As such, there is a need to increase recombinant protein production from host cells and improve long term stability of expression. One way to achieve this objective is to improve the titers of proteins expressed by cell expression systems. The invention described herein provides mutations that contribute to increased stability of piggyBac transposase expressed in host cells. It was found that piggyBac transposases encoded by nucleic acid sequences engineered with such mutations lead to improved titer of the recombinant proteins expressed in cells comprising the engineered piggyBac transposase compared to the titer of the recombinant proteins expressed in cells comprising wild type piggyBac transposase or no piggyBac transposase.

SUMMARY

In one example the disclosure provides a piggyBac transposase comprising an amino acid substitution at one or more of the positions 147, 176, 221, 247, 429, 533, and 573 of SEQ ID NO:2. In one example the piggyBac transposase comprises an amino acid substitution of leucine for the isoleucine at one or more of the positions 147, 176, 221, and 247 of SEQ ID NO:2. In one example the piggyBac transposase comprises an amino acid substitution of threonine for serine at one or more of the positions 429, 533, and 573 of SEQ ID NO:2. In one example the piggyBac transposase comprises an amino acid substitution of leucine for the isoleucine at one or more of the positions 147, 176, 221, and 247 and/or an amino acid substitution of threonine for serine at one or more of the positions 429, 533, and 573 of SEQ ID NO:2. In a related example the piggyBac transposase comprises at least one of the following amino acid substitutions, a leucine for the isoleucine at position 147 of SEQ ID NO:2, a leucine for the isoleucine at position 247 of SEQ ID NO:2, and a threonine for the serine at position 533 of SEQ ID NO:2. In a related example the piggyBac transposase comprises at least two of the following amino acid substitutions, a leucine for the isoleucine at position 147 of SEQ ID NO:2, a leucine for the isoleucine at position 247 of SEQ ID NO:2, and a threonine for the serine at position 533 of SEQ ID NO:2. In another related aspect the piggyBac transposase comprises a leucine for isoleucine substitution at position 147 of SEQ ID NO:2, a leucine for isoleucine substitution at position 247 of SEQ ID NO:2, and a threonine for serine substitution at position 533 of SEQ ID NO:2. In one example the piggyBac transposase comprises the titer of a recombinant protein of interest expressed by a cell transfected with the engineered piggyBac transposase as described herein is improved compared to the titer of the same

protein of interest expressed by a cell transfected with a wild type piggyBac transposase or no piggyBac transposase. In one example, the recombinant protein of interest is an antigen binding protein.

In one example the disclosure provides a piggyBac transposase engineered to increase stability in a host cell, wherein the piggyBac transposase comprises an amino acid substitution at one or more of the positions 147, 176, 221, 247, 429, 533, and 573 of SEQ ID NO:2.

In another aspect the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 8. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO: 10 or 12. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO:4. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO:6. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO:8. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO:10. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO: 12. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO: 14. In another example the disclosure provides a piggyBac transposase having the amino acid sequence of SEQ ID NO:16.

In another aspect the disclosure provides an engineered nucleic acid molecule encoding a piggyBac transposase as described herein. An engineered nucleic acid molecule as described herein, wherein the nucleic acid molecule is selected from DNA or mRNA. In another aspect the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, or SEQ ID NO:18. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7. In another example the

disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 11, SEQ ID NO: 13, or SEQ ID NO: 15. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 9, or SEQ ID NO: 11. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 17, or SEQ ID NO:18. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 3. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 5. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 7. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 9. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 11. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 13. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 15. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 17. In another example the disclosure provides a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO:18.

In another aspect the disclosure provides a vector comprising the nucleic acid molecule encoding a piggyBac transposase as described herein. In one aspect the vector comprises the nucleic acid molecule encoding a piggyBac transposase as described herein, further comprising at least one nucleic acid sequence encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon. In one example the vector comprises one or more nucleic acid sequences encoding one or more proteins of interest.

In another aspect the disclosure provides a cell transfected with a piggyBac transposase encoded by a nucleic acid sequence as described herein.

In another aspect the disclosure provides a cell transfected with a vector as described herein. In one example the cell is transfected with a piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 17 or 18. In one example the cell is a host cell. In one example the cell is a CHO cell. In one example the cell is an immune cell.

In another aspect the disclosure provides a cell co-transfected with an engineered nucleic acid molecule encoding a piggyBac transposase as described herein, and a vector comprising at least one nucleic acid molecule encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon. In another aspect the disclosure provides a cell co-transfected with a vector comprising an engineered nucleic acid molecule encoding a piggyBac transposase as described herein, and a vector comprising at least one nucleic acid molecule encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon. In another aspect the disclosure provides a cell co-transfected with a vector comprising an engineered nucleic acid molecule encoding a piggyBac transposase as described herein, and also comprising at least one nucleic acid molecule encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon.

