

ENHANCED hAT FAMILY TRANSPOSON-MEDIATED GENE TRANSFER AND ASSOCIATED COMPOSITIONS, SYSTEMS, AND METHODS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/435,522, filed December 16, 2016, which application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Transposable genetic elements, also called transposons, are segments of DNA that can be mobilized from one genomic location to another within a single cell. Transposons can be divided into two major groups according to their mechanism of transposition: transposition can occur (1) via reverse transcription of an RNA intermediate for elements termed retrotransposons, and (2) via direct transposition of DNA flanked by terminal inverted repeats (TIRs) for DNA transposons. Active transposons encode one or more proteins that are required for transposition. The natural active DNA transposons harbor a transposase enzyme gene.

[0003] DNA transposons in the hAT family are widespread in plants and animals. A number of active hAT transposon systems have been identified and found to be functional, including but not limited to, the Hermes transposon, Ac transposon, hobo transposon, and the Tol2 transposon. The hAT family is composed of two families that have been classified as the AC subfamily and the Buster subfamily, based on the primary sequence of their transposases. Members of the hAT family belong to Class II transposable elements. Class II mobile elements use a cut and paste mechanism of transposition. hAT elements share similar transposases, short terminal inverted repeats, and an eight base-pairs duplication of genomic target.

SUMMARY

[0004] One example of the present disclosure provides 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. In some examples, the mutant TcBuster transposase has increased transposition efficiency in comparison to a wild-type TcBuster transposase having amino acid sequence SEQ ID NO: 1.

[0004a] One aspect of the present disclosure provides 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.

[0005] Another example of the present disclosure provides 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. In some examples, the mutant TcBuster transposase has increased transposition efficiency in comparison to a wild-type TcBuster transposase having amino acid sequence SEQ ID NO: 1.

[0005a] Another aspect of the present disclosure provides 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.

[0006] Yet another aspect of the present disclosure provides 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**.

[0007] In some examples, a mutant TcBuster transposase 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. In some examples, the mutant TcBuster transposase comprises one or more amino acid substitutions that increase a net charge at a neutral pH in comparison to SEQ ID NO: 1, and the one or more amino acids are located in proximity to D223, D289, or E589, when numbered in accordance to SEQ ID NO: 1. In some examples, the proximity is a distance of about 80, 75, 70, 60, 50, 40, 30, 20, 10, or 5 amino acids. In some examples, the proximity is a distance of about 70 to 80 amino acids.

[0008] In some examples, 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.

[0009] In some examples, the one or more amino acid substitutions comprise a substitution to a lysine or an arginine. In some examples, 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. In some examples, the mutant TcBuster transposase comprises one or more amino acid substitutions from **Table 4**. In some examples, the mutant TcBuster transposase comprises one or more amino acid substitutions from **Table 2**. In some examples, the mutant TcBuster transposase comprises one or more amino acid substitutions from **Table 3**. In some examples, the mutant TcBuster transposase comprises amino acid substitutions V377T, E469K, and

D189A, when numbered in accordance with SEQ ID NO: 1. In some examples, the mutant TcBuster transposase comprises amino acid substitutions K573E and E578L, when numbered in accordance with SEQ ID NO: 1. In some examples, the mutant TcBuster transposase comprises amino acid substitution I452K, when numbered in accordance with SEQ ID NO: 1. In some examples, the mutant TcBuster transposase comprises amino acid substitution A358K, when numbered in accordance with SEQ ID NO: 1. In some examples, the mutant TcBuster transposase comprises amino acid substitution V297K, when numbered in accordance with SEQ ID NO: 1. In some examples, the mutant TcBuster transposase comprises amino acid substitution N85S, when numbered in accordance with SEQ ID NO: 1. In some examples, the mutant TcBuster transposase comprises amino acid substitutions I452F, V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1. In some examples, the mutant TcBuster transposase comprises amino acid substitutions A358K, V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1. In some examples, the mutant TcBuster transposase comprises amino acid substitutions V377T, E469K, D189A, K573E and E578L, when numbered in accordance with SEQ ID NO: 1.

[0010] In some examples, 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.

[0011] Yet another example of the present disclosure provides a fusion transposase comprising a TcBuster transposase sequence and a DNA sequence specific binding domain. In some examples, the TcBuster transposase sequence has at least 70% identity to full-length SEQ ID NO: 1.

[0012] In some examples, the DNA sequence specific binding domain comprises a TALE domain, zinc finger domain, AAV Rep DNA-binding domain, or any combination thereof. In some examples, the DNA sequence specific binding domain comprises a TALE domain.

