

What is claimed is:

1. An engineered *Escherichia coli* (*E. coli*) host cell, wherein the engineered *E. coli* host cell comprises a gene knockout of at least one gene selected from the group consisting of SbcC and SbcD, and wherein the engineered *E. coli* host cell does not include an engineered viability- or yield-reducing mutation in any of sbcB, recB, recD, and recJ.
2. The engineered *E. coli* host cell of claim 1, wherein the engineered *E. coli* host cell does not include any engineered mutations in any of sbcB, recB, recD, and recJ.
3. The engineered *E. coli* host cell of claim 1, wherein the engineered *E. coli* host cell does not include any mutations in any of sbcB, recB, recD, and recJ.
4. The engineered *E. coli* host cell of any of claims 1-3, wherein the engineered *E. coli* host does not include or produce a SbcCD complex.
5. The engineered *E. coli* host cell of any of claims 1-3, wherein the engineered *E. coli* host does not include a functional SbcCD complex.
6. The engineered *E. coli* host cell of any of claims 1-3, wherein the engineered *E. coli* host comprises a SbcCD complex, and wherein the SbcCD complex is non-functional.
7. The engineered *E. coli* host cell of any of claims 1-6, wherein the gene knockout comprises a knockout of SbcC.
8. The engineered *E. coli* host cell of any of claims 1-6, wherein the gene knockout comprises a knockout of SbcD.
9. The engineered *E. coli* host cell of any of claims 1-6, wherein the gene knockout comprises a knockout of SbcC and SbcD.
10. The engineered *E. coli* host cell of any of claims 1-9, wherein the engineered *E. coli* host cell is derived from a cell line selected from the group consisting of DH5 α , DH1, JM107, JM108, JM109, MG1655 and XL1Blue.

11. The engineered *E. coli* host cell of any of claims 1-10, wherein the engineered *E. coli* host cell further comprises a genomic antibiotic resistance marker.

12. The engineered *E. coli* host cell of claim 11, wherein the genomic antibiotic resistance marker comprises a sequence having at least 90%, at least 95%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 23.

13. The engineered *E. coli* host cell of claim 11, wherein the genomic antibiotic resistance marker is kanR comprising a sequence encoding a protein having at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 36.

14. The engineered *E. coli* host cell of any of claims 1-10, wherein the engineered *E. coli* host cell does not include a genomic antibiotic resistance marker.

15. The engineered *E. coli* host cell of any of claims 1-14, wherein the engineered *E. coli* host cell further comprises a Rep protein suitable for culturing a Rep protein dependent plasmid.

16. The engineered *E. coli* host cell of any of claims 1-14, wherein the engineered *E. coli* host cell further comprises a genomic nucleic acid sequence having at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, and SEQ ID NO: 29.

17. The engineered *E. coli* host cell of any of claims 1-14, wherein the engineered *E. coli* host cell further comprises a genomic nucleic acid sequence encoding a Rep protein having at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 34, and SEQ ID NO: 35.

18. The engineered *E. coli* host cell of any of claims 1-14, wherein the engineered *E. coli* host cell further comprises a Rep protein having at least 90%, at least 95%, at least 98%, at least 99% or 100% identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 34, and SEQ ID NO: 35.

19. The engineered *E. coli* host cell of any of claims 1-18, further comprising a genomic nucleic acid sequence encoding a temperature-sensitive lambda repressor.

20. The engineered *E. coli* host cell of claim 19, wherein the temperature-sensitive lambda repressor is cITs857.

21. The engineered *E. coli* host cell of claim 19, wherein the genomic nucleic acid sequence encoding the temperature-sensitive lambda repressor has at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 24.

22. The engineered *E. coli* host cell of claim 19, wherein the genomic nucleic acid sequence encoding the temperature-sensitive lambda repressor encodes an amino acid sequence with at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 37.

23. The engineered *E. coli* host cell of claim 19, wherein the engineered *E. coli* host cell comprises the temperature-sensitive lambda repressor, the temperature-sensitive lambda repressor having an amino acid sequence with at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 37.

