CLAIMS

1. A method of producing a multi-subunit complex, comprising:

   (a) providing a culture comprising yeast cells comprising genes that provide for the expression of the subunits of said multi-subunit complex;

   (b) adding a bolus of ethanol to said culture that results in a concentration of ethanol in said culture of between 0.2% and 2.75%; and

   (c) culturing said culture to produce said multi-subunit complex, wherein said culturing comprises adding a feed comprising a fermentable carbon source to said culture.

2. The method of **claim 1**, wherein the ethanol bolus enhances the formation of stable disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

3. The method of **claim 1**, wherein said multi-subunit complex contains one or more polypeptides comprising at least one disulfide bond.

4. The method of **claim 1**, wherein said multi-subunit complex comprises an antibody.

5. The method of any one of **claims 1 to 4**, which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

6. The method of any one of **claims 1 to 4**, which decreases the relative abundance of product-associated variants having a higher or lower apparent molecular weight than said desired multi-subunit complex as detected by size exclusion chromatography or gel electrophoresis relative to the same method effected in the absence of the bolus of ethanol.

7. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having aberrant stoichiometry relative to the same method effected in the absence of the bolus of ethanol.

8. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having aberrant disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

9. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having reduced cysteines relative to the same method effected in the absence of the bolus of ethanol.

10. The method of any one of **claims 1 to 4**, which decreases the relative abundance of complexes having aberrant glycosylation relative to the same method effected in the absence of the bolus of ethanol.

11. The method of **claim 4**, which modulates the formation or stability of inter-heavy chain disulfide bonds.

12. The method of **claim 4 or 11**, which modulates the formation or stability of disulfide bonds linking the light and heavy chains.

13. The method of any one of **claims 4 or 11 to 12**, which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

14. The method of **claim 13**, wherein said product-associated variants comprise one or more of the H1L1, H2L1, and H4L4 product-associate variants.

15. The method of any one of **claims 4 or 11 to 14**, which increases the purity of said antibody relative to said method effected in the absence of said bolus of ethanol.

16. The method of any one of the **foregoing claims**, wherein step (b) is effected prior to step (c).

17. The method of any one of **claims 1 to 15**, wherein step (b) is effected subsequent to step (c).

18. The method of any one of **claims 1 to 15**, wherein step (b) is effected concurrently with step (c).

19. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture of between 0.01% and 4% (w/v).

20. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture of between 0.4% and 2.25%, between 0.5% and 1.5%, between 0.5% and 2%, between 0.6% and 1.75%, between 0.7% and 1.5%, or between 0.8% and 1.25%.

21. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture that is at least 0.4%, 0.6%, 0.6%, 0.7%, 0.8% or 0.9% (w/v).

22. The method of any one of the **foregoing claims**, wherein step (b) results in a concentration of ethanol in said culture that is at most 2%, 1.8%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.35%, 0.3%, 0.25%, 0.2%, or 0.15% (w/v).

23. The method of any one of the **foregoing claims**, wherein step (b) comprises adding ethanol to said culture, adding a carrier comprising ethanol to said culture, adding said cells to a medium or carrier comprising ethanol, or replacing part of the culture medium.

24. The method of any one of the **foregoing claims**, wherein said bolus of ethanol is added to the culture medium over a period of time between 1 and 20 minutes.

25. The method of any one of the **foregoing claims**, wherein step (c) comprises providing oxygen to said cells.

26. The method of **claim 25**, wherein said providing oxygen comprises agitating said culture.

27. The method of **claim 25 or 26**, wherein said providing oxygen comprises contacting said culture with a gas mixture comprising oxygen.

28. The method of any one of the **foregoing claims**, wherein said fermentable carbon source comprises glucose or galactose.

29. The method of **claim 28**, wherein said feed comprises glucose.

30. The method of **claim 28**, wherein said feed comprises one or more of glucose, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

31. The method of any one of the **foregoing claims**, further comprising maintaining the concentration of ethanol between an upper set point and a lower set point during step (c).

32. The method of **claim 31**, wherein said lower set point is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.2%, 0.3%, 0.4%, 0.6%, 0.6%, 0.7%, 0.8% or 0.9% (w/v).

33. The method of **claim 31 or 32**, wherein said upper set point is 4%, 3.5%, 3%, 2.5%, 2%, 1.8%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.35%, 0.3%, 0.25%, 0.2%, or 0.15% (w/v).

