# CLAIMS

1. A polypeptide comprising a piggyBac transposase or a fragment or a derivative thereof having transposase function comprising at least one amino acid substitution selected from the group consisting of
isoleucine (I) at amino acid position 30 or at an amino acid position corresponding thereto is replaced by alanine (A) (I30A),
glutamine (Q) at amino acid position 118 or at an amino acid position corresponding thereto is replaced by proline (P) (Q118P),
methionine (M) at amino acid position 185 or at an amino acid position corresponding thereto is replaced by valine (V) (M185V),
methionine (M) at amino acid position 282 or at an amino acid position corresponding thereto is replaced by leucine (L) (M282L), and
asparagine (N) at amino acid position 538 or at an amino acid position corresponding thereto is replaced by arginine (R) (N538R).

2. The polypeptide of claim 1, wherein the piggyBac transposase or the fragment or the derivative thereof having transposase function comprises the following amino acid substitutions:
isoleucine (I) at amino acid position 30 or at an amino acid position corresponding thereto is replaced by alanine (A) (I30A),
methionine (M) at amino acid position 282 or at an amino acid position corresponding thereto is replaced by leucine (L) (M282L), and
asparagine (N) at amino acid position 538 or at an amino acid position corresponding thereto is replaced by arginine (R) (N538R).

3. The polypeptide of claims 1 or 2, wherein the piggyBac transposase or the fragment or the derivative thereof having transposase function comprises the following amino acid substitutions:
isoleucine (I) at amino acid position 30 or at an amino acid position corresponding thereto is replaced by alanine (A) (I30A),
glutamine (Q) at amino acid position 118 or at an amino acid position corresponding thereto is replaced by proline (P) (Q118P),

methionine (M) at amino acid position 185 or at an amino acid position corresponding thereto is replaced by valine (V) (M185V),
methionine (M) at amino acid position 282 or at an amino acid position corresponding thereto is replaced by leucine (L) (M282L), and
asparagine (N) at amino acid position 538 or at an amino acid position corresponding thereto is replaced by arginine (R) (N538R).

~~4. The polypeptide of any one of claims 1 to 3, wherein the piggyBac transposase has an amino acid sequence according to SEQ ID NO: 18 and comprises at least one amino acid substitution selected from the group consisting of
isoleucine (I) at amino acid position 30 is replaced by alanine (A) (I30A),
glutamine (Q) at amino acid position 118 is replaced by proline (P) (Q118P),
methionine (M) at amino acid position 185 is replaced by valine (V) (M185V),
methionine (M) at amino acid position 282 is replaced by leucine (L) (M282L), and
asparagine (N) at amino acid position 538 is replaced by arginine (R) (N538R), or
is a variant thereof which is at least 80% identical to said amino acid sequence, wherein said variant comprises at least one amino acid substitution selected from the group consisting of
isoleucine (I) at amino acid position 30 or at an amino acid position corresponding thereto is replaced by alanine (A) (I30A),
glutamine (Q) at amino acid position 118 or at an amino acid position corresponding thereto is replaced by proline (P) (Q118P),
methionine (M) at amino acid position 185 or at an amino acid position corresponding thereto is replaced by valine (V) (M185V),
methionine (M) at amino acid position 282 or at an amino acid position corresponding thereto is replaced by leucine (L) (M282L), and
asparagine (N) at amino acid position 538 or at an amino acid position corresponding thereto is replaced by arginine (R) (N538R).~~

~~5. The polypeptide of any one of claims 1 to 4, wherein the piggyBac transposase has an amino acid sequence according to SEQ ID NO: 22 comprising alanine (A) at amino acid position 30, leucine (L) at amino acid position 282 and arginine (R) at amino acid position 538, or~~

~~is a variant thereof which is at least 80 % identical to said amino acid sequence, wherein said variant comprises alanine (A) at amino acid position 30 or at an amino acid position corresponding thereto, leucine (L) at amino acid position 282 or at an amino acid position corresponding thereto and arginine (R) at amino acid position 538 or at an amino acid positon corresponding thereto.~~