24. The engineered *E. coli* host cell of any of claims 19-23, wherein the temperature-sensitive lambda repressor is a phage ϕ 80 attachment site chromosomally integrated copy of a arabinose inducible CITS857 gene.

25. The engineered *E. coli* host cell of any of claims 1-24, further comprising a genomic nucleic acid sequence encoding a genomically expressed RNA-IN regulated selectable marker.

26. The engineered *E. coli* host cell of claim 24, wherein the genomic nucleic acid sequence encoding the genomically expressed RNA-IN regulated selectable marker comprises a sequence having at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 25.

27. The engineered *E. coli* host cell of claim 24, wherein the genomic nucleic acid sequence that encodes the RNA-IN regulated selectable marker encodes a protein having at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 38.

28. The engineered *E. coli* host cell of claim 24, wherein the RNA-IN regulated selectable marker has at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 38.

29. An engineered *E. coli* host cell having the following genotype: F- ϕ 80lacZ Δ M15 Δ (lacZYA-argF) U169 recA1 endA1 hsdR17 (rk⁻, mk⁺) gal⁻ phoA supE44 λ ⁻ thi-1 gyrA96 relA1 **Δ SbcDC::kanR**.

30. An engineered *E. coli* host cell having the following genotype: F- ϕ 80lacZ Δ M15 Δ (lacZYA-argF) U169 recA1 endA1 hsdR17 (rk⁻, mk⁺) gal⁻ phoA supE44 λ ⁻ thi-1 gyrA96 relA1 **Δ SbcDC**.

31. An engineered *E. coli* host cell having the following genotype: DH5 α att_{HK022}::pL (OL1-G to T) P42L-P106I-F107S P113S (P3⁻), SpecR StrepR; **Δ SbcDC::kanR**.

32. An engineered *E. coli* host cell having the following genotype: DH5 α att_{HK022}::pL (OL1-G to T) P42L-P106I-F107S P113S (P3⁻), SpecR StrepR; **Δ SbcDC**.

33. An engineered *E. coli* host cell having the following genotype: DH5 α att λ :: Pc-RNA-IN- SacB, catR; att_{HK022}::pL (OL1-G to T) P42L-P106I-F107S P113S (P3⁻), SpecR StrepR; att ϕ 80::pARA-CI857ts Pc-RNA-IN- SacB, tetR; **Δ SbcDC::kanR**.

34. An engineered *E. coli* host cell having the following genotype: DH5 α att λ :: Pc-RNA-IN- SacB, catR; att_{HK022}::pL (OL1-G to T) P42L-P106I-F107S P113S (P3⁻), SpecR StrepR; att ϕ 80::pARA-CI857ts Pc-RNA-IN- SacB, tetR; **Δ SbcDC**.

35. An engineered *E. coli* host cell having the following genotype: DH5 α dcm- att λ :: Pc-RNA-IN- SacB, catR; att_{HK022}::pL (OL1-G to T) P42L-P106I-F107S P113S (P3⁻), SpecR StrepR; att ϕ 80::pARA-CI857ts Pc-RNA-IN- SacB, tetR; **Δ SbcDC**.

36. An engineered *E. coli* host cell having the following genotype: DH5 α dcm- att λ :: Pc-RNA-IN- SacB, catR; att_{HK022}::pL (OL1-G to T) P42L-P106I-F107S P113S (P3⁻), SpecR StrepR; att ϕ 80::pARA-CI857ts Pc-RNA-IN- SacB, tetR; **Δ SbcDC::kanR**.

37. An engineered *E. coli* host cell having the following genotype: DH5 α att λ :: Pc-RNA-IN- SacB, catR; att ϕ 80::pARA-CI857ts Pc-RNA-IN- SacB, tetR; Δ SbcDC.

38. An engineered *E. coli* host cell having the following genotype: DH5 α att λ :: Pc-RNA-IN- SacB, catR; att ϕ 80::pARA-CI857ts Pc-RNA-IN- SacB, tetR; Δ SbcDC::kanR.

39. The engineered *E. coli* host cell of any of claims 1-38, wherein the engineered *E. coli* host cell does not include any engineered viability- or yield-reducing mutations in at least one of uvrC, mcrA, mcrBC-hsd-mrr, and combinations thereof.