34. The method of **claim 31 or 32**, wherein said upper set point is at most 1.5%, 1.4%, 1.3, 1.2%, or 1.1% (w/v).

35. The method of any one of **claims 1 to 30**, further comprising maintaining the concentration of ethanol at a set point during step (c).

36. The method of **claim 35**, wherein said set point is 0.1%, 0.2%, 0. 3%, 0. 4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 01.%, 01.1%, 01.2%, 01.3%, 01.4%, or 01.5% (w/v).

37. The method of any one of **claims 1 to 30**, wherein step (c) comprises maintaining the concentration of ethanol in said culture between 0.01% and 4%, between 0.02% and 3.75%, between 0.04% and 3.5%, between 0.08% and 3.25%, between 0.1% and 3%, between 0.2% and 2.75%, between 0.3% and 2.5%, between 0.4% and 2.25%, between 0.5% and 2%, between 0.6% and 1.75%, between 0.7% and 1.5%, or between 0.8% and 1.25%.

38. The method of any one of claims 21 to 27, wherein the concentration of ethanol in said culture is maintained by controlling production of ethanol by said cells or by addition of ethanol to said culture.

39. The method of claim 38, wherein the step of controlling production of ethanol comprises controlling one or more of the concentration of glucose, availability of oxygen, intensity of agitation, gas pressure, flow rate of supplied air or other gas mixture, viscosity of the culture, culture density, concentration of oxygen in the supplied air or other gas mixture, and temperature.

40. The method of any one of the **foregoing claims**, wherein the time between step (a) and step (b) is less than 72 hours, less than 48 hours, less than 24 hours, less than 12 hours, less than 9 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 90 minutes, less than 30 minutes, less than 5 minutes, or less than 1 minute.

41. The method of any one of the **foregoing claims**, wherein the time between step (b) and step (c) is less than 10 hours, less than 9 hours, less than 8 hours, less than 7 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, less than 90 minutes, less than 80 minutes, less than 70 minutes, less than 60 minutes, less than 50 minutes,

less than 40 minutes, less than 30 minutes, less than 20 minutes, less than 10 minutes, less than 5 minutes, or less than 1 minute.

42. The method of any one of the **foregoing claims**, wherein the culture of step (a) is produced by adding a carbon source to said culture, and culturing said culture until the carbon source is depleted.

43. The method of **claim 42**, wherein said carbon source comprises one or more of: glucose, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

44. The method of **claim 42 or 43**, wherein the depletion of the carbon source is determined by detecting a decrease in the metabolic activity of said cells.

45. The method of **claim 44**, wherein said decrease in the metabolic activity of said cells is identified by detecting a decrease in the consumption of oxygen by said cells, by detecting an increase in pH in the culture, by detecting stabilization of the wet cell mass, or by detecting an increase in the concentration of ammonia in the culture.

46. The method of **claim 45**, wherein said decrease in the consumption of oxygen by said cells is identified by detecting an increase in the concentration of dissolved oxygen in said culture.

47. The method of any one of the **foregoing claims**, wherein said bolus of ethanol results in a concentration of ethanol in said culture of between between 1% and 1.5%.

48. The method of **claim 1**, wherein said yeast cells comprise methylotrophic yeast.

49. The method of **claim 48**, wherein said methylotrophic yeast is of the genus *Pichia*.

50. The method of **claim 49**, wherein said methylotrophic yeast of the genus *Pichia* is *Pichia pastoris*.

51. The method of **claim 49**, wherein said methylotrophic yeast of the genus *Pichia* is selected from the group consisting of: *Pichia angusta*, *Pichia guillermordii*, *Pichia methanolica*, and *Pichia inositovera*.

52. The method of **claim 49**, wherein the genes that provide for expression of said multi-subunit complex are integrated into one or more genomic loci.

53. The method of claim 49, wherein at least one of said genomic loci are selected from the group consisting of the pGAP locus, 3' AOX TT locus; PpURA5; OCH1; AOX1; HIS4; GAP; pGAP; 3' AOX TT; ARG; and the HIS4 TT locus.

54. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are expressed under control of an inducible or constitutive promoter.

55. The method of **claim 54**, wherein said inducible promoter is selected from the group consisting of the AOX1, CUP1, tetracycline inducible, thiamine inducible, and FLD1 promoters.

56. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are 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, thiamine inducible promoters, chimeric promoters derived therefrom, yeast promoters, mammalian promoters, insect promoters, plant promoters, reptile promoters, amphibian promoters, viral promoters, and avian promoters.

57. The method of any one of the **foregoing claims**, wherein said cell is a diploid, tetraploid cell, or polyploid.

58. The method of any one of the **foregoing claims**, further comprising purifying said multi-subunit complex from said cells or from the culture medium.

59. The method of **claim 58**, wherein said multi-subunit complex is purified from an intracellular component, cytoplasm, nucleoplasm, or a membrane of said cells.

60. The method of **claim 58**, wherein said cells secrete said multi-subunit complex into the culture medium.

61. The method of **claim 60**, wherein said multi-subunit complex is purified from said culture medium.

62. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises a monospecific or bispecific antibody.

63. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises a human antibody or a humanized antibody or fragment thereof.

64. The method of **claim 63**, wherein said humanized antibody is of mouse, rat, rabbit, goat, sheep, or cow origin.

65. The method of **claim 63**, wherein said humanized antibody is of rabbit origin.

66. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises a monovalent, bivalent, or multivalent antibody.

67. The method of **claim 4 or any one of claims 61 to 66**, wherein said antibody is purified from said culture by protein A and/or protein G affinity.

68. The method of any one of the **foregoing claims**, wherein at least one of the genes that provide for expression of a subunit of said multi-subunit complex is optimized for expression in said cell.

69. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises an antibody and the purity of said antibody is assessed by measuring the fraction of the antibody produced by said cell that is contained in antibody complexes having the expected apparent hydrodynamic radius, is contained in antibody complexes having the expected molecular weight, and / or specifically binds a target of said antibody.

70. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises an antibody and the yield of said antibody is assessed by determining the amount of antibody produced by said cell discounting any product-associated variants that are abnormally glycosylated, contained in antibody complexes other than complexes having the expected apparent hydrodynamic radius, contained in antibody complexes having the expected molecular weight, and / or that fail to specifically bind to the target of said antibody.

71. The method of claim 69 or 70, wherein the molecular weight of said antibody complexes is determined by non-reducing SDS-PAGE.

72. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises an antibody, said method further comprising purifying said antibody.

73. The method of any one of the **foregoing claims**, wherein said culture cell produces a supernatant antibody titer of at least 100 mg / L, at least 150 mg / L, at least 200 mg / L, at least 250 mg / L, at least 300 mg / L, between 100 and 300 mg / L, between 100 and 500 mg / L, between 100 and 1000 mg / L, at least 1000 mg / L, at least 1250 mg/liter, at least 1500 mg/liter, at least 1750 mg/liter, at least 2000 mg/liter, at least 10000 mg/liter, or more.

74. The method of any one of the **foregoing claims**, wherein one or more subunits of said multi-subunit complex are expressed from more than one gene copy.

75. The method of any one of the **foregoing claims**, wherein said multi-subunit complex comprises an antibody which is expressed from between 1-10 copies of a gene encoding the light chain of said antibody and from 1-10 copies of a gene encoding the heavy chain of said antibody.

76. The method of any one of the **foregoing claims**, wherein genes that provide for expression of said multi-subunit complex are integrated into genome of said cells.

77. The method of any one of claims **1-75**, wherein genes that provide for expression of said multi-subunit complex are contained on an extrachromosomal element, plasmid, or artificial chromosome.

78. The method of **claim 76 or 77**, wherein said cells comprise more copies of the gene that provide for the expression of the light chain of said antibody than copies of the gene that provide for expression of the heavy chain of said antibody.

79. The method of **claim 76 or 76**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 2, 2 and 3, 3 and 3, 3 and 4, 3 and 5, 4 and 3, 4 and 4, 4 and 5, 4 and 6, 5 and 4, 5 and 5, 5 and 6, or 5 and 7.

