CLAIMS

What is claimed is:

1. Recombinant cells capable of increased production of isoprene wherein the cells are engineered for increased carbon flux towards isoprene production such that the activity of one or more enzymes from the group consisting of:

(a) citrate synthase,

(b) phosphotransacetylase

(c) acetate kinase,

(d) lactate dehydrogenase,

(e) malate dehydrogenase,

(f) pyruvate dehydrogenase,

(g) phosphogluconolactonase, and

(h) and phosphoenolpyruvate carboxylase

is modulated, and wherein said cells further comprise (i) one or more nucleic acids encoding one or more mevalonate (MVA) pathway polypeptides and (ii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide; and wherein said cells produce increased amounts of isoprene compared to isoprene-producing cells that have not been engineered for increased carbon flux towards isoprene.

2. The cells of claim 1 wherein the one or more nucleic acids encoding MVA pathway polypeptides are from the upper MVA pathway, wherein the upper MVA pathway nucleic acids are selected from the group consisting of AA-CoA thiolase, HMG-CoA synthase, and HMG-CoA reductase nucleic acids.

3. The cells of claim 2 wherein said one or more nucleic acids encoding an upper mevalonate (MVA) pathway polypeptide is an *mvaE* gene and an *mvaS* gene.

4. The cells of claim 3, wherein the *mvaE* gene and the *mvaS* gene is selected from the group consisting of: (a) an *mvaE* gene and an *mvaS* gene from *L. grayi*; (b) an *mvaE* gene and an *mvaS* gene from *E. faecium*; (c) an *mvaE* gene and an *mvaS* gene from *E. gallinarum*; (d) an *mvaE* gene and an *mvaS* gene from *E. casseliflavus*; and (e) an *mvaE* gene and an *mvaS* gene from *E. faecalis*.

5. The cells of claim 1 wherein the one or more nucleic acids encoding MVA pathway polypeptides are from the lower MVA pathway, wherein the lower MVA pathway nucleic acids are selected from the group consisting of MVK, PMK, and MVD nucleic acids.

6. The cells of claim 5, wherein the MVK is selected from the group consisting of *M. mazei* mevalonate kinase, *M. burtonii* mevalonate kinase polypeptide, *Lactobacillus* mevalonate kinase polypeptide, *Lactobacillus sakei* mevalonate kinase polypeptide, yeast mevalonate kinase polypeptide, *Saccharomyces cerevisiae* mevalonate kinase polypeptide, *Streptococcus* mevalonate kinase polypeptide, *Streptococcus pneumoniae* mevalonate kinase polypeptide, *Streptomyces* mevalonate kinase polypeptide, and *Streptomyces* CL190 mevalonate kinase polypeptide.

7. The cells of claim 1, wherein the cells further comprise one or more heterologous nucleic acids encoding one or DXP pathway polypeptides.

8. The cells of claim 1, wherein the isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid, *Populus alba* x *Populus tremula* or variant thereof.

9. The cells of claim 1, wherein the cells are gram-positive bacterial cells, *Streptomyces* cells, gram-negative bacterial cells, *Escherichia* cells, *Pantoea* cells, fungal cells, filamentous fungal cells, *Trichoderma* cells, *Aspergillus* cells, or yeast cells.

10. The cells of claim 1, wherein the activity of citrate synthase is modulated by decreasing the activity of an endogenous citrate synthase gene.

11. The cells of claim 10, wherein the activity of citrate synthase is modulated by chromosomal replacement of an endogenous citrate synthase gene with a transgene encoding an NADH-insensitive citrate synthase.

12. The cells of claim 11, wherein the transgene encoding an NADH-insensitive citrate synthase is derived from *Bacillus subtilis*.

13. The cells of claim 10, wherein the activity of citrate synthase is modulated by replacing the endogenous citrate synthase gene promoter with a synthetic constitutively low expressing promoter.

14. The cells of any one of claims 10-13, wherein decreasing the activity of citrate synthase results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have decreased expression of citrate synthase.

15. The cells claim 1, wherein carbon flux is directed towards mevalonate production by modulating the activity of phosphotransacetylase and/or acetate kinase.

16. The cells of claim 15, wherein the activity of phosphotransacetylase and/or acetate kinase is modulated by attenuating the activity of an endogenous phosphotransacetylase gene and/or an endogenous acetate kinase gene.

17. The cells of claim 16, wherein endogenous phosphotransacetylase and/or endogenous acetate kinase gene expression is attenuated by deletion of the endogenous phosphotransacetylase gene and/or the endogenous acetate kinase gene.

18. The cells of claim 15 or 16, wherein the cells produces decreased amounts of acetate in comparison to microorganisms that do not have attenuated endogenous phosphotransacetylase gene and/or endogenous acetate kinase gene expression.

19. The cells of any one of claims 1-9 or 15-18, wherein attenuating the activity of the endogenous phosphotransacetylase gene and/or the endogenous acetate kinase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have attenuated endogenous phosphotransacetylase gene and/or endogenous acetate kinase gene expression

20. The cells of claim 1, wherein carbon flux is directed towards mevalonate production by modulating the activity of lactate dehydrogenase.

21. The cells of claim 20, wherein the activity of lactate dehydrogenase is modulated by attenuating the activity of an endogenous lactate dehydrogenase gene.

