

## Claims

1. A method of increasing the yield and/or titer of at least one protein of interest in a eukaryotic host cell which comprises at least one polynucleotide encoding the at least one protein of interest, comprising overexpressing in said host cell:
  - a) at least one polynucleotide encoding at least one Sbh1 transport protein;  
and
  - b) (i) at least one polynucleotide encoding at least one Kar2 endoplasmic reticulum (ER) helper protein; or  
(ii) at least one polynucleotide encoding at least one Hac1 transcription factor,  
thereby increasing the yield and/or titer of the protein of interest in comparison to a eukaryotic host cell which does not overexpress the polynucleotides of a) and b).
2. The method of claim 1, comprising:
  - engineering said host cell to overexpress the polynucleotides of a) and b);
  - engineering said host cell to comprise at least one polynucleotide encoding the at least one protein of interest, preferably operably linked to a promoter;
  - culturing said host cell under suitable conditions to overexpress the polynucleotides of a) and b), and to express the at least one polynucleotide encoding the at least one protein of interest, optionally
  - isolating the at least one protein of interest from the cell culture, and optionally
  - purifying the at least one protein of interest.
3. A method of manufacturing at least one protein of interest in a eukaryotic host cell comprising:
  - providing said host cell engineered to overexpress
    - a) at least one polynucleotide encoding at least one Sbh1 transport protein,  
and
    - b) (i) at least one polynucleotide encoding at least one Kar2 endoplasmic reticulum (ER) helper protein; or  
(ii) at least one polynucleotide encoding at least one Hac1 transcription factor,  
wherein the host cell further comprises at least one polynucleotide encoding the at least one protein of interest,
  - culturing said host cell under suitable conditions to overexpress the polynucleotides a) and b) and to express the at least one polynucleotide encoding the at least one protein of interest, optionally
  - isolating the at least one protein of interest from the cell culture, optionally

- purifying the at least one protein of interest, optionally
  - modifying the at least one protein of interest, and optionally
  - formulating the at least one protein of interest.
4. The method of any one of claims 1-3, wherein the host cell is engineered to overexpress at least one polynucleotide encoding at least one Hac1 transcription factor, thereby enabling overexpression of the at least one Kar2 ER helper protein.
  5. The method of any one of claims 1-4, wherein the protein of interest is an enzyme, a therapeutic protein, a food additive or a feed additive.
  6. The method of any one of claims 1-5, wherein the overexpression of said polynucleotides of a) and b) increases the yield and/or titer of at least one of the model protein Fab (SEQ ID NOs: 55 and 56), scFv (SEQ ID NO: 57), or CES, in comparison to a host cell which is not engineered to overexpress said polynucleotides of a) and b), and/or wherein the yield and/or titer of the at least one protein of interest is increased at least about 1.1-fold, at least about 1.25-fold, at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 7.5-fold, or at least about 10-fold.
  7. The method of any one of claims 1-6, wherein at least one of the at least one polynucleotide encoding the at least one protein of interest and the polynucleotides of a) and b) are integrated in the genome of the host cell or contained in a vector or plasmid, which does not integrate into the genome of the host cell.
  8. The method of any one of claims 1-7, wherein the eukaryotic host cell is a fungal host cell.
  9. The method of claim 8, wherein the fungal host cell is a yeast host cell selected from the group consisting of *Komagataella phaffii*, *Hansenula polymorpha*, *Trichoderma reesei*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Yarrowia lipolytica*, *Pichia methanolica*, *Candida boidinii*, and *Schizosaccharomyces pombe*.

10. The method of any one of claims 1-9, wherein the Sbh1 transport protein comprises an amino acid sequence as shown in any one of SEQ ID NOs: 1-9 or a functional homolog thereof comprising at least 90% sequence identity to an amino acid sequence as shown in any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 9; and/or  
wherein the Kar2 ER helper protein comprises an amino acid sequence as shown in any one of SEQ ID NOs: 10-18 or a functional homolog thereof comprising at least 90% sequence identity to an amino acid sequence as shown in any one of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 or SEQ ID NO: 18.
11. The method of any one of claims 1-10, wherein the Hac1 transcription factor comprises at least:
  - a) a DNA binding domain comprising:
    - i) an amino acid sequence as shown in any one of SEQ ID NOs: 19-27, or
    - ii) a functional homolog thereof comprising at least 90% sequence identity to an amino acid sequence as shown in any one of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26 or SEQ ID NO: 27, and
  - b) an activation domain or  
wherein the Hac1 transcription factor comprises an amino acid sequence as shown in any one of SEQ ID NOs: 28-36 or a functional homolog thereof comprising at least 90% sequence identity to an amino acid sequence as shown in any one of SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35 or SEQ ID NO: 36.
12. The method of any one of claims 1-11, further comprising overexpressing in said host cell or engineering said host cell to overexpress at least one polynucleotide encoding at least one additional ER helper protein.
13. The method of claim 12, wherein said at least one additional ER helper protein comprises
  - (i) Lhs1 helper protein, preferably comprising an amino acid sequence as shown in any one of SEQ ID NOs: 37-46 or a functional homolog thereof comprising at least 90% sequence identity to an amino acid sequence as shown in any one of SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ

- ID NO: 44, SEQ ID NO: 45 or SEQ ID NO: 46; or
- (ii) Sil1 helper protein, preferably comprising an amino acid sequence as shown in any one of SEQ ID NOs: 47-54 or a functional homolog thereof comprising at least 90% sequence identity to an amino acid sequence as shown in any one of SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53 or SEQ ID NO: 54.
14. A recombinant eukaryotic host cell for manufacturing at least one protein of interest, which is engineered to overexpress:
- a) at least one polynucleotide encoding at least one Sbh1 transport protein as defined in any one of the preceding claims, and
- b) (i) at least one polynucleotide encoding at least one Kar2 endoplasmic reticulum (ER) helper protein as defined in any one of the preceding claims, or
- (ii) at least one polynucleotide encoding at least one Hac1 transcription factor as defined in any one of the preceding claims.
15. Use of the recombinant eukaryotic host cell of claim 14 for manufacturing at least one protein of interest.