase (HASA)
originating from *Chlorella virus CviK1*

ATGCTTTTCAGTTGTTTTCCTTCGTGATCTGCCATCTTAGTTTTATTTGCGTTATTACAAGAGGCTTCTAAAA
GTGACGATGGGAAAGAACATTATAATAATGGTGAGTTGGTACACAATAATAACTTCCAACATCATCGCCGTCGGT
15 GGGGCGTCTCTGATTTTGGCCCCAGCCATCACTGGATACATCTTGCACTGGAACATCGCCTTGTCACCATCTGG
GGAGTTTCCGCTTATGGGATATTTCGTGTTTCGGATTCTTCTGGCTCAGGTTTTATTTAGTGAGCTAAATCGTAAA
AGACTAAGAAAGTGATTCTCTAAGACCAAAGGGTTGGAACGACGTACGTCTGGCTGTCATCATAGCTGGTTAC
AGGGAAGACCCGTACATGTTTCAGAAATGCCTTGAGTCAGTAAGGGACTCTGACTATGGTAATGTCGCTCGTCTT
ATCTGTGTAATTGACGGAGATGAGGACGACGACATGAAGATGGCAGCCGTTTATAAGGCGATATATAATGACAA
20 ATCAAGAAGCCAGAGTTTGTGTTATGTGAGAGCGATGATAAGGAAGGCGAGAGAATTGACTCAGATTTTAGCAGG
GACATCTGCGTCTTACAACCACACCGTGGCAAAGGGAGTGCTTGTATACCGGCTTTCAGCTGGCCAAAATGGAC
CCGTCGGTAAACGCCGTTGTTCTTATTGACTCTGACACGGTCTTGAAAAGGACGCAATCTTGGAAGTCGTGTAT
CCATTGGCCTGTGACCCGGAAATTCAGCTGTGCGAGGAGAGTGCAAAATATGGAACACAGATACACTTTTGTCTG
TTGTTGGTGGCTTGGAGATATTATTCAGCCTTTTTCGTGGAGAGATCCGCCCAATCATTCTTTAGGACAGTCCAG
25 TGTGTAGGTGGACCCTTGGGCGCTACAAGATAGATATCATCAAAGAGATCAAAGATCCCTGGATCTCCCAACGT
TTCTTAGGTGAGAAGTGACCTACCGTGACGACCGTAGGTTAACAAATGAAATCTTAATGAGGGGTAAGAAGGTC
GTCTTCACTCCTTTTCGAGTTGGCTGGAGTGACTCACCAACCAACGTGTTTTCAGGTACATCGTACAACAGACTAGG
TGGAGTAAATCATGGTGCCGTGAGATATGGTACACTTTGTTTGTCTGCGTGGAAGCACGGACTAAGCGGAATTTGG
TTGGCATTGAGTGCCTTTATCAGATTACGTACTTCTTCTAGTCATATATTTATTTTCGAGACTAGCCGTTGAA
30 GCTGACCCAAGAGCACAGACCGCAACAGTAATCGTGCTTACCACGGTAGCCCTGATTAAGTGTGGTTACTTCTCT
TTTCGTGCGAAAGATATTCGTGCTTTCTACTTCGTCCTGTACACATTTGTCTACTTCTTCTGTATGATCCCAGCA
AGGGTGACAGCCATGATGACACTTTGGGATATCGGTGGGGCACGCGTGCGGAAACGAGAAACCAAGTGTGCGG
ACTAGGGTCGCTCTGTGGCTAAACAGTATTTGATAGCATACATGTGGTGGGCAGCAGTTGTTGGCGCTGGGGTA
TACTCCATTGTTTATAATTGGATGTTTGACTGGAACCTCACTATCCTATAGGTTTCGCACTTGTTAGGTATATGCTCT
35 TACATCGTATTCATCACAATCGTTCTAGTCATCTATTTACAGGAAAGATTACCACATGGAATTTACCAAATTA
CAGAAGGAGTTAATCAAGGATAGGGTCTTATACGATGCTTCCACTAACGCCCAGACTGTATAA