22. The cells of claim 21, wherein endogenous lactate dehydrogenase gene expression is attenuated by deletion of the endogenous lactate dehydrogenase gene.

23. The cells of any one of claims 20-22, wherein the cells produces decreased amounts of lactate in comparison to microorganisms that do not have attenuated endogenous lactate dehydrogenase gene expression.

24. The cells of any one of claims 20-23, wherein attenuating the activity of the endogenous lactate dehydrogenase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have attenuated endogenous lactate dehydrogenase gene expression.

25. The cells of any one of claims 1, wherein carbon flux is directed towards mevalonate production by modulating the activity of NADP-dependent malate dehydrogenase.

26. The cells of claim 25, wherein the activity of NADP-dependent malate dehydrogenase is modulated by increasing the activity of an NADP-dependent malate dehydrogenase gene.

27. The cells of claim 26, wherein the NADP-dependent malate dehydrogenase gene is an endogenous gene.

28. The cells of claim 27, wherein expression of the endogenous NADP-dependent malate dehydrogenase gene is increased by replacing the endogenous NADP-dependent malate dehydrogenase gene promoter with a synthetic constitutively expressing promoter.

29. The cells of claim 25 or 26, wherein the cells further comprises a heterologous nucleic acid encoding an NADP-dependent malate dehydrogenase polypeptide.

30. The cells of any one of claims 25-29, wherein the cells produces increased amounts of pyruvate in comparison to microorganisms that do not have increased expression of an NADP-dependent malate dehydrogenase gene.

31. The cells of any one of claims 25-30, wherein increasing the activity of an NADP-dependent malate dehydrogenase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have increased NADP-dependent malate dehydrogenase gene expression.

32. The cells of any one of claims 1, wherein carbon flux is directed towards mevalonate production by modulating the activity of pyruvate dehydrogenase.

33. The cells of claim 32, wherein the activity of pyruvate dehydrogenase is modulated by increasing the activity of one or more genes of the pyruvate dehydrogenase complex consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

34. The cells of claims 32 or 33, wherein the activity of the pyruvate dehydrogenase complex is modulated by attenuating the activity of an endogenous pyruvate dehydrogenase complex repressor gene.

35. The cells of claim 34, wherein the one or more genes of the pyruvate dehydrogenase complex are endogenous genes.

36. The cells of claim 35, wherein expression of the one or more endogenous genes of the pyruvate dehydrogenase complex is increased by replacing one or more endogenous gene promoters with one or more synthetic constitutively expressing promoters.

37. The cells of claim 32 or 33, wherein the cells further comprises one or more heterologous nucleic acids encoding one or more polypeptides from the group consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

38. The cells of claim 34, wherein the activity of an endogenous pyruvate dehydrogenase complex repressor is attenuated by deletion of the endogenous pyruvate dehydrogenase complex repressor gene.

39. The cells of any one of claims 32-38, wherein the cells produces increased amounts of acetyl Co-A in comparison to microorganisms wherein the activity of pyruvate dehydrogenase is not modulated.

40. The cells of any one of claims 32-39, wherein modulating the activity of pyruvate dehydrogenase results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have modulated pyruvate dehydrogenase expression.

41. The cells of claim 1, wherein carbon flux is directed towards mevalonate production by modulating the activity of phosphogluconolactonase (PGL).

42. The cells of claim 41, wherein the activity of PGL is modulated by attenuating the activity of an endogenous PGL gene.

43. The cells of claim 42, wherein the activity of PGL is attenuated by replacing the endogenous PGL gene promoter with a synthetic constitutively low expressing promoter.

44. The cells of claim 43, wherein the activity of an endogenous PGL is attenuated by deletion of the endogenous PGL gene.

45. The cells of claim 1, wherein carbon flux is directed towards mevalonate production by modulating the activity of phosphoenolpyruvate carboxylase.

46. The cells of claim 45, wherein the activity of phosphoenolpyruvate carboxylase is modulated by attenuating the activity of an endogenous phosphoenolpyruvate carboxylase gene.

47. The cells of claim 46, wherein the activity of phosphoenolpyruvate carboxylase is attenuated by replacing the endogenous phosphoenolpyruvate carboxylase gene promoter with a synthetic constitutively low expressing promoter.

48. The cells of claim 47, wherein the activity of an endogenous phosphoenolpyruvate carboxylase is attenuated by deletion of the endogenous phosphoenolpyruvate carboxylase gene.

49. A method of producing isoprene, comprising: (a) culturing the cells of claim 1 under suitable culture conditions for production of isoprene; and (b) producing the isoprene.

50. Recombinant cells capable of increased production of mevalonate wherein the cells are engineered for increased carbon flux towards mevalonate, production such that the activity of one or more enzymes from the group consisting of:

(a) citrate synthase,

(b) phosphotransacetylase

(c) acetate kinase,

(d) lactate dehydrogenase,

(e) malate dehydrogenase,

(f) pyruvate dehydrogenase,

(g) phosphogluconolactonase, and

(h) and phosphoenolpyruvate carboxylase

is modulated and wherein said cells further comprise one or more nucleic acids encoding one or more upper mevalonate (MVA) pathway polypeptides; and wherein the cells produce increased amounts of mevalonate compared to mevalonate-producing cells that have not been engineered for increased carbon flux towards mevalonate.