80. The method of **claim 76 or 77**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 1, 3 and 1, 4 and 1, 5 and 1, 6 and 1, 7 and 1, 8 and 1, 9 and 1, 10 and 1, 1 and 2, 2 and 2, 3 and 2, 4 and 2, 5 and 2, 6 and 2, 7 and 2, 8 and 2, 9 and 2, 10 and 2, 1 and 3, 2 and 3, 3 and 3, 4 and 3, 5 and 3, 6 and 3, 7 and 3, 8 and 3, 9 and 3, 10 and 3, 1 and 4, 2 and 4, 3 and 4, 4 and 4, 5 and 4, 6 and 4, 7 and 4, 8 and 4, 9 and 4, 10 and 4, 1 and 5, 2 and 5, 3 and 5, 4 and 5, 5 and 5, 6 and 5, 7 and 5, 8 and 5, 9 and 5, 10 and 5, 1 and 6, 2 and 6, 3 and 6, 4 and 6, 5 and 6, 6 and 6, 7 and 6, 8 and 6, 9 and 6, 10 and 6, 1 and 7, 2 and 7, 3

and 7, 4 and 7, 5 and 7, 6 and 7, 7 and 7, 8 and 7, 9 and 7, 10 and 7, 1 and 8, 2 and 8, 3 and 8, 4 and 8, 5 and 8, 6 and 8, 7 and 8, 8 and 8, 9 and 8, 10 and 8, 1 and 9, 2 and 9, 3 and 9, 4 and 9, 5 and 9, 6 and 9, 7 and 9, 8 and 9, 9 and 9, 10 and 9, 1 and 10, 2 and 10, 3 and 10, 4 and 10, 5 and 10, 6 and 10, 7 and 10, 8 and 10, 9 and 10, 10 and 10.

81. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown in a production medium.

82. The method of **claim 81**, wherein said production medium is a minimal medium.

83. The method of **claim 81**, wherein said minimal medium lacks selective agents.

84. The method of **claim 81**, wherein said minimal medium lacks pre-formed amino acids or other complex biomolecules.

85. The method of **claim 81**, wherein the production medium is a complex medium.

86. The method of **claim 85**, wherein the complex medium comprises one or more of yeast extract, soy peptones, and other plant peptones.

87. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown to a high cell density.

88. The method of **claim 87**, wherein said high cell density is at least 50 g/L.

89. The method of **claim 87**, wherein said high cell density is at least 100 g/L.

90. The method of **claim 87**, wherein said high cell density is at least 300 g/L.

91. The method of **claim 87**, wherein said high cell density is at least 400 g/L.

92. The method of **claim 87**, wherein said high cell density is at least 500 g/L.

93. The method of **claim 87**, wherein said high cell density is at least 750 g/L.

94. The method of any one of the **foregoing claims**, wherein the yeast cells are cultured for at least 20 doublings and maintain high levels of expression of said multi-subunit complex after said at least 20 doublings.

95. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 50 doublings and maintain high levels of expression of said multi-subunit complex after said at least 50 doublings.

96. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 100 doublings and maintain high levels of expression of said multi-subunit complex after said at least 100 doublings.

97. The method of any one of the **foregoing claims**, wherein at least one subunit of said multi-subunit complex comprises a secretion signal.

98. The method of **claim 97**, wherein said multi-subunit complex comprises an antibody.

99. The method or composition of **claim 97 or 98**, wherein the secretion signal comprises one or more polypeptides selected from the group consisting of: SEQ ID NOS: 414 to 437 and any combination thereof.

100. The method of any one of the foregoing claims, wherein said multi-subunit complex is not Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, and Ab21-NGF, or an Fab2 or Fab1 fragment thereof.

101. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not contain at least one, at least two, at least three, at least four, at least five, or at least all six of the complementarity determining regions (CDRs) contained in any of the following

antibodies: Ab1-NGF, Ab2-NGF, Ab3-NGF, Ab4-NGF, Ab5-NGF, Ab6-NGF, Ab7-NGF, Ab8-NGF, Ab9-NGF, Ab10-NGF, Ab11-NGF, Ab12-NGF, Ab13-NGF, Ab14-NGF, Ab15-NGF, Ab16-NGF, Ab17-NGF, Ab18-NGF, Ab19-NGF, Ab20-NGF, or Ab21-NGF and optionally having binding specificity for NGF.

102. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not comprise or consist of the light and heavy chain polypeptide sequences of SEQ ID NOs: 51 and 401, respectively, SEQ ID NOs: 53 and 402, respectively, SEQ ID NOs: 405 and 406, respectively, and SEQ ID NOs: 407 and 408, respectively.

103. The method of any one of the **foregoing claims**, wherein said multi-subunit complex does not comprise an antibody containing at least one, at least two, at least three, at least four, at least five, or at least all six of the CDRs of SEQ ID NOs: 55, 56, 57, 58, 59, and 60, and optionally having binding specificity for NGF.

