

WHAT IS CLAIMED IS:

1. A nucleic acid comprising
a 5' - ITR (inverted terminal repeat) sequence;
a 3' -ITR sequence; and
a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-15 and SEQ ID NOs: 35-58.
2. The nucleic acid of claim 1, wherein the 5' ITR sequence comprises or consists of a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 68, and the 3' ITR sequence comprises or consists of a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 60.
3. The nucleic acid of claim 1, wherein the 5' ITR sequence comprises SEQ ID NO: 68 and the 3' ITR sequence comprises SEQ ID NO: 60.
4. The nucleic acid of claims 1, further comprising a 5'-internal domain and a 3'-internal domain, wherein the 5'-internal domain comprise a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 66, wherein the 3'-internal domain comprise a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 67, wherein the 5'-internal domain is immediately adjacent to the 5'-ITR, and the 3'-internal domain is immediately adjacent to the 3'-ITR.
5. The nucleic acid of claim 1, wherein the nucleic acid comprises one or more regulatory element sequences selected from the group consisting of SEQ ID NOs: 1-15 (e.g., SEQ ID NO: 2).
6. The nucleic acid of claim 1, wherein the nucleic acid comprises one or more regulatory element sequences selected from the group consisting of SEQ ID NOs: 35-46 (e.g., SEQ ID NO: 36).

7. The nucleic acid of any one of claims 1-6, further comprising a promoter and a sequence encoding a polypeptide, wherein the sequence encoding the polypeptide is operably linked to the promoter.
8. The nucleic acid of claim 7, wherein the sequence encoding a polypeptide is located between two regulatory element sequences.
9. The nucleic acid of any one of claims 1-6, further comprising a promoter and a sequence encoding two or more polypeptides, wherein the sequence encoding the two or more polypeptides is operably linked to the promoter.
10. The nucleic acid of claim 9, wherein the sequence encodes an antibody heavy chain and an antibody light chain.
11. The nucleic acid of any one of claims 1-10, wherein the nucleic acid further comprises a WXRE sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 35-46.
12. The nucleic acid of any one of claims 1-10, wherein the nucleic acid comprises two or more expression cassettes.
13. The nucleic acid of any one of claims 1-12, wherein the nucleic acid comprises a selection marker.
14. The nucleic acid of claim 13, wherein the selection marker is an antibiotic resistance gene, a sequence encoding a fluorescent protein, or lacZ.
15. A vector comprising the nucleic acid of any one of claims 1-14.
16. A transposon vector comprising from 5' to 3':

- a 5' ITR sequence consisting of a sequence that is at least 95% identical to SEQ ID NO: 68;
- a non-transposon heterologous DNA sequence; and
- a 3'-ITR sequence consisting of a sequence that is at least 95% identical to SEQ ID NO: 60.
17. The transposon vector of claim 16, wherein the 5' ITR sequence consists of SEQ ID NO: 68 and the 3' ITR sequence consists of SEQ ID NO: 60.
18. The transposon vector of claims 16, further comprising a 5'-internal domain and a 3'-internal domain, wherein the 5'-internal domain comprises a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 66, wherein the 3'-internal domain comprises a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 67, wherein the 5'-internal domain is immediately adjacent to the 5'-ITR, and the 3'-internal domain is immediately adjacent to the 3'-ITR.
19. The transposon vector of any one of claims 16-18, wherein the non-transposon heterologous DNA sequence comprises a promoter and a sequence encoding one or more polypeptides, wherein the sequence encoding one or more polypeptides is operably linked to the promoter.
20. The transposon vector of claim 19, wherein the promoter is a cytomegalovirus (CMV) promoter.
21. The transposon vector of any one of claims 16-20, wherein the non-transposon heterologous DNA sequence further comprises a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30.
22. The transposon vector of any one of claims 16-21, wherein the non-transposon heterologous DNA sequence further comprises a WXRE sequence that is at least 80%,

85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 35-46.