~~6. The polypeptide of any one of claims 1 to 5, wherein the piggyBac transposase has an amino acid sequence according to SEQ ID NO: 20 comprising alanine (A) at amino acid position 30, proline (P) at amino acid position 118, valine (V) at amino acid position 185, leucine (L) at amino acid position 282 and arginine (R) at amino acid position 538, or~~

~~is a variant thereof which is at least 80 % identical to said amino acid sequence, wherein said variant comprises alanine (A) at amino acid position 30 or at an amino acid position corresponding thereto, proline (P) at amino acid position 118 or at an amino acid position corresponding thereto, amino acid valine (V) at amino acid position 185 or at an amino acid position corresponding thereto, leucine (L) at amino acid position 282 or at an amino acid position corresponding thereto and arginine (R) at amino acid position 538 or at an amino acid position corresponding thereto.~~

~~7~~4. The polypeptide of any one of claims 1 to ~~6~~ 3, wherein the polypeptide comprises at least one heterologous chromatin reader domain (CRD).

~~8. The polypeptide of claim 7, wherein the at least one heterologous CRD is connected to the transposase or the fragment or the derivative thereof having transposase function, preferably via a linker.~~

~~9. The polypeptide of claims 7 or 8, wherein the CRD recognises histone methylation degree and/or acetylation state of histones.~~

~~10~~5. The polypeptide of claim ~~9~~ 4, wherein the CRD is a plant homeodomain (PHD) type zinc finger.

~~11~~6. The polypeptide of claim ~~10~~ 5, wherein the PHD type zinc finger is a transcription initiation factor TFIID subunit 3 PHD.

~~12. The polypeptide of any one of claims 1 to 11, wherein the polypeptide further comprises at least one heterologous DNA binding domain.~~

~~13. The polypeptide of any one of claims 1 to 12, wherein the polypeptide further comprises a heterologous nuclear localization signal (NLS).~~

~~14~~7. A polynucleotide encoding the polypeptide of any one of claims 1 to ~~13~~ 6.

~~15~~8. A vector comprising the polynucleotide of claim ~~14~~ 7.

~~16~~9. A transposable element comprising a piggyBac or piggyBac-like
left repeat sequence and
left internal repeat sequence,
wherein the left internal repeat sequence comprises at least one nucleotide modification, wherein the at least one nucleotide modification increases the homology of the left internal repeat sequence to the left repeat sequence.

~~17~~10. The transposable element of claim ~~16~~ 9, wherein the at least one nucleotide modification is selected from the group consisting of a nucleotide substitution, a nucleotide deletion, a nucleotide addition, and a nucleotide insertion, or is a combination thereof.

~~18~~11. The transposable element of claim ~~17~~ 10, wherein the piggyBac left internal repeat sequence

(i) has a nucleotide sequence according to SEQ ID NO: 1 and comprises at least one nucleotide substitution selected from the group consisting of
adenosine (A) at nucleotide position 3 is replaced by cytidine (C) (A3C),
adenosine (A) at nucleotide position 9 is replaced by thymidine (T) (A9T),
adenosine (A) at nucleotide position 10 is replaced by thymidine (T) (A10T), and
guanosine (G) at nucleotide position 12 is replaced by thymidine (T) (G12T), or is a variant thereof which is at least 80% identical to said nucleotide sequence, wherein said variant comprises at least one nucleotide substitution selected from the group consisting of

adenosine (A) at nucleotide position 3 or at a nucleotide position corresponding thereto is replaced by cytidine (C) (A3C),

adenosine (A) at nucleotide position 9 or at a nucleotide position corresponding thereto is replaced by thymidine (T) (A9T),

adenosine (A) at nucleotide position 10 or at a nucleotide position corresponding thereto is replaced by thymidine (T) (A10T), and

guanosine (G) at nucleotide position 12 or at a nucleotide position corresponding thereto is replaced by thymidine (T) (G12T)~~.~~,

~~19. The transposable element of claim 17, wherein the piggyBac-like left internal repeat sequence~~

(ii) has a nucleotide sequence according to SEQ ID NO: 2 and comprises a nucleotide insertion, wherein adenosine (A) is introduced between nucleotide positions 7 and 8, or

is a variant thereof which is at least 80% identical to said nucleotide sequence, wherein said variant comprises a nucleotide insertion, wherein adenosine (A) is introduced between nucleotide positions 7 and 8 or between nucleotide positions corresponding thereto~~.~~,