[0013] In some examples, 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. In some examples, 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. In some examples, 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. In some examples, the TcBuster transposase sequence comprises one or more amino acid substitutions from **Table 1**. In some examples, the TcBuster transposase sequence has increased

transposition efficiency in comparison to a wild-type TcBuster transposase having amino acid sequence SEQ ID NO: 1. In some examples, 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. In some examples, 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, and 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. In some examples, the proximity is a distance of about 80, 75, 70, 60, 50, 40, 30, 20, 10, or 5 amino acids. In some examples, the proximity is a distance of about 70 to 80 amino acids. In some examples, the TcBuster transposase sequence comprises one or more amino acid substitutions from **Table 2**. In some examples, the TcBuster transposase sequence comprises one or more amino acid substitutions from **Table 3**. In some examples, the TcBuster transposase sequence comprises amino acid substitutions V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1. In some examples, the TcBuster transposase sequence comprises amino acid substitutions K573E and E578L, when numbered in accordance with SEQ ID NO: 1. In some examples, the TcBuster transposase sequence comprises amino acid substitution I452K, when numbered in accordance with SEQ ID NO: 1. In some examples, the TcBuster transposase sequence comprises amino acid substitution A358K, when numbered in accordance with SEQ ID NO: 1. In some examples, the TcBuster transposase sequence comprises amino acid substitution V297K, when numbered in accordance with SEQ ID NO: 1. In some examples, the TcBuster transposase sequence comprises amino acid substitution N85S, when numbered in accordance with SEQ ID NO: 1. In some examples, the TcBuster transposase sequence comprises amino acid substitutions I452F, V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1. In some examples, the TcBuster transposase sequence comprises amino acid substitutions A358K, V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1. In some examples, the TcBuster transposase sequence comprises amino acid substitutions V377T, E469K, D189A, K573E and E578L, when numbered in accordance with SEQ ID NO: 1. In some examples, the TcBuster transposase sequence has 100% identity to full-length SEQ ID NO: 1.

[0014] In some examples of a fusion transposase, the TcBuster transposase sequence and the DNA sequence specific binding domain are separated by a linker. In some examples, 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. In some examples, the linker comprises SEQ ID NO: 9.

[0015] Yet another example of the present disclosure provides a polynucleotide that codes for the mutant TcBuster transposase as described herein.

[0016] Yet another example of the present disclosure provides a polynucleotide that codes for the fusion transposase as described herein.

[0017] In some examples, the polynucleotide comprises DNA that encodes the mutant TcBuster transposase or the fusion transposase. In some examples, the polynucleotide comprises messenger RNA (mRNA) that encodes the mutant TcBuster transposase or the fusion transposase. In some examples, the mRNA is chemically modified. In some examples, the polynucleotide comprises nucleic acid sequence encoding for a transposon recognizable by the mutant TcBuster transposase or the fusion transposase. In some examples, the polynucleotide is present in a DNA vector. In some examples, the DNA vector comprises a mini-circle plasmid.

[0018] Yet another example of the present disclosure provides a cell producing the mutant TcBuster transposase or fusion transposase as described herein. Yet another example of the present disclosure provides a cell containing the polynucleotide as described herein.

[0019] Yet another example of the present disclosure provides a method comprising: introducing into a cell the mutant TcBuster transposase as described herein and a transposon recognizable by the mutant TcBuster transposase.

[0020] Yet another example of the present disclosure provides a method comprising: introducing into a cell the fusion transposase as described herein and a transposon recognizable by the fusion transposase.

[0021] In some examples of a method, the introducing comprises contacting the cell with a polynucleotide encoding the mutant TcBuster transposase or the fusion transposase. In some examples, the polynucleotide comprises DNA that encodes the mutant TcBuster transposase or the fusion transposase. In some examples, the polynucleotide comprises messenger RNA (mRNA) that encodes the mutant TcBuster transposase or the fusion transposase. In some examples, the mRNA is chemically modified.

[0022] In some examples of a method, the introducing comprises contacting the cell with a DNA vector that contains the transposon. In some examples, the DNA vector comprises a mini-circle plasmid. In some examples, 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. In some examples, the introducing comprises contacting the cell with the mutant TcBuster transposase or the fusion transposase as a purified protein.

