In the course of *P. pastoris* genome sequencing 54 different sequences were listed as predicted signal peptides which include a cleavage site to provide for the secretion of proteins. (De Schutter et al. Nature Biotechnology doi: 10.1038/nbt.1544 2009).

US2011/0021378A1 describes a set of 54 *P. pastoris* genes identified that contain a signal sequence, among them residues 1-21 of SEQ ID 8 as listed in US2011/0021378A1, again including a cleavage site to provide for the secretion of proteins.

EP2258855A1 describes regulatory sequences of a *P. pastoris* derived expression system, wherein a signal and leader sequence of the *P. pastoris* Epx1 protein is used as a 57 amino acid precursing sequence to facilitate expression and secretion of a protein of interest POI. A cleavage site for a signal peptidase is predicted to be following the signal sequence, i.e. between position 20 and 21, indicated as a hyphen in the sequence of the cleavage site: VSA-AP (SEQ ID 6).

It is desirable to provide alternative regulatory elements suitable for expressing a POI in a recombinant eukaryotic host cell, and methods to produce secreted proteins in eukaryotic cells which are simple and efficient, and could preferably lead to a homogeneous N-terminus of the POI.

SUMMARY ~~OF THE INVENTION~~

The object is solved by the subject matter as claimed.

In one aspect, there is provided an isolated nucleic acid encoding a leader, which is selected from the group consisting of

a) a leader peptide with the amino acid sequence of SEQ ID 10 or a functional variant thereof with one or two point mutations, and

b) a leader peptide with the amino acid sequence selected from the group consisting of SEQ ID 11, 12, 13 and 14,

preferably which is a nucleotide sequence encoding a leader peptide, selected from the group consisting of SEQ ID 18, 19 and 20, or a codon optimized variant of any of SEQ ID 18, 19 or 20.

In one aspect, there is provided isolated nucleic acid encoding a leader, which is selected from the group consisting of

a) a signal peptide with the amino acid sequence of SEQ ID 1 or a functional variant thereof with one or two point mutations, and

b) a signal peptide with the amino acid sequence selected from the group consisting of SEQ ID 2, 3, 4 and 5,

preferably which is a nucleotide sequence encoding a signal peptide, selected from the group consisting of SEQ ID 15, 16 and 17, or a codon optimized variant of any of SEQ ID 15, 16, or 17.

The ~~invention~~ disclosure provides for an isolated nucleic acid encoding a leader, which is selected from the group consisting of

a) a leader peptide with the amino acid sequence of SEQ ID 10 or a functional variant thereof with one or two point mutations,

b) a leader peptide with the amino acid sequence selected from the group consisting of SEQ ID 11, 12, 13 and 14,

c) a signal peptide with the amino acid sequence of SEQ ID 1 or a functional variant thereof with one or two point mutations, preferably excluding SEQ ID 2, and

d) a signal peptide with the amino acid sequence selected from the group consisting of SEQ ID 2, 3, 4 and 5, preferably excluding SEQ ID 2.

The ~~invention~~ disclosure further provides for a leader, which is selected from the group consisting of

a) a leader peptide with the amino acid sequence of SEQ ID 10 or a functional variant thereof with one or two point mutations,

b) a leader peptide with the amino acid sequence selected from the group consisting of SEQ ID 11, 12, 13 and 14,

c) a signal peptide with the amino acid sequence of SEQ ID 1 or a functional variant thereof with one or two point mutations, preferably excluding SEQ ID 2, and

d) a signal peptide with the amino acid sequence selected from the group consisting of SEQ ID 2, 3, 4 and 5, preferably excluding SEQ ID 2.

The specific leader peptide is therefore characterized by a 36 amino acid (aa) leader sequence, wherein the N-terminal 20 aa sequence is a specific signal peptide.

The specific signal peptide is therefore characterized by a 20 aa signal sequence, specifically excluding a C-terminally extended sequence, e.g. a 21 aa sequence that includes a C-terminal extension by an additional amino acid, such as Alanine.

