

CLAIMS

WHAT IS CLAIMED IS:

1. A mutant TcBuster transposase comprising an amino acid sequence at least 70% identical to full-length SEQ ID NO: 1 and having one or more amino acid substitutions that increase a net charge at a neutral pH in comparison to SEQ ID NO: 1, wherein the mutant TcBuster transposase has increased transposition efficiency in comparison to a wild-type TcBuster transposase having amino acid sequence SEQ ID NO: 1.
2. The mutant TcBuster transposase of claim 1, wherein the one or more amino acid substitutions comprise a substitution to a lysine or an arginine.
3. The mutant TcBuster transposase of claim 1 or 2, wherein the one or more amino acid substitutions comprise a substitution of an aspartic acid or a glutamic acid to a neutral amino acid, a lysine or an arginine.
4. The mutant TcBuster transposase of any one of claims 1-3, comprising one or more amino acid substitutions from Table 4.
5. A mutant TcBuster transposase comprising an amino acid sequence at least 70% identical to full-length SEQ ID NO: 1 and having one more amino acid substitutions in a DNA Binding and Oligomerization domain; an insertion domain; a Zn-BED domain; or a combination thereof, wherein the mutant TcBuster transposase has increased transposition efficiency in comparison to a wild-type TcBuster transposase having amino acid sequence SEQ ID NO: 1.
6. A mutant TcBuster transposase comprising an amino acid sequence at least 70% identical to full-length SEQ ID NO: 1 and having one or more amino acid substitutions from Table 1.
7. The mutant TcBuster transposase of any one of claims 1-6, comprising one or more amino acid substitutions that increase a net charge at a neutral pH within or in proximity to a catalytic domain in comparison to SEQ ID NO: 1.
8. The mutant TcBuster transposase of any one of claims 1-7, comprising one or more amino acid substitutions that increase a net charge at a neutral pH in comparison to SEQ ID NO: 1, wherein the one or more amino acids are located in proximity to D223, D289, or E589, when numbered in accordance to SEQ ID NO: 1.
9. The mutant TcBuster transposase of claim 7 or 8, wherein the proximity is a distance of about 80, 75, 70, 60, 50, 40, 30, 20, 10, or 5 amino acids.
10. The mutant TcBuster transposase of claim 7 or 8, wherein the proximity is a distance of about 70 to 80 amino acids.

11. The mutant TcBuster transposase of any one of claims 1-10, wherein the amino acid sequence of the mutant TcBuster transposase is at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identical to full-length SEQ ID NO: 1.
12. The mutant TcBuster transposase of any one of claims 1-11, comprising one or more amino acid substitutions from Table 2.
13. The mutant TcBuster transposase of any one of claims 1-12, comprising one or more amino acid substitutions from Table 3.
14. The mutant TcBuster transposase of any one of claims 1-13, comprising amino acid substitutions V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1.
15. The mutant TcBuster transposase of any one of claims 1-14, comprising amino acid substitutions K573E and E578L, when numbered in accordance with SEQ ID NO: 1.
16. The mutant TcBuster transposase of any one of claims 1-15, comprising amino acid substitution I452K, when numbered in accordance with SEQ ID NO: 1.
17. The mutant TcBuster transposase of any one of claims 1-16, comprising amino acid substitution A358K, when numbered in accordance with SEQ ID NO: 1.
18. The mutant TcBuster transposase of any one of claims 1-17, comprising amino acid substitution V297K, when numbered in accordance with SEQ ID NO: 1.
19. The mutant TcBuster transposase of any one of claims 1-18, comprising amino acid substitution N85S, when numbered in accordance with SEQ ID NO: 1.
20. The mutant TcBuster transposase of any one of claims 1-19, comprising amino acid substitutions I452F, V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1.
21. The mutant TcBuster transposase of any one of claims 1-20, comprising amino acid substitutions A358K, V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1.
22. The mutant TcBuster transposase of any one of claims 1-21, comprising amino acid substitutions V377T, E469K, D189A, K573E and E578L, when numbered in accordance with SEQ ID NO: 1.
23. The mutant TcBuster transposase of any one of claims 1-22, wherein the transposition efficiency is measured by an assay that comprises introducing the mutant TcBuster transposase and a TcBuster transposon containing a reporter cargo cassette into a population of cells, and detecting transposition of the reporter cargo cassette in genome of the population of cells.