51. The cells of claim 50, wherein said one or more nucleic acids encoding an upper mevalonate (MVA) pathway polypeptide is an *mvaE* gene and an *mvaS* gene.

52. The cells of claim 50, wherein the *mvaE* gene and the *mvaS* gene is selected from the group consisting of: (a) an *mvaE* gene and an *mvaS* gene from *L. grayi*; (b) an *mvaE* gene and an *mvaS* gene from *E. faecium*; (c) an *mvaE* gene and an *mvaS* gene from *E. gallinarum*; (d) an

mvaE gene and an *mvaS* gene from *E. casseliflavus*; and (e) an *mvaE* gene and an *mvaS* gene from *E. faecalis*.

53. The cells of claim 50, wherein the activity of citrate synthase is modulated by decreasing the activity of an endogenous citrate synthase gene.

54. The cells of claim 53 wherein the activity of citrate synthase is modulated by chromosomal replacement of an endogenous citrate synthase gene with a transgene encoding an NADH-insensitive citrate synthase.

55. The cells of claim 54, wherein the transgene encoding an NADH-insensitive citrate synthase is derived from *Bacillus subtilis*.

56. The cells of claim 53, wherein the activity of citrate synthase is modulated by replacing the endogenous citrate synthase gene promoter with a synthetic constitutively low expressing promoter.

57. The cells of any one of claims 53-54, wherein decreasing the activity of citrate synthase results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have decreased expression of citrate synthase.

58. The cells of claim 50, wherein carbon flux is directed towards mevalonate production by modulating the activity of phosphotransacetylase and/or acetate kinase.

59. The cells of claim 58, wherein the activity of phosphotransacetylase and/or acetate kinase is modulated by attenuating the activity of an endogenous phosphotransacetylase gene and/or an endogenous acetate kinase gene.

60. The cells of claim 59, wherein endogenous phosphotransacetylase and/or endogenous acetate kinase gene expression is attenuated by deletion of the endogenous phosphotransacetylase gene and/or the endogenous acetate kinase gene.

61. The cells of claim 58 or claim 59, wherein the cells produces decreased amounts of acetate in comparison to microorganisms that do not have attenuated endogenous phosphotransacetylase gene and/or endogenous acetate kinase gene expression.

62. The cells of any one of claims 50-52 or 58-61, wherein attenuating the activity of the endogenous phosphotransacetylase gene and/or the endogenous acetate kinase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have attenuated endogenous phosphotransacetylase gene and/or endogenous acetate kinase gene expression.

63. The cells of claim 50, wherein carbon flux is directed towards mevalonate production by modulating the activity of lactate dehydrogenase.

64. The cells of claim 63, wherein the activity of lactate dehydrogenase is modulated by attenuating the activity of an endogenous lactate dehydrogenase gene.

65. The cells of claim 64, wherein endogenous lactate dehydrogenase gene expression is attenuated by deletion of the endogenous lactate dehydrogenase gene.

66. The cells of any one of claims 64-65, wherein the cells produces decreased amounts of lactate in comparison to microorganisms that do not have attenuated endogenous lactate dehydrogenase gene expression.

67. The cells of any one of claims 64-66, wherein attenuating the activity of the endogenous lactate dehydrogenase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have attenuated endogenous lactate dehydrogenase gene expression.

68. The cells of claim 50, wherein carbon flux is directed towards mevalonate production by modulating the activity of NADP-dependent malate dehydrogenase.

69. The cells of claim 68, wherein the activity of NADP-dependent malate dehydrogenase is modulated by increasing the activity of an NADP-dependent malate dehydrogenase gene.

70. The cells of claim 69, wherein the NADP-dependent malate dehydrogenase gene is an endogenous gene.

71. The cells of claim 70, wherein expression of the endogenous NADP-dependent malate dehydrogenase gene is increased by replacing the endogenous NADP-dependent malate dehydrogenase gene promoter with a synthetic constitutively expressing promoter.

72. The cells of claim 68 or 69, wherein the cells further comprises a heterologous nucleic acid encoding an NADP-dependent malate dehydrogenase polypeptide.

73. The cells of any one of claims 68-72, wherein the cells produces increased amounts of pyruvate in comparison to microorganisms that do not have increased expression of an NADP-dependent malate dehydrogenase gene.

74. The cells of any one of claims 68-73, wherein increasing the activity of an NADP-dependent malate dehydrogenase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have increased NADP-dependent malate dehydrogenase gene expression.

75. The cells of claim 50, wherein carbon flux is directed towards mevalonate production by modulating the activity of pyruvate dehydrogenase.

76. The cells of claim 75, wherein the activity of pyruvate dehydrogenase is modulated by increasing the activity of one or more genes of the pyruvate dehydrogenase complex consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

77. The cells of claims 75 or 76, wherein the activity of the pyruvate dehydrogenase complex is modulated by attenuating the activity of an endogenous pyruvate dehydrogenase complex repressor gene.

78. The cells of claim 77, wherein the one or more genes of the pyruvate dehydrogenase complex are endogenous genes.

79. The cells of claim 78, wherein expression of the one or more endogenous genes of the pyruvate dehydrogenase complex is increased by replacing one or more endogenous gene promoters with one or more synthetic constitutively expressing promoters.