SEQ ID 1: MK**X**STNLILAIAAAS**X**VVSA, wherein

X at position 3 is either F or L

X at position 16 is either A or T

For example, the EpxL-A, 20 amino acid (aa) signal peptide, has the amino acid sequence of SEQ ID 1, wherein X at position 3 is L, and X at position 16 is A (SEQ ID 2).

According to a specific example, the EpxL-A, 20 amino acid (aa) signal peptide, has the amino acid sequence of SEQ ID 1, wherein X at position 3 is F, and X at position 16 is A (SEQ ID 3).

Specifically, the ~~invention~~ disclosure provides for a signal peptide or a nucleic acid which encodes the signal peptide which has an amino acid sequence selected from the group consisting of SEQ ID 2, 3, 4 and 5.

SEQ ID 2: MK**L**STNLILAIAAAS**A**VVSA

SEQ ID 3: MK**F**STNLILAIAAAS**A**VVSA

SEQ ID 4: MK**F**STNLILAIAAAS**T**VVSA

SEQ ID 5: MK**L**STNLILAIAAAS**T**VVSA

The amino acid substitutions at position 3 and 16 are indicated bold and underlined.

The nucleic acid molecule encoding the signal peptide according to the ~~invention~~ disclosure is herein also called "signal sequence".

The leader according to the ~~invention~~ disclosure and as defined above specifically has the amino acid sequence of SEQ ID 10.

SEQ ID 10:
MK**X**STNLILAIAAAS**X**VVSAAPVAPAEEAANHLHKR, wherein
X at position 3 is either F or L
X at position 16 is either A or T

Specifically, the ~~invention~~ disclosure provides a leader or a nucleic acid which encodes the leader which has an amino acid sequence selected from the group consisting of SEQ ID 11, 12, 13 and 14.

SEQ ID 11: MK**L**STNLILAIAAAS**A**VVSAAPVAPAEEAANHLHKR
SEQ ID 12: MK**F**STNLILAIAAAS**A**VVSAAPVAPAEEAANHLHKR
SEQ ID 13: MK**F**STNLILAIAAAS**T**VVSAAPVAPAEEAANHLHKR
SEQ ID 14: MK**L**STNLILAIAAAS**T**VVSAAPVAPAEEAANHLHKR

The amino acid substitutions at position 3 and 16 are indicated bold and underlined.

For example, the EpxL-KR 36 amino acid (aa) truncated leader, has the amino acid sequence of SEQ ID 10, wherein X at position 3 is L, and X at position 16 is A.

According to a specific example, the EpxL-KR, 36 amino acid (aa) leader peptide, has the amino acid sequence of SEQ ID 10, wherein X at position 3 is F, and X at position 16 is A (SEQ ID 12).

The leader of the present ~~invention~~ disclosure with the amino acid sequence of SEQ ID 10, 11, 12, 13 or 14, or a functional variant thereof with one or two point mutations, is herein called the "truncated leader".

Specifically, the nucleic acid encoding the truncated leader comprises or consists of a nucleic acid encoding a signal peptide selected from the group consisting of SEQ ID 2, 3, 4 and 5, preferably excluding SEQ ID 2. Specifically, the nucleic acid encoding the truncated leader comprises or consists of a nucleic acid encoding a leader peptide selected from the group consisting of SEQ ID 11, 12, 13, and 14.

Specifically, the truncated leader comprises or consists of a the signal peptide selected from the group consisting of SEQ ID 2, 3, 4 and 5, preferably excluding SEQ ID 2, or of a leader peptide selected from the group consisting of SEQ ID 11, 12, 13, and 14.

In one aspect, there is provided an isolated leader encoded by a nucleic acid as defined herein, preferably with an amino acid sequence acid sequence selected from the group consisting of SEQ ID 1, 2, 3, 4, 5, 10, 11, 12, 13 and 14.

A specifically preferred nucleic acid encodes a signal peptide of the amino acid sequence of SEQ ID 3 or a leader peptide of SEQ ID 12; preferably the nucleic acid sequence encoding the signal peptide or the leader peptide is SEQ ID 16 or SEQ ID 19, respectively. A specifically preferred leader is a signal peptide having the amino acid sequence of SEQ ID 3 or a leader peptide having the amino acid sequence of SEQ ID 12.