24. A fusion transposase comprising a TcBuster transposase sequence and a DNA sequence specific binding domain, wherein the TcBuster transposase sequence has at least 70% identity to full-length SEQ ID NO: 1.
25. The fusion transposase of claim 24, wherein the DNA sequence specific binding domain comprises a TALE domain, zinc finger domain, AAV Rep DNA-binding domain, or any combination thereof.
26. The fusion transposase of claim 24 or 25, wherein the DNA sequence specific binding domain comprises a TALE domain.
27. The fusion transposase of any one of claims 24-26, wherein the TcBuster transposase sequence has at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity to full-length SEQ ID NO: 1.
28. The fusion transposase of any one of claims 24-27, wherein the TcBuster transposase sequence comprises one or more amino acid substitutions that increase a net charge at a neutral pH in comparison to SEQ ID NO: 1.
29. The fusion transposase of claim 28, wherein the one or more amino acid substitutions comprise a substitution with a lysine or an arginine.
30. The fusion transposase of claim 28 or 29, wherein the one or more amino acid substitutions comprise a substitution of an aspartic acid or a glutamic acid with a neutral amino acid, a lysine or an arginine.
31. The fusion transposase of any one of claims 24-30, wherein the TcBuster transposase sequence comprises one or more amino acid substitutions in a DNA Binding and Oligomerization domain; an insertion domain; a Zn-BED domain; or a combination thereof.
32. The fusion transposase of any one of claims 24-31, wherein the TcBuster transposase sequence comprises one or more amino acid substitutions from Table 1.
33. The fusion transposase of any one of claims 24-32, wherein the TcBuster transposase sequence has increased transposition efficiency in comparison to a wild-type TcBuster transposase having amino acid sequence SEQ ID NO: 1.
34. The fusion transposase of any one of claims 24-33, wherein the TcBuster transposase sequence comprises one or more amino acid substitutions that increase a net charge at a neutral pH within or in proximity to a catalytic domain in comparison to SEQ ID NO: 1.
35. The fusion transposase of any one of claims 24-34, wherein the TcBuster transposase sequence comprises one or more amino acid substitutions that increase a net charge at a neutral pH in comparison to SEQ ID NO: 1, wherein the one or more amino acid substitutions are located in proximity to D223, D289, or E589, when numbered in accordance to SEQ ID NO: 1.

36. The fusion transposase of claim 34 or 35, wherein the proximity is a distance of about 80, 75, 70, 60, 50, 40, 30, 20, 10, or 5 amino acids.
37. The fusion transposase of claim 34 or 35, wherein the proximity is a distance of about 70 to 80 amino acids.
38. The fusion transposase of any one of claims 24-37, wherein the TcBuster transposase sequence comprises one or more amino acid substitutions from Table 2.
39. The fusion transposase of any one of claims 24-38, wherein the TcBuster transposase sequence comprises one or more amino acid substitutions from Table 3.
40. The fusion transposase of any one of claims 24-39, wherein the TcBuster transposase sequence comprises amino acid substitutions V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1.
41. The fusion transposase of any one of claims 24-40, wherein the TcBuster transposase sequence comprises amino acid substitutions K573E and E578L, when numbered in accordance with SEQ ID NO: 1.
42. The fusion transposase of any one of claims 24-41, wherein the TcBuster transposase sequence comprises amino acid substitution I452K, when numbered in accordance with SEQ ID NO: 1.
43. The fusion transposase of any one of claims 24-42, wherein the TcBuster transposase sequence comprises amino acid substitution A358K, when numbered in accordance with SEQ ID NO: 1.
44. The fusion transposase of any one of claims 24-43, wherein the TcBuster transposase sequence comprises amino acid substitution V297K, when numbered in accordance with SEQ ID NO: 1.
45. The fusion transposase of any one of claims 24-44, wherein the TcBuster transposase sequence comprises amino acid substitution N85S, when numbered in accordance with SEQ ID NO: 1.
46. The fusion transposase of any one of claims 24-45, wherein the TcBuster transposase sequence comprises amino acid substitutions I452F, V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1.
47. The fusion transposase of any one of claims 24-46, wherein the TcBuster transposase sequence comprises amino acid substitutions A358K, V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1.

