

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 one or more amino acid substitutions, when numbered in accordance with SEQ ID NO: 1, selected from: a) N85S, (b) V377T, E469K, and D189A, and (c) K573E and E578L.

[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.

[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

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 one or more amino acid substitutions, when numbered in accordance with SEQ ID NO: 1, selected from:
 - (a) N85S,
 - (b) V377T, E469K, and D189A , and
 - (c) K573E and E578L.
2. The mutant TcBuster transposase of claim 1, further comprising one or more amino acid substitutions from Table 4.
3. The mutant TcBuster transposase of claim 1 or claim 2, further comprising one or more amino acid substitutions that increase a net charge at a neutral pH within 80, 75, 70, 60, 50, 40, 30, 20, 10, or 5 amino acids of a catalytic domain in comparison to SEQ ID NO: 1.
4. The mutant TcBuster transposase of any one of claims 1-3, further 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 within 80, 75, 70, 60, 50, 40, 30, 20, 10, or 5 amino acids of D223, D289, or E589, when numbered in accordance with SEQ ID NO: 1.
5. The mutant TcBuster transposase of claim 4, wherein the one or more amino acids are located within about 70 to 80 amino acids of D223, D289, or E589.
6. The mutant TcBuster transposase of any one of claims 1-5, 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.
7. The mutant TcBuster transposase of any one of claims 1-6, further comprising one or more amino acid substitutions from Table 2.
8. The mutant TcBuster transposase of any one of claims 1-7, further comprising one or more amino acid substitutions from Table 3.
9. The mutant TcBuster transposase of any one of claims 1-8, comprising amino acid substitutions V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1.
10. The mutant TcBuster transposase of any one of claims 1-9, comprising amino acid substitutions K573E and E578L, when numbered in accordance with SEQ ID NO: 1.
11. The mutant TcBuster transposase of any one of claims 1-10, further comprising amino acid substitution A358K, when numbered in accordance with SEQ ID NO: 1.

12. The mutant TcBuster transposase of any one of claims 1-11, further comprising amino acid substitution V297K, when numbered in accordance with SEQ ID NO: 1.
13. The mutant TcBuster transposase of any one of claims 1-12, comprising amino acid substitution N85S, when numbered in accordance with SEQ ID NO: 1.
14. The mutant TcBuster transposase of any one of claims 1-13, comprising amino acid substitutions I452F, V377T, E469K, and D189A, when numbered in accordance with SEQ ID NO: 1.