Accordingly, a specific embodiment of the ~~invention~~ disclosure refers to a leader or a nucleic acid encoding such leader, which is

a) a signal peptide with the amino acid sequence of SEQ ID 3, preferably wherein the coding nucleic acid consists of a nucleotide sequence of SEQ ID 16, or a codon optimized variant of SEQ ID 16; or

b) a leader peptide with, or comprising or consisting of the amino acid sequence of SEQ ID 12, preferably wherein the coding nucleic acid consists of a nucleotide sequence of SEQ ID 19, or a codon optimized variant of SEQ ID 19.

Specifically, the nucleic acid according to the ~~invention~~ disclosure has

a) a nucleotide sequence encoding a signal peptide, selected from the group consisting of SEQ ID 15, 16 and 17, preferably excluding SEQ ID 15, or

b) a nucleotide sequence encoding a leader peptide, selected from the group consisting of SEQ ID 18, 19 and 20, or

c) a nucleotide sequence which is a codon optimized variant of SEQ ID 15, 16, 17, 18, 19 or 20.

A specific nucleic acid of the ~~invention~~ disclosure encodes a leader, which is a signal peptide with the amino acid sequence of SEQ ID 3, preferably wherein the nucleic acid consists of a nucleotide sequence of SEQ ID 16, or a codon optimized variant of SEQ ID 16.

A specific nucleic acid of the ~~invention~~ disclosure encodes a leader, which is a leader peptide or truncated leader with the amino acid sequence of SEQ ID 12, preferably wherein the nucleic acid consists of a nucleotide sequence of SEQ ID 19, or a codon optimized variant of SEQ ID 19.

SEQ ID 15: nucleotide sequence obtained from *P. pastoris*, strain CBS7435 (CBS-KNAW Fungal Biodiversity Centre, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands):
ATGAAGCTCTCCACCAATTTGATTCTAGCTATTGCAGCAGCTTCCGCCGTTGTCTC
AGCT

SEQ ID 16: nucleotide sequence as used in Example 9, obtained from *P. pastoris* strain CBS7435 by PCR amplification using the primers described in Example 9.
ATGAAGTTCTCTACCAATTTGATTCTAGCTATTGCAGCAGCTTCCGCCGTTGTCTC
AGCT

SEQ ID 17: nucleotide sequence obtained from *P. pastoris*, strain DSMZ70382 (German Collection of Microorganisms and Cell Cultures)
ATGAAGTTCTCTACCAATTTGATCTTAGCTATTGCAGCAGCATCCACTGTTGTCTC
AGCT

The nucleotides differing in the sequences above are underlined.

SEQ ID 18: nucleotide sequence obtained from *P. pastoris*, strain CBS7435 (CBS-KNAW Fungal Biodiversity Centre, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands)
ATGAAGCTCTCCACCAATTTGATTCTAGCTATTGCAGCAGCTTCCGCCGTTGTCTC
AGCTGCTCCAGTTGCTCCAGCCGAAGAGGCAGCAAACCACTTGCACAAGCGT

SEQ ID 19: nucleotide sequence as used in Example 5, obtained from *P. pastoris* strain CBS7435 by PCR amplification using the primers described in Examples 1 and 5

ATGAAGTTCTCTACCAATTTGATTCTAGCTATTGCAGCAGCTTCCGCCGTTGTCTC
AGCTGCTCCAGTTGCTCCAGCCGAAGAGGCAGCAAACCACTTGCACAAGCGT

SEQ ID 20: nucleotide sequence obtained from *P. pastoris*, strain DSMZ70382 (German Collection of Microorganisms and Cell Cultures)

ATGAAGTTCTCTACCAATTTGATCTTAGCTATTGCAGCAGCATCCACTGTTGTCTC
AGCTGCTCCAGTTGCTCCAGCCGAAGAGGCAGCAAACCACTTGCACAAGCGT

Specifically the nucleic acid according to the ~~invention~~ disclosure is a DNA.