40. The engineered *E. coli* host cell of claim 39, wherein the engineered *E. coli* host cell does not include any engineered mutations in at least one of uvrC, mcrA, mcrBC-hsd-mrr, and combinations thereof.

41. The engineered *E. coli* host cell of claim 39, wherein the engineered *E. coli* host cell does not include any mutations in at least one of uvrC, mcrA, mcrBC-hsd-mrr, and combinations thereof.

42. The engineered *E. coli* host cell of any of claims 1-41, wherein sbcB gene comprises a sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 11, wherein the recB gene comprises a sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12, wherein the recD gene comprises a sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 13, and wherein the recJ gene comprises a sequence having at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 65.

43. An engineered *E. coli* host cell, comprising a gene knockout of at least one gene selected from the group consisting of SbcC and SbcD, wherein the *E. coli* host cell is isogenic to the strain from which it is derived, and wherein the strain from which the engineered *E. coli* host cell is derived is selected from the group consisting of DH5 α , DH1, JM107, JM108, JM109 MG1655 and XL1Blue.

44. The engineered *E. coli* host cell of any of claims 1-43, wherein the *E. coli* host cell is derived from a starting *E. coli* cell, wherein the *sbcC* gene of the starting *E. coli* cell comprises a sequence having at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 9, and wherein the *sbcD* gene of the starting *E. coli* cell comprises a sequence having at least 95%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 10.

45. The engineered *E. coli* host cell of any of claims 1-44, further comprising a vector.

46. The engineered *E. coli* host cell of claim 45, wherein the vector comprises a nucleic acid sequence having an inverted repeat.

47. The engineered *E. coli* host cell of claim 46, wherein the inverted repeat comprises an AAV ITR that comprises
ttggccactccctctctgcgcgctcgtcgtcactgaggccggcgaccaaaggtcgcccgacgcccgggctttgccggcggcctc
agtgcgcgagcgagcgcgagagaggagtgccaactccatcactaggggttcct and
aggaaccctagtgtgagtggtggccactccctctctgcgcgctcgtcgtcactgaggccggcgaccaaaggtcgcccgacgccc
ggctttgccggcggcctcagtgagcgagcgagcgcgagagaggagtgccaa.

48. The engineered *E. coli* host cell of claim 45, wherein the vector comprises nucleic acid sequence having at least one direct repeat.

49. The engineered *E. coli* host cell of claim 48, wherein the at least one direct repeat comprises a polyA, polyG, polyC or polyT repeat of between about 40 and about 150 consecutive nucleotides, between about 60 and 120 consecutive nucleotides, or about 90 consecutive nucleotides.

50. The engineered *E. coli* host cell of claim 45, wherein the vector comprises a nucleic acid sequence having at least one inverted repeat.

51. The engineered *E. coli* host cell of claim 45, wherein the vector comprises a nucleic acid sequence that does not include a palindrome, direct repeat or inverted repeat.

52. The engineered *E. coli* host cell of any of claims 45-51, wherein the vector is an AAV vector and, optionally, wherein the AAV vector comprises an AAV ITR.

53. The engineered *E. coli* host cell of any of claims 45-51, wherein the vector is a lentiviral vector, lentiviral envelope vector, or lentiviral packaging vector.

54. The engineered *E. coli* host cell of any of claims 45-51, wherein the vector is a retroviral vector, retroviral envelope vector or retroviral packaging vector.

55. The engineered *E. coli* host cell of any of claims 45-51, wherein the vector is a mRNA vector containing a polyA repeat.

56. The engineered *E. coli* host cell of any of claims 45-55, wherein vector is a plasmid.

57. The engineered *E. coli* host cell of any of claims 45-56, wherein the vector further comprises a RNA selectable marker.

58. The engineered *E. coli* host cell of claim 57, wherein the RNA selectable marker is a RNA-OUT.

59. The engineered *E. coli* host cell of claim 58, wherein the RNA-OUT has at least 95%, at least 98%, at least 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 47 and SEQ ID NO: 49.