CLAIMS

1. A method of producing secreted full length antibodies, comprising:

(a) providing a growth culture comprising *Pichia pastoris* yeast cells comprising genes that provide for the expression and secretion of full length antibodies by said yeast cells , and growing said Pichia yeast for 12-16 hours ("growth phase");

(b) adding ethanol to said culture after said growth phase in a single bolus addition ("bolus") which bolus results in a concentration of ethanol in said culture of between 0.5% and 1.5% w/v, and

(c) culturing said culture to which ethanol bolus has been added to produce said secreted, full length antibodies, wherein said culturing comprises adding a feed comprising a fermentable carbon source to said culture.

2. The method of **claim 1**, wherein the ethanol bolus enhances the formation of stable disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

3. The method of any one of **claims 1 or 2**, which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

4. The method of any one of **claims 1 to 3**, which decreases the relative abundance of product-associated variants having a higher or lower apparent molecular weight than said desired full length antibodies as detected by size exclusion chromatography or gel electrophoresis relative to the same method effected in the absence of the bolus of ethanol.

5. The method of any one of **claims 1 to 3** which decreases the relative abundance of complexes having aberrant stoichiometry relative to the same method effected in the absence of the bolus of ethanol.

6. The method of any one of **claims 1 to 3**, which decreases the relative abundance of complexes having aberrant disulfide bonds relative to the same method effected in the absence of the bolus of ethanol.

7. The method of any one of **claims 1 to 3**, which decreases the relative abundance of complexes having reduced cysteines relative to the same method effected in the absence of the bolus of ethanol.

8. The method of any one of **claims 1 to 3**, which decreases the relative abundance of complexes having aberrant glycosylation relative to the same method effected in the absence of the bolus of ethanol.

9. The method of **claim 1**, which modulates the formation or stability of inter-heavy chain disulfide bonds.

10. The method of **claim 9**, which modulates the formation or stability of disulfide bonds linking the light and heavy chains.

11. The method of any one of **claims 1, 9 or 10** which decreases the relative abundance of one or more product-associated variants relative to the same method effected in the absence of the bolus of ethanol.

12. The method of **claim 11**, wherein said product-associated variants comprise one or more of the H1L1, H2L1, and H4L4 product-associate variants.

13. The method of any one of **claims 1 to 12**, which increases the purity of said antibody relative to said method effected in the absence of said bolus of ethanol.

14. The method of any one of the **foregoing claims**, wherein step (b) is effected about 15-30 minutes, allowing the ethanol bolus containing culture to equilibrate prior to step (c).

15. The method of any one of the **foregoing claims**, wherein step (c) comprises providing oxygen to said cells.

16. The method of **claim 15**, wherein said providing oxygen comprises agitating said culture.

17. The method of **claim 15 or 16**, wherein said providing oxygen comprises contacting said culture with a gas mixture comprising oxygen.

18. The method of any one of the **foregoing claims**, wherein said fermentable carbon source comprises glucose or galactose.

19. The method of **claim 18**, wherein said fermentable carbon source comprises glucose.

20. The method of **claim 18**, wherein said feed comprises one or more of glucose, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

21. The method of any one of the **foregoing claims**, wherein the time between step (b) and step (c) is less than 10 hours, less than 9 hours, less than 8 hours, less than 7 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, less than 90 minutes, less than 80 minutes, less than 70 minutes, less than 60 minutes, less than 50 minutes, less than 40 minutes, less than 30 minutes, less than 20 minutes, less than 10 minutes, less than 5 minutes, or less than 1 minute.

22. The method of any one of the **foregoing claims**, wherein the culture of step (a) is produced by adding a carbon source to said culture, and culturing said culture until the carbon source is depleted.

23. The method of **claim 22**, wherein said carbon source comprises one or more of: glucose, citrate, sorbitol, xylose, trehalose, arabinose, galactose, fructose, melibiose, lactose, maltose, rhamnose, ribose, mannose, mannitol, and raffinose.

24. The method of **claim 22 or 23**, wherein the depletion of the carbon source is determined by detecting a decrease in the metabolic activity of said cells.

25. The method of **claim 24**, wherein said decrease in the metabolic activity of said cells is identified by detecting a decrease in the consumption of oxygen by said cells, by detecting an increase in pH in the culture, by detecting stabilization of the wet cell mass, or by detecting an increase in the concentration of ammonia in the culture.