Specifically the leader according to the ~~invention~~ disclosure is a polypeptide.

According to a certain ~~aspect~~embodiment, the ~~invention~~ disclosure provides for an isolated leader, truncated leader, signal peptide or leader peptide, preferably the leader has an amino acid sequence selected from the group consisting of SEQ ID 1, 2, 3, 4, 5, 10, 11, 12, 13 and 14, preferably excluding SEQ ID 2.

A specifically preferred leader is a peptide consisting of the amino acid sequence of SEQ ID 3 or of SEQ ID 12.

In one aspect, there is provided an expression cassette comprising a nucleic acid encoding a leader operably linked to a nucleic acid sequence encoding a POI, characterized in that the leader is selected from the group consisting of

a) a leader peptide with the amino acid sequence of SEQ ID 10 or a functional variant thereof with one or two point mutations,

b) a leader peptide with the amino acid sequence selected from the group consisting of SEQ ID 11, 12, 13 and 14, preferably which is a nucleotide sequence encoding a leader peptide, selected from the group consisting of SEQ ID 18, 19 and 20, or a codon optimized variant of any of SEQ ID 18, 19, or 20,

c) a signal peptide with the amino acid sequence of SEQ ID 1 or a functional variant thereof with one or two point mutations, and

d) a signal peptide with the amino acid sequence selected from the group consisting of SEQ ID 2, 3, 4 and 5, preferably which is a nucleotide sequence encoding a signal peptide, selected from the group consisting of SEQ ID 15, 16 and 17, or a codon optimized variant of

any of SEQ ID 15, 16, or 17.

According to another ~~aspect~~ embodiment, the ~~invention~~ disclosure further provides for expression cassette comprising a nucleic acid encoding a leader operably linked to a nucleic acid sequence encoding a POI, characterized in that the leader is selected from the group consisting of

a) a leader peptide with the amino acid sequence of SEQ ID 10 or a functional variant thereof with one or two point mutations,

b) a leader peptide with the amino acid sequence selected from the group consisting of SEQ ID 11, 12, 13 and 14,

c) a signal peptide with the amino acid sequence of SEQ ID 1 or a functional variant thereof with one or two point mutations, and

d) a signal peptide with the amino acid sequence selected from the group consisting of SEQ ID 2, 3, 4 and 5,

preferably wherein the fusion of the signal peptide consisting of the amino acid sequence SEQ ID 2 with a polypeptide comprising or consisting of one or more immunoglobulin single variable domains is excluded, such as for example a nanobody.

Yet, according to specific examples, the ~~invention~~ disclosure provides for the fusion of the leader peptide with the amino acid sequence selected from the group consisting of SEQ ID 11, 12, 13 and 14, or the fusion of the signal peptide with the amino acid sequence selected from the group consisting of SEQ ID 2, 3, 4 and 5 with a POI selected from the group consisting of growth factors, hormones, cytokines, antibodies and antibody fragments, in particular wherein an antibody or antibody fragment is selected from the group consisting of an scFv, minibody, diabody, triabody, tetrabody, Fab, Fc-fusion protein and a full-length antibody such as for example IgG, IgA, IgD, IgM or Ig, preferably a full-length antibody, a scFv or a Fab, specifically including the fusion of the signal peptide consisting of the amino acid sequence SEQ ID 2 with any of such antibodies or antibody fragments, specifically any of a full-length antibody, a scFv or a Fab.

A specifically preferred nucleotide sequence encoding the signal or leader peptide as used in the expression cassette consists of a nucleotide sequence encoding the amino acid sequence of SEQ ID 3 or SEQ ID 12, preferably consists of SEQ ID 16 or SEQ ID 19, or a codon optimized variant of SEQ ID 16 or 19. Such signal or leader peptide is preferably fused to any POI, including any of the antibodies or antibody fragments, specifically including the fusion of the signal peptide or leader peptide consisting of the amino acid sequence SEQ ID 3 or SEQ ID 12 with a polypeptide comprising or consisting of one or more immunoglobulin single variable domains, such as for example a nanobody.