80. The cells of claim 75 or 76, wherein the cells further comprises one or more heterologous nucleic acids encoding one or more polypeptides from the group consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

81. The cells of claim 77, wherein the activity of an endogenous pyruvate dehydrogenase complex repressor is attenuated by deletion of the endogenous pyruvate dehydrogenase complex repressor gene.

82. The cells of any one of claims 75-81, wherein the cells produces increased amounts of acetyl Co-A in comparison to microorganisms wherein the activity of pyruvate dehydrogenase is not modulated.

83. The cells of any one of claims 75-82, wherein modulating the activity of pyruvate dehydrogenase results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have modulated pyruvate dehydrogenase expression.

84. The cells of claim 50, wherein carbon flux is directed towards mevalonate production by modulating the activity of phosphogluconolactonase (PGL).

85. The cells of claim 84, wherein the activity of PGL is modulated by attenuating the activity of an endogenous PGL gene.

86. The cells of claim 85, wherein the activity of PGL is attenuated by replacing the endogenous PGL gene promoter with a synthetic constitutively low expressing promoter.

87. The cells of claim 85, wherein the activity of an endogenous PGL is attenuated by deletion of the endogenous PGL gene.

88. The cells of claim 50, wherein carbon flux is directed towards mevalonate production by modulating the activity of phosphoenolpyruvate carboxylase.

89. The cells of claim 88, wherein the activity of phosphoenolpyruvate carboxylase is modulated by attenuating the activity of an endogenous phosphoenolpyruvate carboxylase gene.

90. The cells of claim 89, wherein the activity of phosphoenolpyruvate carboxylase is attenuating by replacing the endogenous phosphoenolpyruvate carboxylase gene promoter with a synthetic constitutively low expressing promoter.

91. The cells of claim 89, wherein the activity of an endogenous phosphoenolpyruvate carboxylase is attenuated by deletion of the endogenous phosphoenolpyruvate carboxylase gene.

92. A method of producing mevalonate, comprising: (a) culturing the cells of claim 50 under suitable culture conditions for production of isoprene; and (b) producing the mevalonate.

93. The cells of any one of claims 50-92, wherein mevalonate production is increased compared to microorganisms that are not grown under conditions of tri-carboxylic acid (TCA) cycle activity, wherein metabolic carbon flux in the cells is directed towards mevalonate production by modulating the activity of one or more enzymes from the group consisting of (a) citrate synthase, (b) phosphotransacetylase and/or acetate kinase, (c) lactate dehydrogenase, (d) malate dehydrogenase, and (e) pyruvate decarboxylase complex.

94. The cells of any one of claims 50-92, wherein the activity of citrate synthase is modulated by replacing the endogenous citrate synthase gene promoter with a synthetic constitutively low expressing promoter, the activity of lactate dehydrogenase is modulated by attenuating the activity of an endogenous lactate dehydrogenase gene, and the activity of acetate kinase is modulated by attenuating the activity of an endogenous acetate kinase gene.

95. Recombinant cells capable of increased production of isoprenoids wherein the cells are engineered for increased carbon flux towards mevalonate, production such that the activity of one or more enzymes from the group consisting of:

(a) citrate synthase,

(b) phosphotransacetylase

(c) acetate kinase,

(d) lactate dehydrogenase,

(e) malate dehydrogenase,

(f) pyruvate dehydrogenase

(g) phosphogluconolactonase, and

(h) and phosphoenolpyruvate carboxylase

is modulated and wherein said cells further comprise (i) one or more nucleic acids encoding one or more mevalonate (MVA) pathway polypeptides and (ii) one or more nucleic acids encoding polyprenyl pyrophosphate synthases; and wherein the cells produce increased amounts of isoprenoids compared to isoprenoid-producing cells that have not been engineered for increased carbon flux towards mevalonate.

96. The cells of claim 95 wherein the one or more nucleic acids encoding MVA pathway polypeptides are from the upper MVA pathway, wherein the upper MVA pathway nucleic acids are selected from the group consisting of AA-CoA thiolase, HMG-CoA synthase, and HMG-CoA reductase nucleic acids.

97. The cells of claim 96 wherein said one or more nucleic acids encoding an upper mevalonate (MVA) pathway polypeptide is an *mvaE* gene and an *mvaS* gene.

98. The cells of claim 97, wherein the *mvaE* gene and the *mvaS* gene is selected from the group consisting of: (a) an *mvaE* gene and an *mvaS* gene from *L. grayi*; (b) an *mvaE* gene and an

mvaS gene from *E. faecium*; (c) an *mvaE* gene and an *mvaS* gene from *E. gallinarum*; (d) an *mvaE* gene and an *mvaS* gene from *E. casseliflavus*; and (e) an *mvaE* gene and an *mvaS* gene from *E. faecalis*.

99. The cells of claim 95 wherein the one or more nucleic acids encoding MVA pathway polypeptides are from the lower MVA pathway, wherein the lower MVA pathway nucleic acids are selected from the group consisting of MVK, PMK, and, MVD nucleic acids.

100. The cells of claim 99, wherein the MVK is selected from the group consisting of *M. mazei* mevalonate kinase, *M. burtonii* mevalonate kinase polypeptide, *Lactobacillus* mevalonate kinase polypeptide, *Lactobacillus sakei* mevalonate kinase polypeptide, yeast mevalonate kinase polypeptide, *Saccharomyces cerevisiae* mevalonate kinase polypeptide, *Streptococcus* mevalonate kinase polypeptide, *Streptococcus pneumoniae* mevalonate kinase polypeptide, *Streptomyces* mevalonate kinase polypeptide, and *Streptomyces* CL190 mevalonate kinase polypeptide.