~~20. The transposable element of claim 17, wherein the piggyBac-like left internal repeat sequence~~

(iii) has a nucleotide sequence according to SEQ ID NO: 3 and comprises at least one nucleotide substitution selected from the group consisting of

guanosine (G) at nucleotide position 7 is replaced by cytidine (C) (G7C), and

thymidine (T) at nucleotide position 9 is replaced by cytidine (C) (T9C), or

is a variant thereof which is at least 80% identical to said nucleotide sequence, wherein said variant comprises at least one nucleotide substitution selected from the group consisting of

guanosine (G) at nucleotide position 7 or at a nucleotide position corresponding thereto is replaced by cytidine (C) (G7C), and

thymidine (T) at nucleotide position 9 or at a nucleotide position corresponding thereto is replaced by cytidine (C) (T9C)~~.~~,

~~21. The transposable element of claim 17, wherein the piggyBac-like left internal repeat sequence~~

(iv) has a nucleotide sequence according to SEQ ID NO: 4 and comprises a nucleotide substitution, wherein thymidine (T) at nucleotide position 7 is replaced by adenosine (A) (T7A), or

is a variant thereof which is at least 80% identical to said nucleotide sequence, wherein said variant comprises a nucleotide substitution, wherein thymidine (T) at nucleotide position 7 or at a nucleotide position corresponding thereto is replaced by adenosine (A) (T7A), or~~.~~

~~22. The transposable element of claim 17, wherein the piggyBac-like left internal repeat sequence~~

(v) has a nucleotide sequence according to SEQ ID NO: 5 and comprises at least one nucleotide substitution selected from the group consisting of

guanosine (G) at nucleotide position 6 is replaced by thymidine (T) (G6T), and

thymidine (T) at nucleotide position 14 is replaced by guanosine (G) (T14G), or is a variant thereof which is at least 80% identical to said nucleotide sequence, wherein said variant comprises at least one nucleotide substitution selected from the group consisting of

guanosine (G) at nucleotide position 6 or at a nucleotide position corresponding thereto is replaced by thymidine (T) (G6T), and

thymidine (T) at nucleotide position 14 or at a nucleotide position corresponding thereto is replaced by guanosine (G) (T14G).

~~23. The transposable element of any one of claims 16 to 22, wherein the left repeat sequence has a nucleotide sequence selected from the group consisting of SEQ ID NO: 23 to SEQ ID NO: 27 or is a variant thereof which is at least 80% identical to said nucleotide sequence.~~

~~24. The transposable element of any one of claims 16 to 23, wherein the left repeat sequence and the left internal repeat sequence are comprised in/part of a 5'-transposon end sequence.~~

~~25. The transposable element of any one of claims 16 to 24, wherein the transposable element comprises a piggyBac or piggyBac-like 3' transposon end sequence.~~

~~26~~12. The transposable element of any one of claims ~~16~~ 9 to ~~25~~ 11, wherein the transposable element comprises

at least one polynucleotide of interest, or

at least one cloning site for inserting at least one polynucleotide of interest.

~~27. The transposable element of claim 26, wherein~~

~~the at least one polynucleotide of interest of is operably linked to the piggyBac or piggyBac-like 5' transposon end sequence and to the piggyBac or piggyBac-like 3' transposon end sequence, or~~

~~the at least one cloning site for inserting the at last one polynucleotide of interest is located between the piggyBac or piggyBac-like 5'-transposon end sequence and the piggyBac or piggyBac-like 3' transposon end sequence.~~

~~28. The transposable element of claims 26 or 27, wherein the at least one polynucleotide of interest is selected from the group consisting of a polynucleotide encoding a polypeptide, a non-coding polynucleotide, a polynucleotide comprising a promoter sequence, a polynucleotide encoding a mRNA, a polynucleotide encoding a tag, and a viral polynucleotide.~~

~~29. The transposable element of any one of claims 16 to 28, wherein the transposable element is circular, is comprised in/part of a plasmid vector, or is comprised in/part of a minicircle DNA vector.~~

