CLAIMS

1. A recombinant host cell ~~comprising~~, wherein an ~~endogenous gene encoding~~ expression of a FLO8 protein ~~comprising the amino acid sequence identified as SEQ ID NO:1 or a homologue thereof, which host cell~~ is ~~engineered by one or more genetic modifications to reduce expression of said gene~~reduced compared to ~~the host cell prior to said one or more genetic modifications, and which host cell comprises~~an endogenous expression thereof, and a heterologous expression cassette comprising a gene of interest ~~(GOI)~~ under the control of an expression cassette promoter ~~(ECP) which ECP~~is introduced, wherein the expression cassette promoter is repressible by a non-methanol carbon source and ~~comprises~~consists of:

(i) any one of SEQ ID NO:10-16, or

(ii) any one of SEQ ID NO:41-45, or

(iii) the nucleotide sequence having at least 85% sequence identity to a region of at least 300 nt including the 3'end of any one of SEQ ID NO:10-16, or SEQ ID NO:41-45.

2. The host cell of claim 1, wherein ~~said endogenous polynucleotide is a gene encoding said~~the FLO8 protein consists of any one of:

a) the amino acid sequence identified as SEQ ID NO:1 in a *Komagataella phaffii* host cell;

b) the amino acid sequence identified as SEQ ID NO:3 in a *Komagataella pastoris* host cell;

c) the amino acid sequence identified as SEQ ID NO:5 or SEQ ID NO:6 in a *Saccharomyces cerevisiae* host cell;

d) the amino acid sequence identified as SEQ ID NO:7 in a *Yarrowia lipolytica* host cell;

e) the amino acid sequence identified as SEQ ID NO:8 in a *Ogataea polymorpha* host cell; and

f) the amino acid sequence identified as SEQ ID NO:9 in a *Aspergillus niger* host cell.

3. The host cell of claim 1 or 2, wherein ~~said~~the expression cassette promoter consists of the nucleotide sequence having at least 90% or at least 95% sequence

identity to a region of at least 300 nt including the 3'end of any one of SEQ ID NO:10-16, or SEQ ID NO:41-45.

4. The host cell of any one of claims 1 to 3, wherein the expression of a FLO8 protein is reduced by one or more genetic modifications ~~comprises~~comprising a disruption, substitution, deletion or knockout of (i) one or more endogenous polynucleotides encoding the FLO8 protein, or a part thereof; or (ii) an expression control sequence~~, preferably~~ .

5. The host cell of claim 4, wherein said expression control sequence is selected from the group consisting of a promoter, a ribosomal binding site, transcriptional or translational start and stop sequences, an enhancer and activator sequence.

~~3~~6. The host cell of ~~claim~~any one of claims 1 ~~or 2~~to 5, wherein ~~said~~an endogenous gene encoding said FLO8 protein ~~or said homologue~~ is knocked out ~~by said one or more genetic modifications~~.

~~4~~7. The host cell of any one of claims 1 to ~~3~~6, wherein the ~~ECP~~expression cassette promoter is inducible in the presence of a growth-limiting amount of up to 1 g/L of a non-methanol carbon source~~, preferably in the absence of methanol~~; and repressible in the presence of an excess amount of a non-methanol carbon source that is higher than the growth-limiting amount.

~~5~~8. The host cell of any one of claims 1 to 6, wherein the non-methanol carbon source selected from glycerol, glucose, or a combination thereof.

9. The host cell of claim ~~4~~8, wherein the ~~ECP comprises or~~expression cassette promoter is inducible in the absence of methanol.

10. The host cell of any one of claims 1 to 9, wherein the expression cassette promoter consists of SEQ ID NO:10 or SEQ ID NO:11.

~~6~~11. The host cell of any one of claims 1 to ~~5~~10, wherein the expression cassette further comprises a nucleotide sequence encoding a signal peptide enabling the

secretion of a protein of interest ~~(POI)~~ which is encoded by the ~~GOI, preferably wherein the nucleotide sequence encoding the signal peptide is fused adjacent to the 5'-end~~gene of ~~the GOI~~interest.

~~7~~12. The host cell of claim 11, wherein the nucleotide sequence encoding the signal peptide is fused to the 5'-end of the gene of interest.