101. The cells of claim 95, wherein the cells further comprise one or more heterologous nucleic acids encoding one or DXP pathway polypeptides.

102. The cells of claim 95, wherein the cells are gram-positive bacterial cells, *Streptomyces* cells, gram-negative bacterial cells, *Escherichia* cells, *Pantoea* cells, fungal cells, filamentous fungal cells, *Trichoderma* cells, *Aspergillus* cells, or yeast cells.

103. The cells of claim 95, wherein the activity of citrate synthase is modulated by decreasing the activity of an endogenous citrate synthase gene.

104. The cells of claim 103, wherein the activity of citrate synthase is modulated by chromosomal replacement of an endogenous citrate synthase gene with a transgene encoding an NADH-insensitive citrate synthase.

105. The cells of claim 104, wherein the transgene encoding an NADH-insensitive citrate synthase is derived from *Bacillus subtilis*.

106. The cells of claim 103, wherein the activity of citrate synthase is modulated by replacing the endogenous citrate synthase gene promoter with a synthetic constitutively low expressing promoter.

107. The cells of any one of claims 103-106, wherein decreasing the activity of citrate synthase results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have decreased expression of citrate synthase.

108. The cells claim 95, wherein carbon flux is directed towards mevalonate production by modulating the activity of phosphotransacetylase and/or acetate kinase.

109. The cells of claim 108, wherein the activity of phosphotransacetylase and/or acetate kinase is modulated by attenuating the activity of an endogenous phosphotransacetylase gene and/or an endogenous acetate kinase gene.

110. The cells of claim 109, wherein endogenous phosphotransacetylase and/or endogenous acetate kinase gene expression is attenuated by deletion of the endogenous phosphotransacetylase gene and/or the endogenous acetate kinase gene.

111. The cells of claim 108 or 109, wherein the cells produces decreased amounts of acetate in comparison to microorganisms that do not have attenuated endogenous phosphotransacetylase gene and/or endogenous acetate kinase gene expression.

112. The cells of any one of claims 95-102 or 108-111, wherein attenuating the activity of the endogenous phosphotransacetylase gene and/or the endogenous acetate kinase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have attenuated endogenous phosphotransacetylase gene and/or endogenous acetate kinase gene expression

113. The cells of claim 95, wherein carbon flux is directed towards mevalonate production by modulating the activity of lactate dehydrogenase.

114. The cells of claim 113, wherein the activity of lactate dehydrogenase is modulated by attenuating the activity of an endogenous lactate dehydrogenase gene.

115. The cells of claim 114, wherein endogenous lactate dehydrogenase gene expression is attenuated by deletion of the endogenous lactate dehydrogenase gene.

116. The cells of any one of claims 113-115, wherein the cells produces decreased amounts of lactate in comparison to microorganisms that do not have attenuated endogenous lactate dehydrogenase gene expression.

117. The cells of any one of claims 113-116, wherein attenuating the activity of the endogenous lactate dehydrogenase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have attenuated endogenous lactate dehydrogenase gene expression.

118. The cells of any one of claims 95, wherein carbon flux is directed towards mevalonate production by modulating the activity of NADP-dependent malate dehydrogenase.

119. The cells of claim 118, wherein the activity of NADP-dependent malate dehydrogenase is modulated by increasing the activity of an NADP-dependent malate dehydrogenase gene.

120. The cells of claim 119, wherein the NADP-dependent malate dehydrogenase gene is an endogenous gene.

121. The cells of claim 119, wherein expression of the endogenous NADP-dependent malate dehydrogenase gene is increased by replacing the endogenous NADP-dependent malate dehydrogenase gene promoter with a synthetic constitutively expressing promoter.

122. The cells of claim 118 or 119, wherein the cells further comprise a heterologous nucleic acid encoding an NADP-dependent malate dehydrogenase polypeptide.

123. The cells of any one of claims 118-122, wherein the cells produces increased amounts of pyruvate in comparison to microorganisms that do not have increased expression of an NADP-dependent malate dehydrogenase gene.

124. The cells of any one of claims 118-123, wherein increasing the activity of an NADP-dependent malate dehydrogenase gene results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have increased NADP-dependent malate dehydrogenase gene expression.

125. The cells of any one of claims 95, wherein carbon flux is directed towards mevalonate production by modulating the activity of pyruvate dehydrogenase.

126. The cells of claim 125, wherein the activity of pyruvate dehydrogenase is modulated by increasing the activity of one or more genes of the pyruvate dehydrogenase complex consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

127. The cells of claims 125 or 126, wherein the activity of the pyruvate dehydrogenase complex is modulated by attenuating the activity of an endogenous pyruvate dehydrogenase complex repressor gene.

128. The cells of claim 127, wherein the one or more genes of the pyruvate dehydrogenase complex are endogenous genes.

129. The cells of claim 128, wherein expression of the one or more endogenous genes of the pyruvate dehydrogenase complex is increased by replacing one or more endogenous gene promoters with one or more synthetic constitutively expressing promoters.