23. The transposon vector of any one of claims 16-22, wherein the non-transposon heterologous DNA sequence comprises a multiple cloning site.
24. An expression system comprising:
 - (a) a first nucleic acid comprising
 - a 5' - ITR sequence,
 - a non-transposon heterologous DNA sequence comprising a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30,
 - a 3'-ITR sequence; and
 - (b) a second nucleic acid encoding a piggyBac transposase.
25. The expression system of claim 24, wherein the second nucleic acid encoding a piggyBac transposase having an amino acid sequence that is at least 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 33.
26. The expression system of claim 24, wherein the non-transposon heterologous DNA sequence comprises a promoter and a sequence encoding one or more polypeptides, wherein the sequence encoding one or more polypeptides is operably linked to the promoter.
27. The expression system of claim 26, wherein the sequence encodes an antibody heavy chain and/or an antibody light chain.
28. The expression system of claim 26, wherein the sequence encodes a monoclonal antibody, a bispecific antibody, a recombinant protein, or a fusion protein.
29. The expression system of claim 26, wherein the promoter is a CMV promoter.

30. The expression system of claim 26, wherein the promoter is an inducible promoter (e.g., a heat shock promoter, a metallothionein promoter, or a glucocorticoid response element).
31. The expression system of any one of claims 24-30, wherein the non-transposon heterologous DNA sequence further comprises a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30.
32. The expression system of any one of claims 24-31, wherein the non-transposon heterologous DNA sequence further comprises a WXRE regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 35-46.
33. An isolated nucleic acid comprising a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30.
34. The isolated nucleic acid of claim 33, wherein the nucleic acid further comprises a promoter and a protein-coding sequence.
35. The isolated nucleic acid of claim 34, wherein the regulatory element sequence is located between the promoter and the protein-coding sequence.
36. The isolated nucleic acid of claim 34, wherein the regulatory element sequence is located at the 3' of the protein-coding sequence.
37. The isolated nucleic acid of any one of claims 33-36, wherein the regulatory element sequence can be transcribed to a 5'-UTR or a 3'-UTR.

38. The isolated nucleic acid of any one of claims 33-37, wherein the isolated nucleic acid further comprises a 5'-ITR and a 3'-ITR, and the regulatory element sequence is located between the 5'-ITR and the 3'-ITR.
39. A vector comprising the nucleic acid of any one of claims 33-38.
40. A vector comprising a piggyBac transposon, the piggyBac transposon comprising the following genetic elements in a 5' to 3' direction:
a 5'-ITR comprising a TR_L, a 5'-ITR spacer, a IR_L;
a promoter;
a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30;
a protein-coding sequence; and
a 3'-ITR comprising a IR_R, a 3'-ITR spacer, a TR_R.
41. A vector comprising a piggyBac transposon, the piggyBac transposon comprising the following genetic elements in a 5' to 3' direction:
a 5'-ITR comprising a TR_L, a 5'-ITR spacer, a IR_L;
a promoter;
a protein-coding sequence;
a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30; and
a 3'-ITR comprising a IR_R, a 3'-ITR spacer, a TR_R.
42. A vector comprising a piggyBac transposon, the piggyBac transposon comprising the following genetic elements in a 5' to 3' direction:
a 3'-ITR comprising a TR_R, a 3'-ITR spacer, a IR_R;
a promoter;
a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30;
a protein-coding sequence; and
a 5'-ITR comprising a IR_L, a 5'-ITR spacer, a TR_L.

43. A vector comprising a piggyBac transposon, the piggyBac transposon comprising the following genetic elements in a 5' to 3' direction:
- a 3'-ITR comprising a TR_R, a 3'-ITR spacer, a IR_R;
 - a promoter;
 - a protein-coding sequence;
 - a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30; and
 - a 5'-ITR comprising a IR_L, a 5'-ITR spacer, a TR_L.
44. The vector of claim 42 or 43, wherein the TR_L comprises or consists of a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a reverse sequence or a reverse complementary sequence of SEQ ID NO: 61; wherein the 5'-ITR spacer comprises or consists of a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a reverse sequence or a reverse complementary sequence of SEQ ID NO: 62; wherein the IR_L comprises or consists of a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a reverse sequence or a reverse complementary sequence of SEQ ID NO: 63; wherein the TR_R comprises or consists of a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a reverse sequence or a reverse complementary sequence of SEQ ID NO: 64; and wherein the IR_R comprises or consists of a sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a reverse sequence or a reverse complementary sequence of SEQ ID NO: 65.
45. The vector of any one of claims 40-44, wherein the vector comprises two or more regulatory element sequences, each of which is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30.
46. The vector of any one of claims 40-45, wherein the vector further comprises a 5'-internal domain sequence and a 3'-internal domain sequence.