48. The fusion transposase of any one of claims 24-47, wherein the TcBuster transposase sequence comprises amino acid substitutions V377T, E469K, D189A, K573E and E578L, when numbered in accordance with SEQ ID NO: 1.
49. The fusion transposase of any one of claims 24-27, wherein the TcBuster transposase sequence has 100% identity to full-length SEQ ID NO: 1.
50. The fusion transposase of any one of claims 24-48, wherein the TcBuster transposase sequence and the DNA sequence specific binding domain are separated by a linker.
51. The fusion transposase of claim 50, wherein the linker comprises at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, or at least 50 amino acids.
52. The fusion transposase of claim 50 or 51, wherein the linker comprises SEQ ID NO: 9.
53. A polynucleotide that codes for the mutant TcBuster transposase of any one of claims 1-22.
54. A polynucleotide that codes for the fusion transposase of any one of claims 24-49.
55. The polynucleotide of claim 53 or 54, wherein the polynucleotide comprises DNA that encodes the mutant TcBuster transposase or the fusion transposase.
56. The polynucleotide of any one of claims 53-55, wherein the polynucleotide comprises messenger RNA (mRNA) that encodes the mutant TcBuster transposase or the fusion transposase.
57. The polynucleotide of claim 56, wherein the mRNA is chemically modified.
58. The polynucleotide of any one of claims 53-57, wherein the polynucleotide comprises nucleic acid sequence encoding for a transposon recognizable by the mutant TcBuster transposase or the fusion transposase.
59. The polynucleotide of any one of claims 53-58, wherein the polynucleotide is present in a DNA vector.
60. The polynucleotide of claim 59, wherein the DNA vector comprises a mini-circle plasmid.
61. A cell producing the mutant TcBuster transposase or fusion transposase of any one of claims 1-49.
62. A cell containing the polynucleotide of any one of claims 53-60.
63. A method comprising: introducing into a cell the mutant TcBuster transposase of any one of claims 1-22 and a transposon recognizable by the mutant TcBuster transposase.
64. A method comprising: introducing into a cell the fusion transposase of any one of claims 24-49 and a transposon recognizable by the fusion transposase.
65. The method of claim 63 or 64, wherein the introducing comprises contacting the cell with a polynucleotide encoding the mutant TcBuster transposase or the fusion transposase.

66. The method of claim 65, wherein the polynucleotide comprises DNA that encodes the mutant TcBuster transposase or the fusion transposase.
67. The method of claim 65, wherein the polynucleotide comprises messenger RNA (mRNA) that encodes the mutant TcBuster transposase or the fusion transposase.
68. The method of claim 67, wherein the mRNA is chemically modified.
69. The method of any one of claims 63-68, wherein the introducing comprises contacting the cell with a DNA vector that contains the transposon.
70. The method of claim 69, wherein the DNA vector comprises a mini-circle plasmid.
71. The method of any one of claims 65-70, wherein the introducing comprises contacting the cell with a plasmid vector that contains both the transposon and the polynucleotide encoding the mutant TcBuster transposase or the fusion transposase.
72. The method of any one of claims 63-71, wherein the introducing comprises contacting the cell with the mutant TcBuster transposase or the fusion transposase as a purified protein.
73. The method of any one of claims 63-72, wherein the transposon comprises a cargo cassette positioned between two inverted repeats.
74. The method of claim 73, wherein a left inverted repeat of the two inverted repeats comprises a sequence having at least 50%, at least 60%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity to SEQ ID NO: 3.
75. The method of claim 73, wherein a left inverted repeat of the two inverted repeats comprises SEQ ID NO: 3.
76. The method of any one of claims 73-75, wherein a right inverted repeat of the two inverted repeats comprises a sequence having at least 50%, at least 60%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity to SEQ ID NO: 4.
77. The method of any one of claims 73-75, wherein a right inverted repeat of the two inverted repeats comprises SEQ ID NO: 4.
78. The method of claim 73, wherein a left inverted repeat of the two inverted repeats comprises a sequence having at least 50%, at least 60%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity to SEQ ID NO: 5.
79. The method of claim 73, wherein a left inverted repeat of the two inverted repeats comprises SEQ ID NO: 5.
80. The method of claim 73, 78, or 79, wherein a right inverted repeat of the two inverted repeats comprises a sequence having at least 50%, at least 60%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity to SEQ ID NO: 6.