130. The cells of claim 125 or 126, wherein the cells further comprises one or more heterologous nucleic acids encoding one or more polypeptides from the group consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

131. The cells of claim 130, wherein the activity of an endogenous pyruvate dehydrogenase complex repressor is attenuated by deletion of the endogenous pyruvate dehydrogenase complex repressor gene.

132. The cells of any one of claims 125-131, wherein the cells produces increased amounts of acetyl Co-A in comparison to microorganisms wherein the activity of pyruvate dehydrogenase is not modulated.

133. The cells of any one of claims 125-132, wherein modulating the activity of pyruvate dehydrogenase results in more carbon flux into the mevalonate dependent biosynthetic pathway in comparison to microorganisms that do not have modulated pyruvate dehydrogenase expression.

134. The cells of claim 95, wherein carbon flux is directed towards mevalonate production by modulating the activity of phosphogluconolactonase (PGL).

135. The cells of claim 134, wherein the activity of PGL is modulated by attenuating the activity of an endogenous PGL gene.

136. The cells of claim 135, wherein the activity of PGL is decreased by replacing the endogenous PGL gene promoter with a synthetic constitutively low expressing promoter.

137. The cells of claim 136, wherein the activity of an endogenous PGL is attenuated by deletion of the endogenous PGL gene.

138. The cells of claim 95, wherein carbon flux is directed towards mevalonate production by modulating the activity of phosphoenolpyruvate carboxylase.

139. The cells of claim 138, wherein the activity of phosphoenolpyruvate carboxylase is modulated by attenuating the activity of an endogenous phosphoenolpyruvate carboxylase gene.

140. The cells of claim 139, wherein the activity of phosphoenolpyruvate carboxylase is decreased by replacing the endogenous phosphoenolpyruvate carboxylase gene promoter with a synthetic constitutively low expressing promoter.

141. The cells of claim 140, wherein the activity of an endogenous phosphoenolpyruvate carboxylase is attenuated by deletion of the endogenous phosphoenolpyruvate carboxylase gene.

142. The cells of claims 93-141, wherein the isoprenoid is selected from group consisting of monoterpenes, diterpenes, triterpenes, tetraterpenes, sequiterpenes, and polyterpenes.

143. The cells of claim 142, wherein the isoprenoid is a sesquiterpene.

144. The cells of claim 142, wherein the isoprenoid is selected from the group consisting of abietadiene, amorphadiene, carene, α-farnesene, β-farnesene, farnesol, geraniol, geranylgeraniol, linalool, limonene, myrcene, nerolidol, ocimene, patchoulol, β-pinene, sabinene, γ-terpinene, terpindene and valencene.

145. A method of producing isoprenoids, comprising: (a) culturing the cells of claim 1 under suitable culture conditions for production of isoprene; and (b) producing the isoprenoids.

146. The method of claim 49, further comprising (c) recovering the isoprene.

147. The method of claim 92, further comprising (c) recovering the mevalonate.

148. The method of claim 145, further comprising (c) recovering the isoprenoids.

149. The cells of claim 1, further comprising one or more nucleic acids encoding an isopentenyl-diphosphate delta-isomerase (IDI) polypeptide.

150. The cells of claim 95, further comprising one or more nucleic acids encoding an isopentenyl-diphosphate delta-isomerase (IDI) polypeptide.

CLAIMS

What is claimed is:

1. Recombinant cells capable of increased production of isoprene wherein said cells comprise:

(i) a modulation of the activity of one or more enzymes from the group consisting of: (a) decreasing the activity of citrate synthase, (b) increasing the activity of malate dehydrogenase, (c) increasing the activity of pyruvate dehydrogenase, (d) attenuating the activity of phosphogluconolactonase (PGL), and (e) attenuating the activity of phosphoenolpyruvate carboxylase, resulting in increased carbon flux toward isoprene production;

(ii) one or more nucleic acids encoding one or more mevalonate (MVA) pathway polypeptides; and

(iii) one or more heterologous nucleic acids encoding an isoprene synthase polypeptide; and wherein said cells produce increased amounts of isoprene compared to isoprene-producing cells that do not comprise (i).

2. The cells of claim 1 wherein the one or more nucleic acids encoding MVA pathway polypeptides are from the upper MVA pathway, wherein the upper MVA pathway nucleic acids are selected from the group consisting of acetyl-CoA acetyltransferase (AA-CoA thiolase), HMG-CoA synthase, and HMG-CoA reductase nucleic acids.

3. The cells of claim 2 wherein said one or more nucleic acids encoding an upper MVA pathway polypeptide is an *mvaE* gene and an *mvaS* gene.

4. The cells of claim 3, wherein the *mvaE* gene and the *mvaS* gene is selected from the group consisting of: (a) an *mvaE* gene and an *mvaS* gene from *L. grayi*; (b) an *mvaE* gene and an *mvaS* gene from *E. faecium*; (c) an *mvaE* gene and an *mvaS* gene from *E. gallinarum*; (d) an *mvaE* gene and an *mvaS* gene from *E. casseliflavus*; and (e) an *mvaE* gene and an *mvaS* gene from *E. faecalis*.

5. The cells of claim 1 wherein the one or more nucleic acids encoding MVA pathway polypeptides are from the lower MVA pathway, wherein the lower MVA pathway nucleic

acids are selected from the group consisting of mevalonate kinase (MVK), phosphomevalonate kinase (PMK), and diphosphomevalonte decarboxylase (MVD) nucleic acids.