~~30. The transposable element of any one of claims 16 to 29, wherein the transposable element is a piggyBac or piggyBac-like transposable element.~~

~~31~~13. A method for producing a transgenic cell comprising the steps of:

(i) providing a cell, and

(ii) introducing

(iia) a transposable element, and

a polypeptide of any one of claims 1 to ~~13~~ 6, or

a polynucleotide of claim ~~14~~ 7, or

a vector of claim ~~15~~ 8, or

(iib) a transposable element of any one of claims ~~16~~ 9 to ~~30~~ 12, and

a transposase or a fragment or a derivative thereof having transposase function, or

a polynucleotide encoding a transposase or a fragment or a derivative thereof having transposase function, or

a vector comprising a polynucleotide encoding a transposase or a fragment or a derivative thereof having transposase function, or

(iic) a transposable element of any one of claims ~~16~~ 9 to ~~30~~ 12, and

a polypeptide of any one of claims 1 to ~~13~~ 6, or

a polynucleotide of claim ~~14~~ 7, or

a vector of claim ~~15~~ 8

into the cell, thereby producing the transgenic cell.

~~32. The method of claim 31, wherein the introduction takes place via electroporation, transfection, injection, lipofection, and/or (viral) infection.~~

~~33. The method of claims 31 or 32, wherein the transposable element of (iia) is comprised in/part of a polynucleotide molecule, preferably a vector.~~

~~34. The method of claims 31 or 32, wherein the transposable element of (iia) and the polynucleotide of claim 14 are comprised in/part of a polynucleotide molecule, preferably a vector.~~

~~35. The method of any one of claims 31 to 34, wherein the transposable element of (iia) comprises terminal repeats (TRs).~~

~~36~~14. The method of ~~any one of claims 31 to 35~~ claim 13, wherein the transposable element of (iia) comprises at least one polynucleotide of interest.

~~37. The method of claim 36, wherein the at least one polynucleotide of interest is flanked by TRs.~~

~~38. The method of claims 36 or 37, wherein the at least one polynucleotide of interest is selected from the group consisting of a polynucleotide encoding a polypeptide, a non-coding polynucleotide, a polynucleotide comprising a promoter sequence, a polynucleotide encoding a mRNA, a polynucleotide encoding a tag, and a viral polynucleotide.~~

~~39. The method of any one of claims 31 to 38, wherein the transposable element of (iia) is a DNA transposable element.~~

~~40. The method of claim 39, wherein the DNA transposable element comprises terminal inverted repeats (TIRs).~~

~~41. The method of any one of claims 31 to 40, wherein the transposase of (iib) is selected from the group consisting of a wild-type piggyBac transposase, a hyperactive piggyBac transposase, a wild-type piggyBac-like transposase, and a hyperactive piggyBac-like transposase.~~

~~42. The method of claim 41, wherein the piggyBac-like transposase is selected from the group consisting of piggyBat transposase, piggyBac-like transposase from *Xenopus tropicalis*, and piggyBac-like transposase from *Bombyx mori*.~~

~~43. The method of any one of claims 31 to 42, wherein the cell is an eukaryotic cell.~~

~~44. The method of claim 43, wherein the eukaryotic cell is a vertebrate, yeast, fungus, insect cell.~~

~~45. The method of claim 44, wherein the vertebrate cell is a mammalian cell.~~

~~46~~15. A transgenic cell obtainable by the method of ~~any one of claims 31 to 45~~ claim 13 or 14.

~~47~~16. Use of a transgenic cell of claim ~~46~~ 15 for the production of a protein or virus.

~~48~~17. A kit comprising

(i) a transposable element, and

a polypeptide of any one of claims 1 to ~~13~~ 6, or

a polynucleotide of claim ~~14~~ 7, or

a vector of claim ~~15~~ 8; or

(ii) a transposable element of any one of claims ~~16~~ 9 to ~~30~~ 12, and

a transposase or a fragment or a derivative thereof having transposase function, or

a polynucleotide encoding a transposase or a fragment or a derivative thereof having transposase function, or

a vector comprising a polynucleotide encoding a transposase or a fragment or a derivative thereof having transposase function; or

(iii) a transposable element of any one of claims ~~16~~ 9 to ~~30~~ 12, and

a polypeptide of any one of claims 1 to ~~13~~ 6, or

a polynucleotide of claim ~~14~~ 7, or

a vector of claim ~~15~~ 8.