- SEQ ID NO: 102** is a reencoded nucleic acid sequence of hyaluronan synthase (HASA)
originating from *Chlorella virus IL-5-2s1*

ATGGCTGCTTCATTCTATGTTATCTTCTTGTTCGTAATATGCCACCTGTCCTTCGTCTTATATCGTCAAGTTCTA
TACATAATTTTGGGTAGATACTGGAGTGTTAACAAGCTGACAATGATCTCTTGGTATACCATTATATCATCTAAT
CTAATCGCAATCGGTGGGGCTTCCCTTATTTTGGCACCGGCCATAACCGGTACATTTTGCATTGGAACATAGCT
CTAAGCACAAATTTGGGGTGTGTTCAGCCTATGGCATTTCGTGTTTCGGGTTCTTTCTTGCTCAGGTTTTGTTTCAGT
5 GAGCTTAACAGGAAGAGGTTGAGAAAATGGATCTCTTTGAGACCAGATAACTGGAACGCGGTGAGAGTTGCCGTG
ATAATCGCTGGTTACCGTGAAGACCCATACATGTTTCAAAAGTGTTTGAATCGGTGAGAGACTCCGATTATGGT
AACATTGCGAGGCTTATATGCGTTATAGATGGGGACGAAGATGACGACATGAAGATGGCAGATGTATACAAGGCT
ATTTACAATGACAATATAAAGAAGCCGAAATTATCCTATGTGAGTCCGACGACAAAGAGGGAGAAAGGATAGAC
AGTGATTTTAGCCGTGATATTTGCGTGCTTCAGCCTCACAGAGGTAAGCGTGAATGTTTATATACTGGCTTTCAG
10 TTAGCTAAAATGGACCCCTCTGTACACGCGGTTGTGCTTATTGACTCGGATACAGTTTTGGAGAAGGATGCCATC
CTGGAAGTGGTCTACCCACTTGCTTGCGACCATGAGATTCAAGCAGTCGCAGGTGAATGCAAGATATGGAATACG
GACACCTTATTATCCATATTAGTCGCCTGGCGTTACTACTCAGCTTTCGTGCGTTGAGCGTAGCGCTCAGTCCCTC
TTCCGTACTGTGCAATGCGTCGGCGGTCCGTTAGGGGCATACAAAATAGACATAATTAAAGAGATAAAGGAGCCG
TGGATTTACAAAGATTTCTGGGTCAGAAGTGACGTACGAGATGACAGAAGATTAACTAACGAAGTGTTGATG
15 CGTGGAAGAAAGTAGTCTTCACACCTTTTGCGGTAGGATGGAGCGACTCGCCTACTAATGTCTTCCGTTATATT
GTGCAGCAGACGAGGTGGAGTAAGAGCTGGTGCAGGGAGATTTGGTATACACTGTTTCGCGCGGTGGAAGCATGGG
TTATCAGGCATTTGGTTGGCATTGTGAGTGCTTGATCAGATCACATACTTCTTTCTAGTGATATATCTGTTTCAGT
AGACTAGCAGTCGAGGCTGACCCGAGAGCGCAAACCTGCAACGGTTATAGTCAGCACAATGGTTTCACTAATCAAG
TGTGGATATTTCTCATTCCGTGCTAAGGACATTAGAGCGTTTTATTTCTGACTATATACTTTTGTGTACTTCTTT
20 TGCATGATCCCAGCAAGAGTCACTGCTATGATGACCTTGTGGGATATTGGGTGGGGTACTAGGGGTGGAACGTTG
AAACCATCGATCGGAAGTAGGATCTCTTTGTGGGCTAAGCAGTACTTAATCGCGTACATGTGGTGGGCAGCTGTG
ATCGCCGCGAGGAGTCTATAGTATAATACATAATTGGATGTTTGATTGGAACCTTGTCTATCGTTTCGCGTTA
ATAGGCATCTGCTCGTATATCGTATTCAATTGCCATTGTTATAGTGATATACTTCACAGGTAAGATCACCCTTGG
AATTTTACCAAGCTACAGAAAGAGTTAATTGAAGATAGAGTCTTGCATGATGCCGATGTGGACATACAGAACGTA
25 TAA