13. The host cell of any one of claims 1 to ~~6~~12, wherein the ~~GOI~~gene of interest encodes a protein of interest ~~(POI)~~ which is a peptide or protein selected from the group consisting of an antigen-binding protein, a therapeutic protein, an enzyme, a peptide, a protein antibiotic, a toxin fusion protein, a carbohydrate - protein conjugate, a structural protein, a regulatory protein, a vaccine antigen, a growth factor, a hormone, a cytokine, a process enzyme, and a metabolic enzyme.

~~8~~14. The host cell of ~~any one of claims~~claim 1 ~~to 7~~, which is

a) a yeast cell of a genus selected from the group consisting of Pichia, Hansenula, Komagataella, Saccharomyces, Kluyveromyces, Candida, Ogataea, Yarrowia, and Geotrichum, such as *Pichia pastoris, Komagataella phaffii, Komagataella pastoris, Komagataella pseudopastoris, Saccharomyces cerevisiae, Ogataea minuta, Kluyveromces lactis, Kluyveromes marxianus, Yarrowia lipolytica* or *Hansenula polymorpha*; or

b) a cell of filamentous fungi, such as *Aspergillus awamori* or *Trichoderma reesei*.

~~9~~15. A method of increasing the yield of a protein of interest ~~(POI)~~ produced by a host cell expressing a gene of interest ~~(GOI)~~ encoding said ~~POI~~protein of interest under the control of a promoter which is repressible by a non-methanol carbon source, by reducing in said host cell an expression of a ~~gene encoding a~~ FLO8 protein ~~comprising the amino acid sequence identified as SEQ ID NO:1 or a homologue~~compared to an endogenous expression thereof~~.~~

, wherein the promotor consists of:

(i) any one of SEQ ID NO:10-16, or

(ii) any one of SEQ ID NO:41-45, or

(iii) ~~for producing~~the nucleotide sequence having at least 85% sequence identity to a region of at least 300 nt including the 3'end of any one of SEQ ID NO:10-16, or SEQ ID NO:41-45.

~~17~~16. The method of claim 15 ~~or 16~~, wherein the FLO8 protein ~~of interest (POI) encoded by~~ consists of any one of:

a ~~gene of interest (GOI) by culturing the~~ ) the amino acid sequence identified as SEQ ID NO:1 in a *Komagataella phaffii* host cell;

b) the amino acid sequence identified as SEQ ID NO:3 in a *Komagataella pastoris* host cell;

c) the amino acid sequence identified as SEQ ID NO:5 or SEQ ID NO:6 in a *Saccharomyces cerevisiae* host cell;

d) the amino acid sequence identified as SEQ ID NO:7 in a *Yarrowia lipolytica* host cell;

e) the amino acid sequence identified as SEQ ID NO:8 in a *Ogataea polymorpha* host cell; and

f) the amino acid sequence identified as SEQ ID NO:9 in a *Aspergillus niger* host cell.

~~18~~17. The method of any one of claims ~~1 to 8 under conditions~~15 to ~~produce said POI.~~ ~~17~~16, wherein the non-methanol carbon source selected from glycerol, glucose, or a combination thereof.

~~19~~18. The method of any one of claims 15 to ~~18~~17, wherein the promoter consists of the nucleotide sequence having at least 90% or at least 95% sequence identity to a region of at least 300 nt including the 3'end of any one of SEQ ID NO:10-16, or SEQ ID NO:41-45.

~~20~~19. The method of any one of claims 15 to ~~19~~18, comprising the steps:

a) culturing the host cell under growing conditions; and

b) culturing the host cell under growth-limiting conditions ~~in~~which is the presence of up to 1 g/L of a second non-methanol carbon source, resulting in expression of said ~~GOI~~gene of interest to produce said ~~POI, preferably~~protein of interest.

~~21~~20. The method of any one of claims 15 to ~~20~~19, wherein said step a) culturing is performed in a batch phase; and said step b) culturing is performed in fed-batch or a continuous cultivation phase.

~~122~~221. The method of ~~claim 11~~any one of claims 15 to ~~21~~20, wherein said first or second carbon source is selected from saccharides, polyols, alcohols, or mixtures of any one or more of the foregoing.