81. The method of claim 73, 78, or 79, wherein a right inverted repeat of the two inverted repeats comprises SEQ ID NO: 6.
82. The method of any one of claims 73-81, wherein the cargo cassette comprises a promoter selected from the group consisting of: CMV, EFS, MND, EF1 α , CAGCs, PGK, UBC, U6, H1, and Cumate.
83. The method of any one of claims 73-82, wherein the cargo cassette comprises a CMV promoter.
84. The method of any one of claims 73-83, wherein the cargo cassette is present in a forward direction.
85. The method of any one of claims 73-83, wherein the cargo cassette is present in a reverse direction.
86. The method of any one of claims 63-85, wherein the introducing comprises transfecting the cell with the aid of electroporation, microinjection, calcium phosphate precipitation, cationic polymers, dendrimers, liposome, microprojectile bombardment, fugene, direct sonic loading, cell squeezing, optical transfection, protoplast fusion, impalefection, magnetofection, nucleofection, or any combination thereof.
87. The method of any one of claims 63-86, wherein the introducing comprises electroporating the cell.
88. The method of any one of claims 63-87, wherein the cell is a primary cell isolated from a subject.
89. The method of claim 88, wherein the subject is a human.
90. The method of claim 88 or 89, wherein the subject is a patient with a disease.
91. The method of any one of claims 88-90, wherein the subject has been diagnosed with cancer or tumor.
92. The method of any one of claims 88-91, wherein the cell is isolated from blood of the subject.
93. The method of any one of claims 63-92, wherein the cell comprises a primary immune cell.
94. The method of any one of claims 63-93, wherein the cell comprises a primary leukocyte.
95. The method of any one of claims 63-94, wherein the cell comprises a primary T cell.
96. The method of claim 95, wherein the primary T cell comprises a gamma delta T cell, a helper T cell, a memory T cell, a natural killer T cell, an effector T cell, or any combination thereof.
97. The method of any one of claims 93-96, wherein the primary immune cell comprises a CD3+ cell.