According to another ~~aspect~~embodiment, the ~~invention~~ disclosure further provides for an expression cassette comprising

a) a nucleotide sequence encoding a signal peptide, selected from the group consisting of SEQ ID 15, 16 and 17, or

b) a nucleotide sequence encoding a leader peptide, selected from the group consisting of SEQ ID 18, 19 and 20, or

c) a nucleotide sequence which is a codon optimized variant of SEQ ID 15, 16, 17, 18, 19 or 20,

preferably wherein the fusion construct of the nucleotide sequence of SEQ ID 15 fused to a nucleotide sequence encoding one or more immunoglobulin single variable domains such as for example a nanobody is excluded.

Yet, according to a specific example, the ~~invention~~ disclosure provides for the fusion construct of the nucleotide sequence of SEQ ID 15 fused to a nucleotide sequence encoding an antibody or antibody fragment selected from the group consisting of an scFv, minibody, diabody, triabody, tetrabody, Fab, Fc-fusion protein and a full-length antibody such as for example IgG, IgA, IgD, IgM or IgE, preferably a full-length antibody, a scFv or a Fab.

A specifically preferred nucleotide sequence is SEQ ID 16, encoding the signal peptide of SEQ ID 3.

Another specifically preferred nucleotide sequence is SEQ ID 19, encoding the leader peptide of SEQ ID 12.

Therefore, according to specific examples, the ~~invention~~ disclosure provides for the fusion construct of the nucleotide sequence of SEQ ID 16 or 19 fused to a nucleotide sequence encoding any protein of interest, e.g. specifically a POI as described herein, including, but not limited to, any of the antibodies or antibody fragments, specifically including the fusion constructs wherein the POI is a polypeptide comprising or consisting of one or more immunoglobulin single variable domains, such as for example a nanobody.

By the expression cassette according to the ~~invention~~ disclosure it was the first time possible to provide for the expression of a POI, and specifically a secreted mature POI, with a correct, native N-terminal amino acid residue, in particular without an additional Alanine at the N-terminus.

Specifically, the POI is selected from therapeutic proteins, including antibodies or fragments thereof, enzymes and peptides, protein antibiotics, toxin fusion proteins, carbohydrate - protein conjugates, structural proteins, regulatory proteins, vaccines and vaccine-like proteins or particles, process enzymes, growth factors, hormones and cytokines, or wherein said POI mediates the production of a host cell metabolite, preferably selected from the group consisting of antibodies or fragments thereof, growth factors, hormones and cytokines.

According to specific examples, the antibody or fragments thereof are selected from the group consisting of an scFv, minibody, diabody, triabody, tetrabody, Fab, Fc-fusion protein and a full-length antibody such as for example IgG, IgA, IgD, IgM or IgE, preferably a full-length antibody, a scFv or a Fab.

Specifically, the expression cassette according to the ~~invention~~ disclosure is a fusion of a nucleic acid according to the ~~invention~~ disclosure with a nucleic acid encoding a POI and as such is a non-naturally occurring nucleic acid. The ~~invention~~ disclosure further provides a fusion protein of a leader according to the ~~invention~~ disclosure with a POI and as such is a non-naturally occurring fusion protein.

Therefore, the leader is engineered for the improved production of a POI, e.g. for an increased secretion yield and improved quality such as the correct N-terminus, and employs a leader different from the leaders of the prior art.

The expression cassette according to the ~~invention~~ disclosure specifically encodes a leader sequence consisting either of the signal peptide, or the signal peptide prolonged by the pro-sequence consisting of APVAPAEEAANHLHKR (SEQ ID 7), which pro-sequence is part of the native full-length leader of the *P. pastoris* Epx1 protein, i.e. a 16 amino acid sequence identical to amino acids 21-36 of the 57 amino acid sequence of

SEQ ID 8:
MKFSTNLILAIAAASTVVSAAPVAPAEEAANHLHKRAYYTDTTKTHTFTEVVTVYRT.

Both leaders, the signal peptide and the truncated leader of the ~~invention~~disclosure, surprisingly turned out to have unexpected improved properties over the leaders of the prior art, e.g.