~~132~~322. A method for producing a protein of interest ~~(POI) in a~~ encoded by a gene of interest by culturing the host cell of any one of claims 1 to 14 under conditions to produce said protein of interest.

~~24~~23. The method of claim ~~23~~22, wherein the FLO8 protein consists of any one of:

a) the amino acid sequence identified as SEQ ID NO:1 in a *Komagataella phaffii* host cell;

b) the amino acid sequence identified as SEQ ID NO:3 in a *Komagataella pastoris* host cell;

c) the amino acid sequence identified as SEQ ID NO:5 or SEQ ID NO:6 in a *Saccharomyces cerevisiae* host cell;

d) the amino acid sequence identified as SEQ ID NO:7 in a *Yarrowia lipolytica* host cell;

e) the amino acid sequence identified as SEQ ID NO:8 in a *Ogataea polymorpha* host cell; and

f) the amino acid sequence identified as SEQ ID NO:9 in a *Aspergillus niger* host cell.

~~25~~24. The method of claim ~~23~~22 or ~~24~~23, wherein the expression cassette promoter consists of the nucleotide sequence having at least 90% or at least 95% sequence identity to a region of at least 300 nt including the 3'end of any one of SEQ ID NO:10-16, or SEQ ID NO:41-45.

~~26~~25. The method of any one of claims ~~23~~22 to ~~25~~24, comprising the steps:

a) culturing the host cell under growing conditions; and

b) culturing the host cell under growth-limiting conditions which is the presence of up to 1 g/L of a second non-methanol carbon source, resulting in expression of said gene of interest to produce said protein of interest,

2726. The method of claim 2625, wherein said step a) culturing is performed in a batch phase; and said step b) culturing is performed in fed-batch or a continuous cultivation phase.

2827. The method of any one of claims 2524 to 2726, wherein said first or second carbon source is selected from saccharides, polyols, alcohols, or mixtures of any one or more of the foregoing.

2928. A method for producing a protein of interest in a host cell, comprising the steps:

a) genetically engineering the host cell to reduce an expression of a FLO8 protein compared to an endogenous ~~gene encoding a FLO8 protein comprising the amino acid sequence identified as SEQ ID NO:1 or a homologue~~expression thereof;

introducing into the host cell a heterologous expression cassette comprising a gene of interest ~~(GOI)~~ expressing said ~~POI~~protein of interest under the control of an expression cassette promoter ~~(ECP)~~ that is operably linked to the ~~GOI, which ECP~~gene of interest, wherein the expression cassette promoter is repressible by a non-methanol carbon source and ~~comprises~~consists of:

(i) any one of SEQ ID NO:10-16, or

(ii) any one of SEQ ID NO:41-45, or

~~(i)~~(iii) the nucleotide sequence having at least 85% sequence identity to a region of at least 300 nt including the 3'end of any one of SEQ ID NO:10-16, or SEQ ID NO:41-45; and

b) culturing said host cell under conditions to produce said ~~POI;~~ protein of interest. ~~optionally~~

3029. The method of claim 2928, wherein the FLO8 protein consists of any one of:

a) the amino acid sequence identified as SEQ ID NO:1 in a *Komagataella phaffii* host cell;

b) the amino acid sequence identified as SEQ ID NO:3 in a *Komagataella pastoris* host cell;

c) the amino acid sequence identified as SEQ ID NO:5 or SEQ ID NO:6 in a *Saccharomyces cerevisiae* host cell;

d) the amino acid sequence identified as SEQ ID NO:7 in a *Yarrowia lipolytica* host cell;

e) the amino acid sequence identified as SEQ ID NO:8 in a *Ogataea polymorpha* host cell; and

f) the amino acid sequence identified as SEQ ID NO:9 in a *Aspergillus niger* host cell.

~~31~~30. The method of claim ~~29~~28 or ~~30~~29, wherein the expression cassette promoter consists of the nucleotide sequence having at least 90% or at least 95% sequence identity to a region of at least 300 nt including the 3'end of any one of SEQ ID NO:10-16, or SEQ ID NO:41-45.

~~32~~31. The method of any one of claims ~~29~~28 to ~~31~~30, further comprising:

step c) isolating said ~~POI~~protein of interest from the cell culture; ~~and optionally~~step b) purifying said ~~POI.~~protein of interest; or

both of the steps c) and b).