60. The engineered *E. coli* host cell of claim 57, wherein the vector further includes a RNA-OUT antisense repressor RNA.

61. The engineered *E. coli* host cell of claim 60, wherein the RNA-OUT antisense repressor RNA has at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 48.

62. The engineered *E. coli* host cell of any of claims 45-61, wherein the vector further comprises a bacterial origin of replication.

63. The engineered *E. coli* host cell of claim 62, wherein the bacterial origin of replication is selected from the group consisting of R6K, pUC and ColE2.

64. The engineered *E. coli* host cell of claim 63, wherein the bacterial origin of replication is selected from the group consisting of a sequence having at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, and SEQ ID NO: 22.

65. The engineered *E. coli* host cell of any of claims 45-64, wherein the vector is a Rep protein dependent plasmid.

66. The engineered *E. coli* host cell of any of claims 45-65, wherein the vector is a eukaryotic pUC-free minicircle expression vector that can include: (i) a eukaryotic region sequence encoding a gene of interest and having 5' and 3' ends; and (ii) a spacer region having a length of less than 1000, preferably less than 500, basepairs that links the 5' and 3' ends of the eukaryotic region sequence and that comprises a R6K bacterial replication origin and a RNA selectable marker.

67. The engineered *E. coli* host cell of any of claims 45-65, wherein the vector is a covalently closed circular plasmid having a backbone including a Pol III-dependent R6K origin of replication and an RNA-OUT selectable marker, where the backbone is less than 1000 bp, and an insert including a structured DNA sequence.

68. The engineered *E. coli* host cell of claim 67, wherein the structured DNA sequence is selected from the group consisting of an inverted repeat sequence, a direct repeat sequence, a homopolymeric repeat sequence, an eukaryotic origin of replication, and a eukaryotic promoter enhancer sequence.

69. The engineered *E. coli* host cell of claim 67, wherein the structured DNA sequence is selected from the group consisting of a polyA repeat, a SV40 origin of replication, a viral LTR, a Lentiviral LTR, a Retroviral LTR, a transposon IR/DR repeat, a Sleeping Beauty transposon IR/DR repeat, an AAV ITR, a CMV enhancer, and a SV40 enhancer.

70. The engineered *E. coli* host cell of any of claims 67-69, wherein the PolIII-dependent R6K origin of replication has at least 90%, at least 95%, at least 98%, at least 99% or 100%

sequence identity to a sequence selected from the group consisting of SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, and SEQ ID NO: 60.

71. The engineered *E. coli* host cell of any of claims 67-70, wherein the RNA-OUT selectable marker is an RNA-IN regulating RNA-OUT functional variant with at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 47 or SEQ ID NO: 49.

72. The engineered *E. coli* host cell of any of claims 67-70, wherein the RNA-OUT antisense repressor RNA can have a sequence having at least 90%, at least 95%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 48.

73. A method for producing an engineered *Escherichia coli* (*E. coli*) cell, comprising:

knocking out at least one gene selected from the group consisting of SbcC and SbcD in a starting *E. coli* cell that does not include an engineered viability- or yield-reducing mutation in any of sbcB, recB, recD, and recJ to yield the engineered *E. coli* cell.

74. The method of claim 73, wherein the starting *E. coli* cell does not include any engineered mutations in any of sbcB, recB, recD, and recJ.

75. The method of claim 74, wherein the starting *E. coli* cell does not include any mutations in any of sbcB, recB, recD, and recJ.

76. The method of any of claims 73-75, wherein the step of knocking out the at least one gene does not result in any mutations in any of sbcB, recB, recD, and recJ in the engineered *E. coli* cell.

77. The method of any of claims 73-76, wherein starting *E. coli* cell does not include an engineered viability- or yield-reducing mutation in at least one of uvrC, mcrA, mcrBC-hsd-mrr, and combinations thereof.

78. The method of claim 77, wherein the starting *E. coli* cell does not include any engineered mutations in at least one of uvrC, mcrA, mcrBC-hsd-mrr, and combinations thereof.

79. The method of claim 78, wherein the starting *E. coli* cell does not include any mutations in at least one of *uvrC*, *mcrA*, *mcrBC-hsd-mrr*, and combinations thereof.