In another example the disclosure provides a cell as described herein, wherein the titer of a protein of interest expressed by the cell transfected with the engineered piggyBac transposase is improved compared to the titer of the same protein of interest expressed by a cell transfected with a wild type piggyBac transposase or no piggyBac transposase. In one aspect is provided a recombinant protein of interest expressed by the cell. In a related example is a pharmaceutical composition comprising the recombinant protein of interest.

In another example the disclosure provides a method for improving the titer of a recombinant protein of interest expressed by a host cell comprising engineering a nucleic acid molecule encoding a piggyBac transposase to increase stability in the host cell; co-transfecting the host cell the engineered nucleic acid molecule encoding the piggyBac transposase and with a vector comprising the nucleic acid sequence encoding the protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon; and culturing the cells to express the recombinant protein of interest, wherein the titer of the recombinant protein of interest expressed by the host cell transfected with the engineered piggyBac transposase is improved compared to the titer of the recombinant protein of interest expressed by a host cell transfected with a wild type piggyBac transposase or no piggyBac transposase. In one example the host cell is transfected with a first vector comprising the engineered nucleic acid molecule encoding the piggyBac transposase and with a second vector comprising the nucleic acid sequence encoding the protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon. In one example the host cell is transfected with a single vector comprising the

engineered nucleic acid molecule encoding the piggyBac transposase and a nucleic acid sequence encoding the protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon.

In another example the disclosure provides a method for increasing recombinant protein production in a mammalian cell culture expressing a recombinant protein comprising establishing a cell culture in a bioreactor using a host cell that has been co-transfected with a nucleic acid molecule encoding a piggyBac transposase engineered to increase stability in the host cell, and a vector comprising the nucleic acid molecule encoding the protein of interest flanked by at least the inverted repeat elements of a piggyBac transposon; and expressing the recombinant protein of interest; wherein the titer of the recombinant protein of interest expressed by the host cell transfected with the engineered piggyBac transposase is improved compared to the titer of the recombinant protein of interest expressed by the host cell transfected with a wild type piggyBac transposase or no piggyBac transposase. In one example the host cell has been co-transfected with a vector comprising a nucleic acid molecule encoding a piggyBac transposase engineered to increase stability in the host cell, and a vector comprising the nucleic acid molecule encoding the protein of interest flanked by at least the inverted repeat elements of a piggyBac transposon. In one example the host cell is transfected with a single vector comprising the engineered nucleic acid molecule encoding the piggyBac transposase and a nucleic acid sequence encoding the protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon.

In another example the disclosure provides a method for producing an isolated, purified, recombinant protein of interest comprising establishing a cell culture in a bioreactor with a host cell expressing a recombinant protein of interest, wherein the cell line has been co-transfected with a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in the host cell, and a vector comprising the nucleic acid sequence encoding the protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon; culturing the cells to express the recombinant protein of interest; harvesting the recombinant protein of interest, processing the recombinant protein of interest through one or more unit operations, and obtaining an isolated, purified, recombinant protein of interest. In one example the cell line has been co-transfected with a vector comprising a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in the host cell, and a vector comprising the nucleic acid sequence

encoding the protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon. In one example the host cell is transfected with a single vector comprising the engineered nucleic acid molecule encoding the piggyBac transposase and a nucleic acid sequence encoding the protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon. In one example at least one unit operation is a capture chromatography step selected from affinity chromatography, ion exchange chromatography, anion exchange chromatography, cation exchange chromatography, multi-modal chromatography, hydrophobic interaction chromatography, and hydroxyapatite chromatography. In a related at least one unit operation is a polish chromatography step selected from ion exchange chromatography, anion exchange chromatography, cation exchange chromatography, multi-modal chromatography, hydrophobic interaction chromatography, and hydroxyapatite chromatography. In one example at least one unit operation is selected from virus inactivation, virus filtration, depth filtration, and UF/DF. In one example the titer of the recombinant protein of interest expressed by the host cell transfected with the engineered piggyBac transposase is improved compared to the titer of the recombinant protein of interest expressed by the host cell transfected with a wild type piggyBac transposase or no piggyBac transposase. In one example is the isolated, purified, recombinant protein of. In a related example is a pharmaceutical composition comprising the isolated protein of interest.

In one example the disclosure provides a kit for transfecting a cell comprising a vector comprising a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in the host cell; and a vector comprising at least the 5' and 3' inverted repeat elements of a piggyBac transposon into which at least one nucleic acid sequence encoding at least one protein of interest is inserted.