47. The vector of claim 46, wherein the 5'-internal domain sequence comprises or consists of a sequence that is at least or about 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 66 and the 3'-internal domain sequence comprises or consists of a sequence that is at least or about 80%, 85%, 90%, 95% or 100% identical to SEQ ID NO: 67.
48. The vector of any one of claims 40-47, wherein the regulatory element sequence can be transcribed to a 5'-UTR or a 3'-UTR.
49. The vector of any one of claims 40-48, wherein the vector further comprises a sequence encoding a piggyBac transposase, wherein the sequence encoding the piggyBac transposase is outside a region between the 5'-ITR and the 3'-ITR.
50. A method of generating a cell in culture for expressing a polypeptide of interest, comprising:
(a) introducing into a cell:
a transposon vector comprising:
a 5'- ITR (inverted terminal repeat) sequence;
a 3'-ITR sequence;
a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30 and SEQ ID NOs: 35-58; and
a sequence encoding the polypeptide of interest; and
(b) culturing the cell under an appropriate condition, wherein the piggyBac transposon is integrated into the genome of the cell, thereby generating a cell for expressing the polypeptide of interest.
51. The method of claim 50, wherein the method further comprises introducing a vector comprising a sequence encoding a piggyBac transposase to the cell.

52. The method of claim 50, wherein the transposon vector comprises a sequence encoding a piggyBac transposase.
53. The method of any one of claims 50-52, wherein the transposon vector is introduced into the cell by microinjection, high velocity propulsion, permeabilization, fusion, or electroporation.
54. The method of any one of claims 50-53, wherein the cell is a Chinese hamster ovary (CHO) cell.
55. The method of any one of claims 50-53, wherein the cell is a mammalian cell or an insect cell.
56. A cell comprising the nucleic acid of any one of claims 1-14 and 33-38, the vector of any one of claims 15-23, 39-49, or the expression system of any one of claims 24-32.
57. The cell of claim 56, wherein the cell is a Chinese hamster ovary (CHO) cell.
58. A method of expressing a protein, comprising:
culturing the cell of claim 56 or 57 under conditions that allow the cell to express the protein; and
collecting and purifying the protein.
59. A protein expressed by the cell of claim 56 or 57, or produced by the method of claim 58.
60. A pharmaceutical composition comprising the protein of claim 59 and a pharmaceutically acceptable carrier.
61. A nucleic acid comprising
a 5' - ITR sequence;
a 3'-ITR sequence; and

one or more regulatory element sequences derived from CHO.

62. An expression system comprising:

- (a) a first nucleic acid comprising a piggyBac transposon comprising the following genetic elements in a 5' to 3' direction:
 - a first TTAA sequence;
 - a 5'-ITR comprising a TR_L, a 5'-ITR spacer and an IR_L;
 - a 5'-internal domain (ID);
 - a sequence of interest;
 - a 3'-ID;
 - a 3'-ITR comprising an IR_R, a 3'-ITR spacer and a TR_R; and
 - a second TTAA sequence; and
- (b) a second nucleic acid encoding a piggyBac transposase.

63. A method of generating a cell for expressing a polypeptide of interest, comprising:

- (a) introducing into a cell:
 - a transposon vector comprising the following genetic elements in a 5' to 3' direction:
 - a first TTAA sequence;
 - a 5'-ITR comprising a TR_L, a 5'-ITR spacer and an IR_L;
 - a 5'-internal domain (ID);
 - a sequence of interest;
 - a 3'-ID;
 - a 3'-ITR comprising an IR_R, a 3'-ITR spacer and a TR_R; and
 - a second TTAA sequence; and
- (b) culturing the cell under an appropriate condition.

64. A cell line whose genome is stably integrated with a piggyBac transposon comprising the following genetic elements:

- a 5'-ITR sequence;
- a regulatory element sequence that is at least 80%, 85%, 90%, 95% or 100% identical to a sequence selected from the group consisting of SEQ ID NOs: 1-30 and 35-58; and

a 3'-ITR sequence.

65. A cell line whose genome is stably integrated with a piggyBac transposon comprising the following genetic elements:

a 5'-ITR comprising a TR_L, a 5'-ITR spacer and an IR_L;

a 5'-internal domain (ID);

a sequence of interest;

a 3'-ID;

a 3'-ITR comprising an IR_R, a 3'-ITR spacer and a TR_R.