SEQ ID NO: 103 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CZ-2*

ATGACATCTTGGAGAACGATTGTGTTCAGCTAACCTTTTCGCAGTTGGTGGGGCTCTTCTAATGTTAGCCCCAGCT
30 ATCGTGGGTATGTATTTTCAGTGAATATAGGCGTGTCCGAGTCTGGGGCATCTCCGTATACGGCGTCTTTGTG
TTGGGATTTTATATCGCTCAAATCGTCTTCTCTGAGTTCAACAGGATGAGATTATCCGATTGGATAAGTCTTAGA
CCAGACAACCTGGAACGCCACAAGGGTTGCGGTTCATTATAGCTGGATACAGAGAAGATCCTTTTATGTTCAAGAAG
TGCTTAGAGAGTGTGAGGGATTGGAATACGGCAACGTAGCAAGGCTTATATGCGTAATTGACGGAGACGAAGAA
GAGGACCTAAAGATGGCTGAGATTTACAAGCAGGTTTACAATGACAACGTGAAGAAACCAGGTGTAGTGTTATGC
35 GAATCGGAGAACAAGAATGGATCGACTATAGATTCGGACGTATCTAAGAATATATGCATACTGCAACCCACCGT
GGGAAACGTGAGAGCCTATATACCGGTTTCCAATTGGCAAGCATGGACCCTTCCGTACATGCGGTTGTTCTTATA
GATTCCGACACTGTATTAGAGAAGAATGCTATTCTTGAGGTCGTATACCCTCTATCTTGTGACCCCAACATTAAG
GCCGTCGCCGGGAGTGCAAGATATGGAATACAGACACCATCTTGTCCATGCTTGTCTCTTGGAGATACTTCTCT
GCCTTCAATGTCGAGCGTGGAGCCAGTCATTATGAAGACTGTGCAATGCGTAGGCGGGCCACTTGGCGCATAC

ACTATCGACATCATAAATGAAATTAAGACCCGTGGATTACCCAGACATTCTGGGCAACAAGTGCACTTATGGC
GACGATAGACGTCTTACCAATGAGGTTTAAATGAGGGGCAAGAAGATTGTATACTCCGTTTGCCGTCGGTTGG
AGCGACAGTCCGACGAATGTCATGAGGTACATTGTGCAGCAAACCTAGATGGAGTAAGTCATGGTGCAGAGAGATA
TGGTACACGTTAGGCAGTGCCTGGAAACATGGATTCTCCGGCATATACCTTGCGTTTGAGTGTATGTACCAGATA
5 ATGTACTTCTTCTTAGTCATGTACCTGTTTTTCGTATATCGCAATAAAGGCAGACATCCGTGCTCAGACTGCAACT
GTGTTGGTTTCTACTCTAGTCACCATAATAAAGAGCAGTTACCTAGCGCTGAGGGCCAAGAACCTGAAGGCTTTC
TATTTTCGTTCTGTATACCTATGTCTATTTCTTTTGCATGATACCTGCTAGGATTACCGCTATGTTTACAATGTTT
GATATAGCTTGGGGTACACGTGGTGGTAATGCGAAAATGACAATCGGGGCTAGAGTTTGGCTGTGGGCGAAGCAG
TTCTTAATAACTTATATGTGGTGGGCAGGAGTACTAGCGGCGGGAGTTTACAGTATAGTTGACAAATTGGTACTTC
10 GATTGGGCTGATATACAGTACAGGTTTGCATTGGTAGGTATTTGCTCGTATTTGGTCTTTGTCAGTATTGTACTG
GTAATCTACTTGATCGGTAAGATAACAACGTGGAACCTACACACCGCTACAGAAGGAACTTATTGAGGAGAGATAC
TTGCATAACGCCTCTGAGAATGCCCGGAAGTTTAA

SEQ ID NO: 104 is a reencoded nucleic acid sequence of hyaluronan synthase (HASA)
15 originating from *Chlorella virus CVG-1*