6. The cells of claim 5, wherein the MVK is selected from the group consisting of *M. mazei* mevalonate kinase, *M. burtonii* mevalonate kinase polypeptide, *Lactobacillus* mevalonate kinase polypeptide, *Lactobacillus sakei* mevalonate kinase polypeptide, yeast mevalonate kinase polypeptide, *Saccharomyces cerevisiae* mevalonate kinase polypeptide, *Streptococcus* mevalonate kinase polypeptide, *Streptococcus pneumoniae* mevalonate kinase polypeptide, *Streptomyces* mevalonate kinase polypeptide, and *Streptomyces* CL190 mevalonate kinase polypeptide.

7. The cells of claim 1, wherein the cells further comprise one or more heterologous nucleic acids encoding one or more 1-deoxy-d-xylulose 5-phosphate (DXP) pathway polypeptides.

8. The cells of claim 1, further comprising one or more nucleic acids encoding an isopentenyl-diphosphate delta-isomerase (IDI) polypeptide.

9. The cells of claim 1, wherein the isoprene synthase polypeptide is a polypeptide from *Pueraria* or *Populus* or a hybrid, *Populus alba* x *Populus tremula* or variant thereof.

10. The cells of claim 1, wherein the cells are gram-positive bacterial cells, *Streptomyces* cells, gram-negative bacterial cells, *Escherichia* cells, *Pantoea* cells, fungal cells, filamentous fungal cells, *Trichoderma* cells, *Aspergillus* cells, or yeast cells.

11. The cells of claim 1, wherein the activity of citrate synthase is decreased by decreasing the expression of a citrate synthase gene.

12. The cells of claim 1, wherein the activity of said malate dehydrogenase is increased by increasing the expression of an NADP-dependent malate dehydrogenase gene.

13. The cells of claim 1, wherein the activity of pyruvate dehydrogenase is increased by increasing the expression of one or more genes of the pyruvate dehydrogenase complex consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

14. The cells of claim 1, wherein the activity of PGL is attenuated by attenuating the expression of a PGL gene.

15. The cells of claim 1, wherein the activity of phosphoenolpyruvate carboxylase is attenuated by attenuating the expression of a phosphoenolpyruvate carboxylase gene.

16. The cells of claim 1, wherein the modulation of the activity of one or more enzymes is from the group consisting of (a) decreasing the activity of citrate synthase and (c) increasing the activity of pyruvate dehydrogenase.

17. A method of producing isoprene, comprising: (a) culturing the cells of claim 1 under suitable culture conditions for production of isoprene; and (b) producing the isoprene.

18. The method of claim 17 further comprising (c) recovering the isoprene.

19. Recombinant cells capable of increased production of mevalonate wherein said cells comprise:

(i) a modulation of the activity of one or more enzymes from the group consisting of: (a) decreasing the activity of citrate synthase, (b) increasing the activity of malate dehydrogenase, (c) increasing the activity of pyruvate dehydrogenase, (d) attenuating the activity of phosphogluconolactonase (PGL), and (e) attenuating the activity of phosphoenolpyruvate carboxylase, resulting in increased carbon flux toward mevalonate production; and

(ii) one or more nucleic acids encoding one or more upper MVA pathway polypeptides; and wherein said cells produce increased amounts of mevalonate compared to mevalonate-producing cells that do not comprise (i).

20. The cells of claim 19, wherein said one or more nucleic acids encoding an upper MVA pathway polypeptide is an *mvaE* gene and an *mvaS* gene.

21. The cells of claim 20 wherein the *mvaE* gene and the *mvaS* gene is selected from the group consisting of: (a) an *mvaE* gene and an *mvaS* gene from *L. grayi*; (b) an *mvaE* gene and an *mvaS* gene from *E. faecium*; (c) an *mvaE* gene and an *mvaS* gene from *E. gallinarum*; (d) an *mvaE* gene and an *mvaS* gene from *E. casseliflavus*; and (e) an *mvaE* gene and an *mvaS* gene from *E. faecalis*.

22. The cells of claim 19, wherein the activity of citrate synthase is decreased by decreasing the expression of a citrate synthase gene.

23. The cells of claim 19, wherein the activity of phosphotransacetylase and/or acetate kinase is attenuated by attenuating the expression of a phosphotransacetylase gene and/or an acetate kinase gene.

24. The cells of claim 19, wherein the activity of lactate dehydrogenase is attenuated by attenuating the expression of a lactate dehydrogenase gene.

25. The cells of claim 19, wherein the activity of said malate dehydrogenase is increased by increasing the expression of an NADP-dependent malate dehydrogenase gene.

26. The cells of claim 19, wherein the activity of pyruvate dehydrogenase is increased by increasing the expression of one or more genes of the pyruvate dehydrogenase complex consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

27. The cells of claim 19, wherein the activity of PGL is attenuated by attenuating the expression of a PGL gene.

28. The cells of claim 19, wherein the activity of phosphoenolpyruvate carboxylase is attenuated by attenuating the activity of a phosphoenolpyruvate carboxylase gene.

29. The cells of claim 19, wherein the modulation of the activity of one or more enzymes is from the group consisting of (a) decreasing the activity of citrate synthase and (c) increasing the activity of pyruvate dehydrogenase.