In one example the disclosure provides a method of generating non-viral genetically modified cells, comprising: (a) establishing a vector comprising a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in a cell and at least one nucleic acid sequence encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon; (b) isolating native immune cells from a donor or subject; (c) transfecting the cells with the vector and a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in a cell; and (d) expanding the cells by cell culture into a larger population of non-viral, genetically modified cells. In one example the cells are transfected with the vector comprising at least

one nucleic acid sequence encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon, and a vector comprising a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in a cell. In one example the cells are transfected with a vector comprising at least one nucleic acid sequence encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon, and a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in a cell.

In one example the disclosure provides a method of treating a subject with a non-viral genetically modified cell, comprising: (a) establishing a vector comprising a least one nucleic acid sequence encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon; (b) isolating native immune cells from a subject or donor; (c) transfecting the cells with the vector and a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in a cell; (d) expanding the cells by cell culture into a larger population of genetically modified cells; (e) isolating the transformed cells from the cell culture to obtain a cell population comprising the genetically modified cells; and (f) reintroducing the non-viral, genetically modified cells into the subject. In one example the cells are transfected with the vector comprising at least one nucleic acid sequence encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon, and a vector comprising a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in a cell. In one example the cells are transfected with a vector comprising at least one nucleic acid sequence encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon, and a nucleic acid sequence encoding a piggyBac transposase engineered to increase stability in a cell. In one example the native immune cell is a mononuclear cell. In one example the native immune cell is a T cell. In one example at least one protein of interest is an antigen receptor, a T cell receptor, or a chimeric antigen receptor. In one example the cell is also transfected with a nucleic acid molecule encoding a suicide gene, an inducible on or accelerator switch, or both. In one example are non-viral genetically modified cells, cell populations, or cell cultures. In one example is a formulation comprising the genetically modified cells or cell. In another example is a method of treating or preventing a disease or disorder in a donor or subject in need thereof comprising administering to the donor or subject an effective amount of the genetically modified cells or cell populations.

What is claimed is:

1. A piggyBac transposase, comprising a leucine for isoleucine substitution at position 147 of SEQ ID NO:2, a leucine for isoleucine substitution at position 247 of SEQ ID NO:2, and a threonine for serine substitution at position 533 of SEQ ID NO:2.

2. A piggyBac transposase having the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16.

3. An engineered nucleic acid molecule encoding a piggyBac transposase according to claims 1 or 2.

4. An engineered nucleic acid molecule according to claim 3, wherein the nucleic acid molecule is selected from DNA or mRNA.

5. A piggyBac transposase encoded by the nucleic acid sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, or SEQ ID NO:18.

6. A vector comprising the nucleic acid molecule encoding a piggyBac transposase according claims 3 or 5.

7. A vector comprising the nucleic acid molecule encoding a piggyBac transposase according claim 6, further comprising at least one nucleic acid sequence encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon.

8. The vector according to claim 7, wherein the vector comprises one or more nucleic acid sequences encoding one or more proteins of interest.

9. A cell transfected with a piggyBac transposase encoded by a nucleic acid sequence according to claim 3 or 5.

10. A cell transfected with a vector according to claim 6.

11. The cell according to claim 9 or 10, wherein the cell is a host cell.

12. The cell according to claim 9 or 10, wherein the cell is a CHO cell.

13. The cell according to claim 9 or 10, wherein the cell is an immune cell.

14. A cell co-transfected with an engineered nucleic acid molecule encoding a piggyBac transposase according to claim 3 or 5, and a vector comprising at least one nucleic acid molecule encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon.

15. A cell co-transfected with a vector comprising an engineered nucleic acid molecule encoding a piggyBac transposase according to claim 6, and a vector comprising at least one nucleic acid molecule encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon.

16. A cell co-transfected with a vector comprising an engineered nucleic acid molecule encoding a piggyBac transposase according to claim 6 and comprising at least one nucleic acid molecule encoding at least one protein of interest flanked by at least the 5' and 3' inverted repeat elements of a piggyBac transposon.

17. The cell according to claims 9, 10, 14, 15, or 16, wherein the titer of a protein of interest expressed by the cell transfected with the engineered piggyBac transposase is improved compared to the titer of the same protein of interest expressed by a cell transfected with a wild type piggyBac transposase or no piggyBac transposase.

18. The cell according to claim 14, 15, 16, or 17, wherein the protein of interest is an antigen binding protein.

19. The cell according to claim 14, 15, 16, or17, wherein the protein of interest is a T cell receptor.

20. A protein of interest expressed by the cell according to claim 14. 15. 16, or 17.