ATGACTTCTTGGCGTACAATTGTTTCTGCTAACTTGTTTCGCAGTTGGTGGCGCCTTGTTGATGTTAGCTCCCGCG
ATAGCCGGTTATGTATTTAAATGGAACATAGGCGTTTCTGCCGTTTGGGGTATTTTCAGTGTACGGGGTCTTCGTG
CTTGTTTTCTATATTGCACAGGTAGTCTTCAGCGAGTTTAAATAGGATGCAGCTGTCAGACTGGATCTCATTGAGA
CCCGACAACCTGGAACGCCACCAGAGTAGCGGTAATTATTGCTGGTTACAGGGAAGACCCCTTTATGTTCAAGAAG
20 TGCTTAGAATCTGTGAGAGACTCAGAATACGGGAACATAGCGAGGCTAATCTGTGTTATCGACGGCGATGAAGAG
GAAGACCTTAAGATGGCTGAGATATACAAGCAAGTATACAACGACAACGTCAAGACCCCCGGTGTGTGCTATGC
GAGAATGAAAAATAAGAATGGTAGCACCATTGACCCAGATTTTATAGTAAGAACATCTGCATTCTGCAACCACACAGG
GGCAAACGTGAATCTTTGTACACCGGATTCCAGATGGCTGGCATGGACCCATCAGTGCACGCAGTTGTACTTATA
GACAGTGATACTGTTCTTGAAAAGAACGCTATCCTAGAGGTTGTGTACCCTCTATCTTGTGATCCAAATATCAAA
25 GCTGTGCGGGGAGAGTGTAAAACTGGAACACAGATACCATTTTGTCTATGCTAGTTTCATGGCGTTATTTCTCG
GCTTTCAACGTGGAGAGAGGCGCACAGTCCCTTTGGAAAACAGTCCAGTGTGTCGGCGGGCCTTTGGGAGCATAC
ACTATAGACATCATAAACGAGATAAAAGACCCCTGGATTACTCAGACATTCTTAGGAAACAAATGTACGTATGGT
GACGACAGAAGGTTAACCAATGAAGTGCTTATGAGGGGAAAGAAGATTGTCTATACTCCCTTTGCTGTGCGTTGG
TCCGATTGCCCCACAAACGTTATGAGGTACATCGTCCAGCAGACAAGGTGGTCTAAGAGCTGGTGCCGTGAGATT
30 TGGTACACCTTGGGATCAGCATGGAACATGGTTTCTCCGGAATCTACTTAGCGTTCGAGTGCATGTACCAGATT
ATGTATTTCTTTATTGTGATGTATTTGTTTCAGTTACATTGCCATTAAAGCCAACATCAGGGCACAAGCAGCCACG
GTATTGGTCAGTACGCTTGTGCGAGTAATCAAATCTAGTTATTTGGCATTAAAGAGCGAAGAACCTAAAAGCTTTG
TATTTGTCCTGTATACCTACGTCTACTTCTTCTGCATGATTCCAGCTAGAATAACAGCGATGTTACAGATGTTT
GATATTGCATGGGGTACTAGAGGCGGTAACGCAAAGATGACGATCGGTGCTAGAGTCTGGTTGTGGGCTAAACAA
35 TTCTTAATTACATACATGTGGTGGGTGGGAGTTTGGCCGCTGGGGTCTACAGTATAGTCGATAACTGGTACTTC
GACTGGGCGGACATCCAGTACAGATTCGCGCTAGTGGGGATTGTCAGTTACTTAGGTTTCGTTTCCATAATGCTG
GTATTCTATCTGATTGGTAAATAACTACGTGGAATTACACTCCTCTACAAAAGGAGCTGATTAAAGAGAGGTTA
CACGCCGCTAATGCAACAGAGGTCTAA

SEQ ID NO: 105 is an amino acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CviKI*