SEQ ID 21: MKLSTNLILAIAAASAVVSAA,

herein also called EpxL-AA (21 amino acids): according to US2011/0021378A1.

Moreover, improved properties could be shown compared to the full-length leader of the *P. pastoris* Epx1 protein as described in EP2258855A1, which is the 57 amino acid sequence of SEQ ID 8 or a variant thereof with a Leucine at position 3.

This was the more surprising, because according to the ~~invention~~ disclosure the 20 amino acid signal peptide without a pro-sequence or the truncated leader has a length of only 35% and 63% of the full-length leader, respectively.

The leader of the ~~invention~~ disclosure would have specific advantages when fused to a POI, such as an increased secretion of the POI and/or an improved quality such as the correct N-terminus as compared to the fusion with the alpha mating factor (aMF) leader, specifically when the POI is a hormone, a cytokine, an antibody or antibody fragment, e.g. selected from the group consisting of an scFv, minibody, diabody, triabody, tetrabody, Fab, Fc-fusion protein and a full-length antibody such as for example IgG, IgA, IgD, IgM or IgE, preferably a full-length antibody, a scFv or a Fab.

The expression cassette according to the ~~invention~~ disclosure explicitly excludes a nucleic acid encoding the native leader of the *P. pastoris* Epx1 protein as described in EP2258855A1 (SEQ ID 8).

The expression cassette according to the ~~invention~~ disclosure further explicitly excludes a nucleic acid encoding the leader with the amino acid 1-21 of the full-length leader of the *P. pastoris* Epx1 protein, i.e. SEQ ID 11, as described in US2011/0021378A1.

According to a specific ~~aspect~~ embodiment of the ~~invention~~disclosure, the expression cassette is optionally incorporated in an expression construct, such as a vector.

According to further specific ~~aspect~~embodiment of the ~~invention~~disclosure, the expression cassette or the expression construct comprises a promoter operably linked to the nucleic acid encoding the leader.

In one aspect, there is provided a recombinant yeast host cell or a vector comprising the expression cassette as defined herein.

According to a specific embodiment, the ~~invention~~ disclosure provides for a recombinant yeast host cell comprising an expression cassette according to the ~~invention~~ disclosure, specifically a yeast host cell line, more specifically a production cell line. According to a specific ~~aspect~~ embodiment of the ~~invention~~ disclosure the expression cassette is a vector, or part of a vector.

Preferably the yeast is selected from the genus group consisting of Pichia, Candida, Torulopsis, Arxula, Hansenula, Ogatea, Yarrowia, Kluyveromyces, Saccharomyces, Komagataella, preferably a methylotrophic yeast, and specifically preferred *P. pastoris, Komagataella pastoris*, *K. phaffii*, or *K. pseudopastoris.*

~~According to another specific embodiment, the invention provides for~~ In one aspect, there is provided a method of producing a POI in a yeast host cell, comprising:

- providing a host cell ~~of the invention~~ as defined herein,
- cultivating said host cell to express said POI, and
- purifying the POI to obtain a preparation of a purified POI.

According to the ~~invention~~disclosure, the expression cassette is preferably employed to facilitate the secretion of recombinant genes in yeast host cells, thereby increasing the yield of secreted products.

Therefore the expression cassette according to the ~~invention~~ disclosure provides for the efficient expression and secretion of a POI in a host cell transformed

with said expression cassette. In this regard, the expression cassette according to the ~~invention~~ disclosure is understood as a yeast expression cassette.

Specifically the POI is a recombinant protein, which term herein always is understood to include both polypeptides and proteins, such as produced by the recombinant host cell. The POI may be a heterologous protein, such as heterologous to yeast, e.g., a protein derived from a higher eukaryote such as human or from a yeast species other than *P. pastoris* or an artificial polypeptide or protein. Alternatively, the POI may be a homologous protein derived from yeast such as e.g. *P. pastoris*, which would, however, not be expressed or not expressed in desirable amounts by a native yeast not transformed with the vector according to the ~~invention~~disclosure, but which would be expressed in desirable amounts, e.g. overexpressed to yield significant amounts of the POI or metabolites by the recombinant yeast host cell according to the ~~invention~~disclosure.