30. A method of producing mevalonate, comprising: (a) culturing the cells of claim 19 under suitable culture conditions for production of mevalonate; and (b) producing the mevalonate.

31. The method of claim 30 further comprising (c) recovering the mevalonate.

32. Recombinant cells capable of increased production of isoprenoids wherein said cells comprise:

(i) a modulation of the activity of one or more enzymes from the group consisting of: (a) decreasing the activity of citrate synthase, (b) increasing the activity of malate

dehydrogenase, (c) increasing the activity of pyruvate dehydrogenase, (d) attenuating the activity of phosphogluconolactonase (PGL), and (e) attenuating the activity of phosphoenolpyruvate carboxylase, resulting in increased carbon flux toward mevalonate production;

(ii) one or more nucleic acids encoding one or more MVA pathway polypeptides; and

(iii) one or more nucleic acids encoding polyprenyl pyrophosphate synthases; and wherein said cells produce increased amounts of isoprenoids compared to isoprenoid-producing cells that do not comprise (i).

33. The cells of claim 32 wherein the one or more nucleic acids encoding MVA pathway polypeptides are from the upper MVA pathway, wherein the upper MVA pathway nucleic acids are selected from the group consisting of acetyl-CoA acetyltransferase (AA-CoA thiolase), HMG-CoA synthase, and HMG-CoA reductase nucleic acids.

34. The cells of claim 33 wherein said one or more nucleic acids encoding an upper MVA pathway polypeptide is an *mvaE* gene and an *mvaS* gene.

35. The cells of claim 34, wherein the *mvaE* gene and the *mvaS* gene is selected from the group consisting of: (a) an *mvaE* gene and an *mvaS* gene from *L. grayi*; (b) an *mvaE* gene and an *mvaS* gene from *E. faecium*; (c) an *mvaE* gene and an *mvaS* gene from *E. gallinarum*; (d) an *mvaE* gene and an *mvaS* gene from *E. casseliflavus*; and (e) an *mvaE* gene and an *mvaS* gene from *E. faecalis*.

36. The cells of claim 32 wherein the one or more nucleic acids encoding MVA pathway polypeptides are from the lower MVA pathway, wherein the lower MVA pathway nucleic acids are selected from the group consisting of mevalonate kinase (MVK), phosphomevalonate kinase (PMK), and diphosphomevalonate decarboxylase (MVD) nucleic acids.

37. The cells of claim 36, wherein the MVK is selected from the group consisting of *M. mazei* mevalonate kinase, *M. burtonii* mevalonate kinase polypeptide, *Lactobacillus* mevalonate kinase polypeptide, *Lactobacillus sakei* mevalonate kinase polypeptide, yeast mevalonate kinase polypeptide, *Saccharomyces cerevisiae* mevalonate kinase polypeptide, *Streptococcus* mevalonate kinase polypeptide, *Streptococcus pneumoniae* mevalonate kinase

polypeptide, *Streptomyces* mevalonate kinase polypeptide, and *Streptomyces* CL190 mevalonate kinase polypeptide.

38. The cells of claim 32, wherein the cells further comprise one or more heterologous nucleic acids encoding one or more 1-deoxy-d-xylulose 5-phosphate (DXP) pathway polypeptides.

39. The cells of claim 32, further comprising one or more nucleic acids encoding an isopentenyl-diphosphate delta-isomerase (IDI) polypeptide.

40. The cells of claim 32, wherein the cells are gram-positive bacterial cells, *Streptomyces* cells, gram-negative bacterial cells, *Escherichia* cells, *Pantoea* cells, fungal cells, filamentous fungal cells, *Trichoderma* cells, *Aspergillus* cells, or yeast cells.

41. The cells of claim 32, wherein the activity of citrate synthase is decreased by decreasing the expression of a citrate synthase gene.

42. The cells of claim 32, wherein the activity of phosphotransacetylase and/or acetate kinase is attenuated by attenuating the activity of a phosphotransacetylase gene and/or an acetate kinase gene.

43. The cells of claim 32, wherein the activity of lactate dehydrogenase is attenuated by attenuating the activity of a lactate dehydrogenase gene.

44. The cells of claim 32, wherein the activity of said malate dehydrogenase is increased by increasing the expression of an NADP-dependent malate dehydrogenase gene.

45. The cells of claim 32, wherein the activity of pyruvate dehydrogenase is increased by increasing the expression of one or more genes of the pyruvate dehydrogenase complex consisting of (a) pyruvate dehydrogenase (E1), (b) dihydrolipoyl transacetylase, and (c) dihydrolipoyl dehydrogenase.

46. The cells of claim 32, wherein the activity of PGL is attenuated by attenuating the expression of a PGL gene.

47. The cells of claim 32, wherein the activity of phosphoenolpyruvate carboxylase is attenuated by attenuating the expression of a phosphoenolpyruvate carboxylase gene.

48. The cells of claim 32, wherein the modulation of the activity of one or more enzymes is from the group consisting of (a) decreasing the activity of citrate synthase and (c) increasing the activity of pyruvate dehydrogenase.

49. A method of producing isoprenoids, comprising: (a) culturing the cells of claim 32 under suitable culture conditions for production of isoprenoids; and (b) producing the isoprenoids.