MLFSCLSFVICHLSFILRYKRLLKVTMGKNIIIMVSWYTIITSNIIAVGGASLILAPAITGYILHWNIALSTIW
 5 GVSAYGIFVFGFFLAQVLFSELNRRKRLRKWISLRPKGWNVDRLAVIIAGYREDPYMFQKCLESVRDSYGNVARL
 ICVIDGDEDDDMKMAAVYKAIYNDNIKKPEFVLCESDDEKEGERIDSDFSRDICVLQPHRGKRECLYTGFQLAKMD
 PSVNAVVLIDSDTVLEKDAILEVVYPLACDPEIQAVAGECKIWNTDTLLSLLVAWRYYSAFCEVERSAQSFFRTVQ
 CVGGPLGAYKIDIKEIKDPWISQRFLGQKCTYGDDRRLTNEILMRGKKVVFPPFAVGWSDSPTNVFRIYVQQTR
 WSKSWCREIWTFLFAAWKHGLSGIWLAFECYQITYFFLVIYLFSLAVEADPRAQTATVIVSTTVALIKCGYFS
 10 FRAKDIRAFYFVLYTFVYFFCMIPARVTAMMTLWDIGWGTRGGNEKPSVGTRVALWAKQYLIAYMWWAAVVGAGV
 YSIVHNWMDWNSLSYRFALVGICSYIVFITIVLVIYFTGKITTNFTKLQKELIKDRVLYDASTNAQTV

SEQ ID NO: 106 is an amino acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus IL-5-2s1*

MAASFYVIFLFVICHLSFVLYRQVLYIILGRYWSVNKLTMISWYTISSNLIAGGASLILAPAITGYILHWNIA
 15 LSTIWGVSAYGIFVFGFFLAQVLFSELNRRKRLRKWISLRPDNWNNAVVRVAVIIAGYREDPYMFQKCLESVRDSYD
 NIARLICVIDGDEDDDMKMAVYKAIYNDNIKKPEIILCESDDKEGERIDSDFSRDICVLQPHRGKRECLYTGFQ
 LAKMDPSVHAVVLIDSDTVLEKDAILEVVYPLACDHEIQAVAGECKIWNTDTLLSILVAWRYYSAFCEVERSAQSF
 FRTVQCVGGPLGAYKIDIKEIKEPWISQRFLGQKCTYGDDRRLTNEVLMRGKKVVFPPFAVGWSDSPTNVFRIY
 20 VQQTRWSKSWCREIWTFLFAAWKHGLSGIWLAFECYQITYFFLVIYLFSLAVEADPRAQTATVIVSTMVSLIK
 CGYFSFRAKDIRAFYFVLYTFVYFFCMIPARVTAMMTLWDIGWGTRGGNVKPSIGTRISLWAKQYLIAYMWWAAV
 IAAGVYSIIHNWMDWNSLSYRFALIGICSYIVFIAIVIVYFTGKITTNFTKLQKELIEDRVLHDADVDIQNV

SEQ ID NO: 107 is an amino acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CZ-2*

MTSWRTIVSANLFAVGGALLMLAPAIAGYVFQWNIGVSAVWGISVYGVFVLGFYIAQIVFSEFNRMRLSDWISLR
 25 PDNWNATRVAVIIAGYREDPFMFKKCLESVRDSEYGNVARLICVIDGDEEEDLKMAEIKQVYNDNVKKPGVVLC
 ESENKNGSTIDSDVSKNICILQPHRGKRESLYTGFQLASMDPSVHAVVLIDSDTVLEKNAILEVVYPLSCDPNIK
 AVAGECKIWNTDTILSMLVSWRYFSAFNVERGAQSLWKTVCVGGPLGAYTIDIINEIKDPWITQTFGLGNKCTYG
 30 DDRRLTNEVLMRGKKIVYTPFAVGWSDSPTNVMRYIVQQTRWSKSWCREIWTFLGSAWKHGFSGIYLAFECMYQI
 MYFFLVMYLFSYIAIKADIRAQTATVLVSTLVTIIKSSYLALRAKNLKAFFVLYTYVYFFCMIPARITAMFTMF
 DIAWGTRGGNAKMTIGARVWLWAKQFLITYMWWAGVLAAGVYSIVDNWYFDWADIQYRFALVGICSYLVFVSIVL
 VIYLIGKITTNWYTPQLQKELIEERYLHNASENAPEV