[0023] In some examples of a method, the transposon comprises a cargo cassette positioned between two inverted repeats. In some examples, 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. In some examples, a left inverted repeat of the two inverted repeats comprises SEQ ID NO: 3. In some examples, 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. In some examples, a right inverted repeat of the two inverted repeats comprises SEQ ID NO: 4. In some examples, 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. In some examples, a left inverted repeat of the two inverted repeats comprises SEQ ID NO: 5. In some examples, 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. In some examples, a right inverted repeat of the two inverted repeats comprises SEQ ID NO: 6. In some examples, the cargo cassette comprises a promoter selected from the group consisting of: CMV, EFS, MND, EF1 α , CAGCs, PGK, UBC, U6, H1, and Cumate. In some examples, the cargo cassette comprises a CMV promoter. In some examples, the cargo cassette is present in a forward direction. In some examples, the cargo cassette is present in a reverse direction. In some examples, the cargo cassette comprises a transgene. In some examples, 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. In some examples, 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. In some examples, 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. In some examples, the introducing comprises electroporating the cell.

[0024] In some examples of a method, the cell is a primary cell isolated from a subject. In some examples, the subject is a human. In some examples, the subject is a patient with a disease. In some examples, the subject has been diagnosed with cancer or tumor. In some examples, the cell is isolated from blood of the subject. In some examples, the cell comprises a primary immune cell. In some examples, the cell comprises a primary leukocyte. In some examples, the cell comprises a primary T cell. In some examples, 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. In some examples, the primary immune cell comprises a CD3+ cell. In some examples, the cell comprises a stem cell. In some examples, 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. In some examples, the stem cell comprises induced pluripotent stem cell.

[0025] Yet another example of the present disclosure provides a method of treatment, comprising: (a) introducing into a cell a transposon and the mutant TcBuster transposase or the fusion transposase as described herein, which recognize the transposon, thereby generating a genetically modified cell; (b) administering the genetically modified cell to a patient in need of the treatment. In some examples, the genetically modified cell comprises a transgene introduced by the transposon. In some examples, the patient has been diagnosed with cancer or tumor. In some examples, the administering comprises transfusing the genetically modified cell into blood vessels of the patient.

[0026] Yet another example of the present disclosure provides a system for genome editing, comprising: the mutant TcBuster transposase or fusion transposase as described herein, and a transposon recognizable by the mutant TcBuster transposase or the fusion transposase.

[0027] Yet another example of the present disclosure provides a system for genome editing, comprising: the polynucleotide encoding a mutant TcBuster transposase or fusion transposase as described herein, and a transposon recognizable by the mutant TcBuster transposase or the fusion transposase.

[0028] In some examples of a system, the polynucleotide comprises DNA that encodes the mutant TcBuster transposase or the fusion transposase. In some examples, the polynucleotide comprises messenger RNA (mRNA) that encodes the mutant TcBuster transposase or the fusion transposase. In some examples, the mRNA is chemically modified. In some examples, the transposon is present in a DNA vector. In some examples, the DNA vector comprises a mini-circle plasmid. In some examples, the polynucleotide and the transposon are present in a same plasmid. In some examples, the transposon comprises a cargo cassette positioned between two inverted repeats. In some examples, 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. In some examples, a left inverted repeat of the two inverted repeats comprises SEQ ID NO: 3. In some examples, 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. In some examples, a right inverted repeat of the two inverted repeats comprises SEQ ID NO: 4. In some examples, 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. In some examples, a left inverted repeat of the two inverted repeats comprises SEQ ID NO: 5. In some examples, 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. In some examples, a right inverted repeat of the two inverted repeats comprises SEQ ID NO: 6. In some examples, the cargo cassette comprises a promoter selected from the group consisting of: CMV, EFS, MND, EF1 α , CAGCs, PGK, UBC, U6, H1, and Cumate. In some examples, the cargo cassette comprises a CMV promoter. In some examples, the cargo cassette comprises a transgene. In some examples, 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. In some examples, 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. In some examples, the cargo cassette is present in a forward direction. In some examples, the cargo cassette is present in a reverse direction.

INCORPORATION BY REFERENCE

[0029] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent that a term incorporated by reference conflicts with a term defined herein, this specification controls.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0031] **Fig. 1** shows the transposition efficiency of several exemplary TcBuster transposon vector constructs, as measured by percent of mCherry positive cells in cells that were transfected with wild-type (WT) TcBuster transposase and the exemplary TcBuster transposons.

[0032] **Fig. 2** shows nucleotide sequence comparison of exemplary TcBuster IR/DR sequence 1 and sequence 2.

[0033] **Fig. 3A** shows representative bright-field and fluorescent images of HEK-293T cells 2 weeks after transfection with exemplary TcBuster transposon Tn-8 (containing puro-mCherry

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.