80. The method of any of claims 73-79, wherein the step of knocking out the at least one gene does not result in any mutations in at least one of *uvrC*, *mcrA*, *mcrBC-hsd-mrr*, and combinations thereof.

81. A method for improved vector production, comprising:

transfecting an engineered *Escherichia coli* (*E. coli*) host cell with a vector to yield a transfected host cell; and

incubating the transfected host cell under conditions sufficient to replicate the vector,

wherein the engineered *E. coli* host cell comprises a gene knockout of at least one gene selected from the group consisting of *SbcC*, *SbcD* and *SbcCD*, and wherein the host cell does not include a viability- or yield-reducing mutation in any of *sbcB*, *recB*, *recD*, and *recJ*.

82. The method of claim 81, wherein the engineered *E. coli* cell is an engineered *E. coli* cell according to any of claims 1-44.

83. The method of any of claims 81-82, further comprising after incubating the transfected host cell under conditions sufficient to replicate the engineered vector:

isolating the vector from the engineered *E. coli* cell.

84. The method of any of claims 81-83, wherein the step of incubating the transfected host cell under conditions sufficient to replicate the engineered vector is performed by a fed-batch fermentation, wherein the fed-batch fermentation comprises growing the engineered *E. coli* cells at a reduced temperature during a first portion of the fed-batch phase, followed by a temperature up-shift to a higher temperature during a second portion of the fed-batch phase.

85. The method of claim 84, wherein the reduced temperature is about 30°C.

86. The method of any of claims 84-85, wherein the higher temperature is about 37-42°C.

87. The method of any of claims 84-86, wherein the first portion is about 12 hours.

88. The method of any of claims 84-87, wherein the second portion is about 8 hours.

89. The method of any of claims 84-88, wherein the plasmid yield after incubating the transfected host cell under conditions sufficient to replicate the engineered vector is higher than for the cell line from which the engineered *E. coli* cell was derived treated under the same conditions.

90. The method of any of claims 84-89, wherein the plasmid yield after incubating the transfected host cell under conditions sufficient to replicate the engineered vector is higher than for SURE2, SURE, Stbl2, Stbl3, or Stbl4 cells treated under the same conditions.

91. A method for improved vector production, comprising:

providing a transfected host cell comprising a gene knockout of at least one gene selected from the group consisting of SbcC, SbcD and SbcCD, and wherein the transfected host cell does not include a viability- or yield-reducing mutation in any of sbcB, recB, recD, and recJ, wherein the transfected host cell is an engineered *Escherichia coli* (*E. coli*) host cell comprising a vector;

incubating the transfected host cell under conditions sufficient to replicate the vector.

92. The method of claim 91, wherein the transfected, engineered *E. coli* host cell is the engineered *E. coli* host cell of any of claims 45-72.

93. The method of any of claims 91-92, further comprising after incubating the transfected host cell under conditions sufficient to replicate the vector:

isolating the vector from the transfected host cell.

94. The method of any of claims 91-93, wherein the step of incubating the transfected host cell under conditions sufficient to replicate the engineered vector is performed by a fed-batch fermentation, wherein the fed-batch fermentation comprises growing the engineered *E. coli* cells

at a reduced temperature during a first portion of the fed-batch phase, followed by a temperature up-shift to a higher temperature during a second portion of the fed-batch phase.

95. The method of claim 94, wherein the reduced temperature is about 30°C.

96. The method of any of claims 91-95, wherein the higher temperature is about 37-42°C.

97. The method of any of claims 91-96, wherein the first portion is about 12 hours.

98. The method of any of claims 91-97, wherein the second portion is about 8 hours.

99. The method of any of claims 91-98, wherein the plasmid yield after incubating the transfected host cell under conditions sufficient to replicate the engineered vector is higher than for the cell line from which the engineered *E. coli* cell was derived treated under the same conditions.

100. The method of any of claims 91-99, wherein the plasmid yield after incubating the transfected host cell under conditions sufficient to replicate the engineered vector is higher than for SURE2, SURE, Stbl2, Stbl3, or Stbl4 cells treated under the same conditions.