SEQ ID NO: 108 is an amino acid sequence of hyaluronan synthase (HASA) originating from *Chlorella virus CVG-1*

MTSWRTIVSANLFAVGGALLMLAPAIAGYVFKWNIGVSAVWGISVYGVFVLGFYIAQVVFSEFNRMQLSDWISLR
 PDNWNATRVAVIIAGYREDPFMFKKCLESVRDSEYGNARLICVIDGDEEEDLKMAEIKQVYNDNVKTPGVVLC

ENENKNGSTIDPDFSKNICILQPHRGKRESLYTGFMAGMDPSVHAVVLIDSDTVLEKNAILEVVYPLSCDPNIK
AVAGECKIWNTDTILSMLVSWRYFSAFNVERGAQSLWKTVCVGGPLGAYTIDIINEIKDPWITQTFLGNKCTYG
DDRRLTNEVLMRGKKIVYTPFAVGWSDSPTNVMRYIVQQTRWSKSWCREIWYTLGSAWKHGFSGIYLAFECMYQI
MYFFIVMYLFSYIAIKANIRAQAATVLVSTLVAVIKSSYLALRAKNLKALYFVLYTYVYFFCMIPARITAMFTMF
5 DIAWGTRGGNAKMTIGARVWLWAKQFLITYMWWVGVLAAGVYSIVDNWYFDWADIQYRFALVGICSYLGFVSIML
VFYLGKITTWNYTPLQKELIKERLHAANATEV

CLAIMS

1. A recombinant yeast cell producing hyaluronic acid (HA) wherein the recombinant cell comprises:

5 (a) one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity;

(b) one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity;

10 (c) one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity wherein the polypeptide having hyaluronidase activity comprises a secretion signal so that hyaluronic acid, in particular of a desired molecular weight (HAMW) is produced by the recombinant yeast cell, and

(d) (i) one or more recombinant nucleic acids encoding a polypeptide having a glutamine synthetase (GLN1) activity; and/or

15 (ii) one or more disrupted endogenous nucleic acids encoding a glutamate synthase (GLT1);

wherein said recombinant yeast cell belongs to the *Saccharomyces* genus, or to the *Candida* genus, or to the *Kluyveromyces* genus, or to the *Ogataea* genus, or to the *Yarrowia* genus, or to the *Debaryomyces* genus, or to the *Ashbya* genus.

20

2. A recombinant host cell producing hyaluronic acid (HA) wherein the recombinant host cell comprises:

(a) one or more recombinant nucleic acids encoding a polypeptide having hyaluronan synthase activity;

25 (b) one or more recombinant nucleic acids encoding a polypeptide having UDP-Glucose dehydrogenase (UDP-GlcDH or HASB) activity;

(c) one or more recombinant nucleic acids encoding a polypeptide having hyaluronidase activity wherein the polypeptide having hyaluronidase activity comprises a secretion signal and an anchoring signal so that hyaluronic acid, in particular of a desired
30 molecular weight (HAMW) is produced by the host cell; and

(d) (i) one or more recombinant nucleic acids encoding a polypeptide having a glutamine synthetase (GLN1) activity; and/or

(ii) one or more disrupted endogeneous nucleic acids encoding a glutamate synthase (GLT1).

3. The recombinant cell according to claim 1 or 2, wherein the molecular weight
5 of the HA is in the range of less than 50kDa, preferably in the range of about 20kDa to about 50kDa.

4. The recombinant cell according to claim 1 or 2, wherein the molecular weight
10 of the HA is in the range of greater than 50kDa, preferably in the range of about 50kDa to about 250kDa.

5. The recombinant cell according to claim 1 or 2, wherein the molecular weight
15 of the HA is in the range of greater than 100kDa, preferably in the range of about 100kDa to about 1500kDa.

6. The recombinant cell according to any one of claims 1 to 5, wherein the
nucleic acid encoding a polypeptide having a glutamine synthetase activity is obtained or
derived from *Saccharomyces cerevisiae*.