Specifically, the POI has an amino acid sequence with a native N-terminal amino acid sequence. Said POI preferably comprises an amino acid sequence that does not comprise an additional Alanine as N-terminal amino acid residue. Specifically, the POI has no additional N-terminal amino acid residues originating from the leader sequence. This is of particular importance for the quality of recombinant protein production and ease of production.

Specifically said POI is a secreted polypeptide or protein, including soluble, extracellular molecules or membrane-bound molecules.

Specifically, said POI is selected from therapeutic proteins, including antibodies or fragments thereof, enzymes and peptides, protein antibiotics, toxin fusion proteins, carbohydrate - protein conjugates, structural proteins, regulatory proteins, vaccines and vaccine-like proteins or particles, process enzymes, growth factors, hormones and cytokines, or wherein said POI mediates the production of a host cell metabolite. The POI may also be an expression product that mediates the production of a host cell metabolite.

Specifically, the POI is selected from the group consisting of growth factors, hormones, cytokines, antibody and antibody fragments, preferably selected from the group consisting of a full-length antibody, such as for example IgG, IgA, IgD, IgM or IgE, an scFv, minibody, diabody, triabody, tetrabody, Fab and a Fc-fusion protein, preferably a full-length antibody, a scFv or a Fab.

Specifically, the POI comprises an N-terminal amino acid residue other than Alanine. Therefore, there is no signal peptidase cleavage site of VSA-AP (SEQ ID 6) as predicted in the prior art (EP2258855A1).

Specifically, the POI may be a secreted protein, such as a mature protein which could be an active form of a protein or a pro-form.

The method according to the ~~invention~~ disclosure preferably provides for *cultivation of the host cell in a cell culture, and said POI or metabolite is obtained, e.g.* as secreted POI, including membrane-bound or soluble or extracellular proteins or metabolites, which are optionally purified from the cell culture supernatant.

In one aspect, there is provided the use of the nucleic acid as defined herein or of the leader as defined herein, for the secretion of a POI from a host cell and/or to increase the secretion of a POI from a host cell.

According to a further ~~aspect~~embodiment, the ~~invention~~disclosure provides for the use of the nucleic acid or leader of the ~~invention~~disclosure, in particular the nucleic acid encoding a signal peptide or a truncated leader of the ~~invention~~disclosure, for the secretion of a POI from a host cell and/or to increase the secretion of a POI from a host cell, preferably, wherein at least 60, 65, 70, 75, 80, 85, 90, 95, 98, or 100% of the secreted POI comprises a native N-terminal amino acid sequence.

FIGURES

Figure 1: promoter sequence pG1: SEQ ID 9.

Figure 2.1: Silver-stained SDS-PAGE of the reduced supernatants of *P. pastoris* secreting pTRP with EpxL-RT or MFα, respectively.

Figure 2.2: Silver-stained SDS-PAGE of the reduced supernatants of *P. pastoris* secreting eGFP with EpxL-RT or MFα.

Figure 2.3: anti-HSA Western Blot of the reduced supernatants of *P. pastoris* secreting HSA with EpxL-RT or MFα, respectively.

Figure 3: Coomassie-stained Western Blot of the supernatants of *P. pastoris* secreting HSA with EpxL-RT for N-terminal sequencing.

Figure 4.1: Silver-stained SDS-PAGE of the reduced supernatants of *P. pastoris* secreting pTRP with EpxL-RT, EpxL-KR or MFα, respectively.

Figure 4.2: Silver-stained SDS-PAGE of the reduced supernatants of *P. pastoris* secreting eGFP with EpxL-KR or MFα, respectively.

Figure 4.3: Supernatants of *P. pastoris* secreting HyHEL heavy chain or HyHEL light chain with EpxL-KR, respectively. HC: Westernblot using anti-IgG gamma chain antibody; LC: SDS-PAGE and silver-stain.

Figure 5.1: Silver-stained SDS-PAGE of the reduced supernatants of *P. pastoris* secreting eGFP with EpxL-KR, EpxL-AA or EpxL-A, respectively.