26. The method of **claim 25**, wherein said decrease in the consumption of oxygen by said cells is identified by detecting an increase in the concentration of dissolved oxygen in said culture.

27. The method of any one of the **foregoing claims**, wherein said bolus of ethanol results in a concentration of ethanol in said culture of between 1% and 1.5% w/v.

28. The method of **claim 1**, wherein the genes that provide for expression of said full length antibody are integrated into one or more genomic loci.

29. The method of **claim 28**, wherein at least one of said genomic loci are selected from the group consisting of the pGAP locus, 3' AOX TT locus; PpURA5; OCH1; AOX1; HIS4; GAP; pGAP; 3' AOX TT; ARG; and the HIS4 TT locus.

30. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are expressed under control of an inducible or constitutive promoter.

31. The method of **claim 30**, wherein said inducible promoter is selected from the group consisting of the AOX1, CUP1, tetracycline inducible, thiamine inducible, and FLD1 promoters.

32. The method of any one of the **foregoing claims**, wherein at least one of the genes encoding said subunits of the multi-subunit complex are 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, thiamine inducible promoters, chimeric promoters derived therefrom, yeast promoters, mammalian promoters, insect promoters, plant promoters, reptile promoters, amphibian promoters, viral promoters, and avian promoters.

33. The method of any one of the **foregoing claims**, wherein said *Pichia pastoris* cells are diploid, tetraploid cell, or polyploid.

34. The method of any one of the **foregoing claims**, further comprising purifying said full length antibody from said cells or from the culture medium.

35. The method of **claim 34**, wherein said cells secrete said full length antibody into the culture medium.

36. The method of **claim 35**, wherein said full length antibody is purified from said culture medium.

37. The method of any one of the **foregoing claims**, wherein said full length antibody comprises a monospecific or bispecific antibody.

38. The method of any one of the **foregoing claims**, wherein said full length antibody comprises a human antibody or a humanized antibody or fragment thereof.

39. The method of **claim 38**, wherein said humanized antibody is of mouse, rat, rabbit, goat, sheep, or cow origin.

40. The method of **claim 38**, wherein said humanized antibody is of rabbit origin.

41. The method of any one of the **foregoing claims**, wherein said full length antibody comprises a monovalent, bivalent, or multivalent antibody.

42. The method of any one of the **foregoing claims**, wherein said antibody is purified from said culture by protein A and/or protein G affinity.

43. The method of any one of the **foregoing claims**, wherein at least one of the genes that provide for expression of a subunit of said antibody is optimized for expression in said cell.

44. The method of any one of the **foregoing claims**, wherein the purity of said antibody is assessed by measuring the fraction of the antibody produced by said cell that is contained in antibody complexes having the expected apparent hydrodynamic radius, is contained in antibody complexes having the expected molecular weight, and / or specifically binds a target of said antibody.

45. The method of any one of the **foregoing claims**, wherein the yield of said antibody is assessed by determining the amount of antibody produced by said cell discounting any product-associated variants that are abnormally glycosylated, contained in antibody complexes other than complexes having the expected apparent hydrodynamic radius, contained in antibody complexes having the expected molecular weight, and / or that fail to specifically bind to the target of said antibody.

46. The method of **claim 44 or 45**, wherein the molecular weight of said antibody complexes is determined by non-reducing SDS-PAGE.

47. The method of any one of the **foregoing claims**, further comprising purifying said antibody.

48. The method of any one of the **foregoing claims**, wherein said culture cell produces a supernatant antibody titer of at least 100 mg / L, at least 150 mg / L, at least 200 mg / L, at

least 250 mg / L, at least 300 mg / L, between 100 and 300 mg / L, between 100 and 500 mg / L, between 100 and 1000 mg / L, at least 1000 mg / L, at least 1250 mg/liter, at least 1500 mg/liter, at least 1750 mg/liter, at least 2000 mg/liter, at least 10000 mg/liter, or more.

49. The method of any one of the **foregoing claims**, wherein one or more subunits of said multi-subunit complex are expressed from more than one gene copy.

50. The method of any one of the **foregoing claims**, wherein said antibody is expressed from between 1-10 copies of a gene encoding the light chain of said antibody and from 1-10 copies of a gene encoding the heavy chain of said antibody.