20 7. The recombinant cell according to any one of claims 1 to 6, wherein the
nucleic acid encoding a polypeptide having hyaluronidase activity is obtained or derived from
at least one of *Cupiennius salei*, *Loxosceles intermedia*, *Hirudo nipponia*, *Bothrops atrox* or
Tityus serrulatus.

25 8. The recombinant cell according to any one of claims 1 to 7, wherein the
nucleic acid encoding a polypeptide having hyaluronan synthase activity is obtained or
derived from *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Chlorella virus CviK1*,
Chlorella virus IL-5-2s1, *Chlorella virus CZ-2*, *Chlorella virus CVG-1*, *Xenopus laevis* or
Pasteurella multocida, and is in particular obtained or derived from at least one of
30 *Streptococcus zooepidemicus*, *Chlorella virus PBCV1*, *Chlorella virus CviK1*, *Chlorella virus*
IL-5-2s1, *Chlorella virus CZ-2*, *Chlorella virus CVG-1* or *Xenopus laevis*.

9. The recombinant cell according to any one of claims 1 to 8, wherein the
nucleic acid encoding a polypeptide having UDP-Glucose dehydrogenase activity is obtained

or derived from at least one of *Arabidopsis thaliana*, *Chlorella virus PBCV1* or *Streptococcus zooepidemicus*, and in particular from *Arabidopsis thaliana* or *Chlorella virus PBCV1*.

10 10. The recombinant cell according to any one of claims 1 to 9, wherein the
5 recombinant cell comprises a recombinant nucleic acid encoding one or more of:

 (i) a polypeptide having glutamine-fructose-6-phosphate amidotransferase
(GFA1) activity; and/or

 (ii) a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase (QR11)
activity.

10

 11. The recombinant cell according to any one of claims 1 to 10, wherein the
recombinant cell comprises at least one recombinant nucleic acid encoding one or more of:

 (i) a polypeptide having Phosphoglucomutase-1 (PGM1) activity; and/or

15 (ii) a polypeptide having UTP-glucose-1-phosphate uridylyltransferase (UGP1)
activity; and/or

 (iii) a polypeptide having Glucosamine-6-phosphate N-acetyltransferase
(GNA1) activity; and/or

 (iv) a polypeptide having phosphoacetylglucosamine mutase (PCM1) activity.

20

 12. The recombinant yeast cell according to any one of claims 1 and 3 to 11,
wherein the recombinant yeast cell is selected from the group consisting of *Saccharomyces
cerevisiae*, *Saccharomyces boulardii*, *Saccharomyces bayanus*, *Saccharomyces paradoxus*,
Saccharomyces mikatae, *Saccharomyces castelli*, *Candida albicans*, *Candida glabrata*,
Candida tropicalis, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Kluyveromyces*
25 *polysporus*, *Kluyveromyces thermotolerans*, *Ogataea polymorpha*, *Yarrowia lipolytica*,
Debaryomyces hansenii, and *Ashbya gossypii*, and is preferably *Saccharomyces cerevisiae*.

30

 13. The recombinant host cell according to any one of claims 2 to 11 wherein the
recombinant host cell belongs to the *Saccharomycetales* order, and is in particular selected
from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces boulardii*,
Saccharomyces bayanus, *Saccharomyces paradoxus*, *Saccharomyces mikatae*,
Saccharomyces castelli, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*,
Kluyveromyces lactis, *Kluyveromyces marxianus*, *Kluyveromyces polysporus*, *Kluyveromyces*

thermotolerens, *Ogataea polymorpha*, *Yarrowia lypolytica*, *Debaryomyces hansenii*, and *Ashbya gossypii*, and is preferably *Saccharomyces cerevisiae*.

14. A method of producing hyaluronic acid (HA) of a desired molecular weight
5 (HAMW) comprising:

(a) cultivating a recombinant cell as defined in any one of claims 1 to 13 in a cultivation medium for a time sufficient to produce hyaluronic acid (HA) of the desired molecular weight; and

(b) optionally isolating or recovering the hyaluronic acid (HA) from the
10 recombinant cell and/or from the cultivation medium.