98. The method of any one of claims 63-97, wherein the cell comprises a stem cell.
99. The method of claim 98, wherein the stem cell is selected from the group consisting of: embryonic stem cell, hematopoietic stem cell, epidermal stem cell, epithelial stem cell, bronchoalveolar stem cell, mammary stem cell, mesenchymal stem cell, intestine stem cell, endothelial stem cell, neural stem cell, olfactory adult stem cell, neural crest stem cell, testicular cell, and any combination thereof.
100. The method of claim 98, wherein the stem cell comprises induced pluripotent stem cell.
101. The method of any one of claims 73-100, wherein the cargo cassette comprises a transgene.
102. The method of claim 101, wherein the transgene codes for a protein selected from the group consisting of: a cellular receptor, an immunological checkpoint protein, a cytokine, and any combination thereof.
103. The method of claim 101 or 102, wherein the transgene codes for a cellular receptor selected from the group consisting of: a T cell receptor (TCR), a B cell receptor (BCR), a chimeric antigen receptor (CAR), or any combination thereof.
104. A method of treatment, comprising:
- (a) introducing into a cell a transposon and the mutant TcBuster transposase or the fusion transposase of any one of claims 1-47, which recognize the transposon, thereby generating a genetically modified cell; and
 - (b) administering the genetically modified cell to a patient in need of the treatment.
105. The method of claim 104, wherein the genetically modified cell comprises a transgene introduced by the transposon.
106. The method of claim 104 or 105, wherein the patient has been diagnosed with cancer or tumor.
107. The method of any one of claims 104-106, wherein the administering comprises transfusing the genetically modified cell into blood vessels of the patient.
108. A system for genome editing, comprising: the mutant TcBuster transposase or fusion transposase of any one of claims 1-49, and a transposon recognizable by the mutant TcBuster transposase or the fusion transposase.
109. A system for genome editing, comprising: the polynucleotide encoding a mutant TcBuster transposase or fusion transposase of any one of claims 1-49, and a transposon recognizable by the mutant TcBuster transposase or the fusion transposase.
110. The system of claim 109, wherein the polynucleotide comprises DNA that encodes the mutant TcBuster transposase or the fusion transposase.

111. The system of claim 109 or 110, wherein the polynucleotide comprises messenger RNA (mRNA) that encodes the mutant TcBuster transposase or the fusion transposase.
112. The system of claim 111, wherein the mRNA is chemically modified.
113. The system of any one of claims 108-112, wherein the transposon is present in a DNA vector.
114. The system of claim 113, wherein the DNA vector comprises a mini-circle plasmid.
115. The system of any one of claims 109-114, wherein the polynucleotide and the transposon are present in a same plasmid.
116. The system of any one of claims 108-115, wherein the transposon comprises a cargo cassette positioned between two inverted repeats.
117. The system of claim 116, wherein a left inverted repeat of the two inverted repeats comprises a sequence having at least 50%, at least 60%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity to SEQ ID NO: 3.
118. The system of claim 116, wherein a right inverted repeat of the two inverted repeats comprises SEQ ID NO: 3.
119. The system of any one of claims 116-118, wherein a right inverted repeat of the two inverted repeats comprises a sequence having at least 50%, at least 60%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity to SEQ ID NO: 4.
120. The system of any one of claims 116-118, wherein a left inverted repeat of the two inverted repeats comprises SEQ ID NO: 4.
121. The system of claim 116, wherein a left inverted repeat of the two inverted repeats comprises a sequence having at least 50%, at least 60%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity to SEQ ID NO: 5.
122. The system of claim 116, wherein a right inverted repeat of the two inverted repeats comprises SEQ ID NO: 5.
123. The system of claim 116, 121, or 122, wherein a right inverted repeat of the two inverted repeats comprises a sequence having at least 50%, at least 60%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identity to SEQ ID NO: 6.
124. The system of claim 116, 121, or 122, wherein a left inverted repeat of the two inverted repeats comprises SEQ ID NO: 6.
125. The system of any one of claims 116-124, wherein the cargo cassette comprises a promoter selected from the group consisting of: CMV, EFS, MND, EF1 α , CAGCs, PGK, UBC, U6, H1, and Cumate.

126. The system of any one of claims 116-116, wherein the cargo cassette comprises a CMV promoter.

127. The system of any one of claims 116-126, wherein the cargo cassette comprises a transgene.

128. The system of claim 127, wherein the transgene codes for a protein selected from the group consisting of: a cellular receptor, an immunological checkpoint protein, a cytokine, and any combination thereof.

129. The system of claim 127 or 128, wherein the transgene codes for a cellular receptor selected from the group consisting of: a T cell receptor (TCR), a B cell receptor (BCR), a chimeric antigen receptor (CAR), or any combination thereof.

130. The system of any one of claims 116-129, wherein the cargo cassette is present in a forward direction.

131. The system of any one of claims 116-129, wherein the cargo cassette is present in a reverse direction.