CLAIMS

1. A method of producing a desired antibody protein in genus Pichia, comprising:

(a) first culturing the Pichia cells comprising one or more genes that provide for the expression of said desired protein at a first temperature which is 27°C–28.5°C; and

(b) next, culturing said Pichia cells at a second temperature which is 30°C–32°C, and allowing said Pichia cells to produce said desired antibody protein.

2. The method as claimed in claim 1, wherein said first temperature is 27.5°C–28.5°C.

3. The method as claimed in claim 1, wherein said second temperature is 30°C–31.5°C.

4. The method as claimed in claim 1, wherein said first temperature is 27.5°C–28.5°C, and the second temperature is 30°C–31.5°C.

5. The method as claimed in any one of claims 1–4, wherein said desired antibody protein comprises a humanized or human antibody.

6. The method as claimed in any one of claims 1–5, providing one or more of the following benefits:

(i) said method increases the yield of said desired antibody protein;

(ii) said method decreases the relative abundance of one or more antibody-associated variants relative to the same method effected without culturing at said first temperature and said second temperature;

(iii) said method decreases the relative abundance of antibody-associated variants having a higher or lower apparent molecular weight than said desired antibody protein as detected by size exclusion chromatography or gel electrophoresis relative to the same method effected without culturing at said first temperature and said second temperature;

(iv) said method decreases the relative abundance of antibody protein complexes having aberrant disulfide bonds relative to the same method effected without culturing at said first temperature and said second temperature;

(v) said method decreases the relative abundance of antibody protein complexes having reduced cysteines relative to the same method effected without culturing at said first temperature and said second temperature;

(vi) said method decreases the relative abundance of antibody protein complexes having aberrant glycosylation relative to the same method effected without culturing at said first temperature and said second temperature;

(vii) said method decreases the relative abundance of one or more antibody-associated variants relative to the same method effected without culturing at said first temperature and said second temperature;

(viii) said Pichia cells are selected from the group consisting of: Pichia angusta, Pichia guilliermondii, Pichia methanolica, and Pichia inositovera;

(ix) the genes that provide for expression of said desired antibody protein are integrated into one or more genomic loci in the Pichia cells;

(x) the genes that provide for expression of said desired antibody protein are integrated into one or more genomic loci in the Pichia cells, wherein at least one of said genomic loci is selected from the group consisting of the pGAP locus, 3' AOX TT locus; PpURA5; OCH1; AOX1; HIS4; GAP; pGAP; 3' AOX TT; ARG; and HIS4 TT locus;

(xi) at least one of the genes encoding subunits of said desired antibody protein are expressed under control of an inducible or constitutive promoter; and

(xii) at least one of the genes encoding subunits of said desired antibody protein are expressed under control of an inducible promoter selected from the group consisting of the AOX1, CUP1, tetracycline inducible, thiamine inducible, and FLD1 promoters.

7. The method as claimed in any one of claims 1–6, wherein step (a) comprises culturing said Pichia cells in a culture medium comprising glycerol as a carbon source until said glycerol is exhausted.

8. The method as claimed in any one of claims 1–7, wherein said desired antibody protein is expressed under control of a promoter selected from the group consisting of: yeast promoters, mammalian promoters, insect promoters, plant promoters, reptile promoters, amphibian promoters, viral promoters, and avian promoters.

9. The method as claimed in any one of claims 1–7, wherein said desired antibody protein is expressed under control of a promoter selected from the group consisting of: the CUP1, AOX1, ICL1, glyceraldehyde-3-phosphate dehydrogenase (GAP), FLD1, ADH1, alcohol dehydrogenase II, GAL4, PHO3, PHO5, and Pyk promoters, tetracycline inducible promoters, and thiamine inducible promoters.

10. The method as claimed in any one of claims 1–9, wherein said Pichia cell is a haploid, diploid, tetraploid cell, or polyploid cell.

11. The method as claimed in any one of claims 1–10, further comprising purifying said desired antibody protein from said Pichia cells or from a Pichia culture medium.

12. The method as claimed in claim 11, wherein said desired antibody protein is purified from an intracellular component, cytoplasm, nucleoplasm, or a membrane of said Pichia cells.

13. The method as claimed in any one of claims 1–11, wherein said Pichia cells secrete said desired antibody protein into the culture medium.

14. The method as claimed in any one of claims 1–13, comprising one or more of the following:

(i) step (a) comprises a batch phase, wherein, optionally, said batch phase comprises culturing the Pichia cells in a medium comprising a carbon source, and, optionally, the end of said batch phase is determined by exhaustion of the carbon source in the culture medium; and

(ii) step (b) comprises a fed-batch phase.

15. The method as claimed in any one of claims 1–14, the promoter or promoters which regulate the transcription of the gene or genes encoding the desired protein are not temperature inducible.