15. The method according to claim 14, wherein the HA has a molecular weight of from about 20kDa to about 50kDa, and preferably from about 20 kDa to about 30 kDa.

16. The method according to claim 15, wherein the HA has a molecular weight
15 of from about 30 kDa to about 50 kDa.

17. The method according to claim 14, wherein the molecular weight of the HA is of from about 50 kDa to about 150 kDa.

20

18. The method according to claim 14, wherein the molecular weight of the HA is of from about 150 kDa to about 1500 kDa.

19. The method according to any one of claims 14 to 18, wherein the
25 recombinant cell comprises at least one recombinant nucleic acid encoding one or more of:

(i) a polypeptide having glutamine-fructose-6-phosphate amidotransferase (GFA1) activity; and/or

(ii) a polypeptide having UDP-N-acetylglucosamine pyrophosphorylase (QRI1)
activity.

30

20. The method according to any one of claims 14 to 19, wherein the recombinant cell comprises at least one recombinant nucleic acid encoding one or more of:

(i) a polypeptide having Phosphoglucomutase-1 (PGM1) activity;

(ii) a polypeptide having UTP-glucose-1-phosphate uridylyltransferase (UGP1) activity;

(iii) a polypeptide having Glucosamine-6-phosphate N-acetyltransferase (GNA1) activity; and/or

5 (iv) a polypeptide having phosphoacetylglucosamine mutase (PCM1) activity.

21. The method according to any one of claims 14 to 20, wherein the recombinant cell is a member of the genus *Saccharomyces*, and in particular is *Saccharomyces cerevisiae*.

10

22. The method according according to any one of claims 14 to 21, wherein the time sufficient to produce hyaluronic acid (HA) of the desired molecular weight is a period of from about 35 hours to about 50 hours, preferably from about 40 hours to about 50 hours, preferably about 48 hours.

15

23. The method according according to any one of claims 14 to 22, wherein the molecular weight of the hyaluronic acid is controlled by regulating the pH of the cultivation medium.

20

24. The method according to any one of claims 14 to 23, wherein the method is carried out on an industrial scale, preferably where the cultivation medium is at least about 100L, more preferably in the range of about 1000L to about 3000L, even more preferably about 10,000L, even more preferably about 100,000L, or even about 250,000L.

25

25. Hyaluronic acid (HA) obtainable from a recombinant cell of any one of claims 1 to 11 or from the method according to any one of claims 14 to 24.

26. A cultivation medium comprising the HA according to claim 25.

30

27. A composition comprising the Hyaluronic acid (HA) according to claim 25.

28. An industrial product or a consumer product or a consumable comprising (i) the HA according to claim 25, (ii) the cultivation medium of claim 26 or (iii) the composition of claim 27.

5 29. The industrial product or the consumer product or the consumable of claim 28, wherein the industrial product or the consumer product or the consumable is a cosmetic product, a flavour product, a fragrance product, a food product, a food, a beverage, a texturant, a pharmaceutical composition, a dietary supplement, a nutraceutical, a cleaning product and/or a dental and/or an oral hygiene composition.

10

30. Use of a recombinant cell as defined in any one of claims 1 to 13 for the production of hyaluronic acid (HA) having a molecular weight in the range of from about 20kDa to about 50kDa or from about 50kDa to about 1000 kDa.

15 31. A method for producing hyaluronic acid comprising the steps of:
 (a) culturing a recombinant yeast as defined in any one of claims 1 and 3 to 12 in a culture medium; and
 (b) recovering the hyaluronic acid from said culture medium,
 wherein the hyaluronic acid recovered in step (b) has a molecular weight
20 controlled through the selection of:
 - the nature and origin of the nucleic acid encoding the hyaluronidase of the recombinant yeast,
 - the nature and origin of the promoter controlling the expression of the nucleic acid encoding the hyaluronidase(s) of the recombinant yeast,
25 - the presence of an anchoring and/or of a secretion signal associated to the encoded hyaluronidase(s) of the recombinant yeast,
 - the pH of the culture medium during the step of culturing the recombinant yeast, and/or
 - the duration of the culturing of the recombinant yeast.