51. The method of any one of the **foregoing claims**, wherein genes that provide for expression of said antibody are integrated into genome of said cells.

52. The method of any one of the **foregoing claims**, wherein genes that provide for expression of said antibody are contained on an extrachromosomal element, plasmid, or artificial chromosome.

53. The method of **claim 51 or 52**, wherein said cells comprise more copies of the gene that provide for the expression of the light chain of said antibody than copies of the gene that provide for expression of the heavy chain of said antibody.

54. The method of **claim 51 or 52**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 2, 2 and 3, 3 and 3, 3 and 4, 3 and 5, 4 and 3, 4 and 4, 4 and 5, 4 and 6, 5 and 4, 5 and 5, 5 and 6, or 5 and 7.

55. The method of **claim 51 or 52**, wherein the respective number of copies of the gene encoding the heavy chain of said antibody and the number of copies of the gene encoding the light chain of said antibody in said cells are: 2 and 1, 3 and 1, 4 and 1, 5 and 1, 6 and 1, 7 and

1, 8 and 1, 9 and 1, 10 and 1, 1 and 2, 2 and 2, 3 and 2, 4 and 2, 5 and 2, 6 and 2, 7 and 2, 8 and 2, 9 and 2, 10 and 2, 1 and 3, 2 and 3, 3 and 3, 4 and 3, 5 and 3, 6 and 3, 7 and 3, 8 and 3, 9 and 3, 10 and 3, 1 and 4, 2 and 4, 3 and 4, 4 and 4, 5 and 4, 6 and 4, 7 and 4, 8 and 4, 9 and 4, 10 and 4, 1 and 5, 2 and 5, 3 and 5, 4 and 5, 5 and 5, 6 and 5, 7 and 5, 8 and 5, 9 and 5, 10 and 5, 1 and 6, 2 and 6, 3 and 6, 4 and 6, 5 and 6, 6 and 6, 7 and 6, 8 and 6, 9 and 6, 10 and 6, 1 and 7, 2 and 7, 3 and 7, 4 and 7, 5 and 7, 6 and 7, 7 and 7, 8 and 7, 9 and 7, 10 and 7, 1 and 8, 2 and 8, 3 and 8, 4 and 8, 5 and 8, 6 and 8, 7 and 8, 8 and 8, 9 and 8, 10 and 8, 1 and 9, 2 and 9, 3 and 9, 4 and 9, 5 and 9, 6 and 9, 7 and 9, 8 and 9, 9 and 9, 10 and 9, 1 and 10, 2 and 10, 3 and 10, 4 and 10, 5 and 10, 6 and 10, 7 and 10, 8 and 10, 9 and 10, 10 and 10.

56. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown in a production medium.

57. The method of **claim 56**, wherein said production medium is a minimal medium.

58. The method of **claim 56**, wherein said minimal medium lacks selective agents.

59. The method of **claim 58**, wherein said minimal medium lacks pre-formed amino acids or other complex biomolecules.

60. The method of **claim 58**, wherein the production medium is a complex medium.

61. The method of **claim 60**, wherein the complex medium comprises one or more of yeast extract, soy peptones, and other plant peptones.

62. The method of any one of the **foregoing claims**, wherein the culture of step (c) is grown to a high cell density.

63. The method of **claim 62**, wherein said high cell density is at least 50 g/L.

64. The method of **claim 62**, wherein said high cell density is at least 100 g/L.

65. The method of **claim 62**, wherein said high cell density is at least 300 g/L.

66. The method of **claim 62**, wherein said high cell density is at least 400 g/L.

67. The method of **claim 62**, wherein said high cell density is at least 500 g/L.

68. The method of **claim 62**, wherein said high cell density is at least 750 g/L.

69. The method of any one of the **foregoing claims**, wherein the yeast cells are cultured for at least 20 doublings and maintain high levels of expression of said full length antibodies after said at least 20 doublings.

70. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 50 doublings and maintain high levels of expression of said full length antibodies after said at least 50 doublings.

71. The method of any one of the **foregoing claims**, wherein the cells of step (c) are cultured for at least 100 doublings and maintain high levels of expression of said full length antibodies after said at least 100 doublings.

72. The method of any one of the **foregoing claims**, wherein at least one subunit of said full length antibodies comprises a secretion signal.

73. The method of **claim 72**, wherein said antibody is humanized.