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~~ example 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~~ example 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 aspect the disclosure provides an engineered nucleic acid molecule encoding a piggyBac transposase, wherein the piggyBac transposase is 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~~ example 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~~ example 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 an engineered nucleic acid molecule 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~~ example 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 aspect the disclosure provides a cell transfected with a vector comprising an engineered nucleic acid molecule encoding a piggyBac transposase as described herein 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.

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~~ example 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

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

~~4~~3. An engineered nucleic acid molecule according to claim ~~3~~2, wherein the nucleic acid molecule is selected from DNA or mRNA.

~~5~~4. An engineered nucleic acid molecule encoding a~~A~~ piggyBac transposase, wherein the piggyBac transposase is 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~~5. A vector comprising the nucleic acid molecule encoding a piggyBac transposase according ~~claims 3 or 5~~to claims 2 or 4.

~~7~~6. ~~A~~ The vector ~~comprising the nucleic acid molecule encoding a piggyBac transposase~~ according to claim ~~6~~5, 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~~7. The vector according to claim ~~7~~6, wherein the vector comprises ~~one~~ two or more nucleic acid sequences encoding ~~one~~ two or more proteins of interest.

~~9~~8. A cell transfected with an engineered ~~a piggyBac transposase encoded by a~~ nucleic acid ~~sequence~~ molecule according to claim ~~3~~ 2 or ~~5~~4.

~~10~~9. A cell transfected with a vector according to claim ~~6~~5.

~~11~~10. The cell according to claim ~~9~~ 8 or ~~10~~9, wherein the cell is a host cell.

~~12~~11. The cell according to claim ~~9~~ 8 or ~~10~~9, wherein the cell is a CHO cell.

~~13~~12. The cell according to claim ~~9~~ 8 or ~~10~~9, wherein the cell is an immune cell.

~~14~~13. A cell co-transfected with an engineered nucleic acid molecule encoding a piggyBac transposase according to claim ~~3~~ 2 or ~~5~~4, 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~~14. A cell co-transfected with a vector comprising an engineered nucleic acid molecule encoding a piggyBac transposase according to claim ~~6~~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.

~~16~~15. A cell ~~co~~ transfected with a vector comprising an engineered nucleic acid molecule encoding a piggyBac transposase according to claim ~~6~~ 5 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~~16. The cell according to claims ~~9, 10, 14, 15, or 16~~13, 14, or 15, wherein the titer of ~~a~~ the at least one protein of interest expressed by the cell transfected with the engineered nucleic acid molecule encoding a 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~~17. The cell according to claim 13, 14, 15, or 16~~14, 15, 16, or 17~~, wherein the protein of interest is an antigen binding protein.

~~19~~18. The cell according to claim 13, 14, 15, or 16~~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.~~