~~49. The kit of claim 48, wherein the transposable element of (i) is comprised in/part of a polynucleotide molecule, preferably a vector.~~

~~50. The kit of claim 48, wherein the transposable element of (i) and the polynucleotide of claim 14 are comprised in/part of a polynucleotide molecule, preferably a vector.~~

~~51. The kit of any one of claims 48 to 50, wherein the transposable element of (i) comprises terminal repeats (TRs).~~

~~52~~18. The kit of ~~any one of claims 48 to 51~~ claim 17, wherein the transposable element of (i) comprises

at least one polynucleotide of interest, or

at least one cloning site for inserting at least one polynucleotide of interest.

~~53. The kit of claim 52, wherein~~

~~the at least one polynucleotide of interest is flanked by TRs, or~~

~~the at least one cloning site for inserting the at least one polynucleotide of interest is located between the TRs.~~

~~54. The kit of claims 52 or 53, wherein the at least one polynucleotide of interest is selected from the group consisting of a polynucleotide encoding a polypeptide, a non-coding polynucleotide, a polynucleotide comprising a promoter sequence, a polynucleotide encoding a mRNA, a polynucleotide encoding a tag, and a viral polynucleotide.~~

~~55. The kit of any one of claims 48 to 54, wherein the transposable element of (i) is a DNA transposable element.~~

~~56. The kit of claim 55, wherein the DNA transposable element comprises terminal inverted repeats (TIRs).~~

~~57. The kit of any one of claims 48 to 56, wherein the transposase of (ii) is selected from the group consisting of a wild-type piggyBac transposase, a hyperactive piggyBac transposase, a wild-type piggyBac-like transposase, and a hyperactive piggyBac-like transposase.~~

~~58. The kit of claim 57, wherein the PiggyBac-like transposase is selected from the group consisting of piggyBat transposase, piggyBac-like transposase from *Xenopus tropicalis*, and piggyBac-like transposase from *Bombyx mori*.~~

~~59. The kit of any one of claims 48 to 58, wherein the kit is for the generation of a transgenic cell.~~

~~60. The kit of claim 59, wherein the kit further comprises instructions on how to generate the transgenic cell.~~

~~61~~19. A targeting system comprising

(i) a transposable element, and

a polypeptide of any one of claims 1 to ~~13~~ 6, or

a polynucleotide of claim ~~14~~ 7, or

a vector of claim ~~15~~ 8; or

(ii) a transposable element of any one of claims ~~16~~ 9 to ~~30~~ 12, and
a transposase or a fragment or a derivative thereof having transposase function, or
a polynucleotide encoding a transposase or a fragment or a derivative thereof having transposase function, or
a vector comprising a polynucleotide encoding a transposase or a fragment or a derivative thereof having transposase function; or

(iii) a transposable element of any one of claims ~~16~~ 9 to ~~30~~ 12, and
a polypeptide of any one of claims 1 to ~~13~~ 6, or
a polynucleotide of claim ~~14~~ 7, or
a vector of claim ~~15~~ 8.

~~62. The targeting system of claim 61, wherein the transposable element of (i) is comprised in/part of a polynucleotide molecule, preferably a vector.~~

~~63. The targeting system of claim 61, wherein the transposable element of (i) and the polynucleotide of claim 14 are comprised in/part of a polynucleotide molecule, preferably a vector.~~

~~64. The targeting system of any one of claims 61 to 63, wherein the transposable element of (i) comprises terminal repeats (TRs).~~

~~65~~20. The targeting system of ~~any one of claims 61 to 64~~ claim 19, wherein the transposable element of (i) comprises at least one polynucleotide of interest.

~~66. The targeting system of claim 65, wherein the at least one polynucleotide of interest is flanked by TRs.~~

~~67. The targeting system of claims 65 or 66, wherein the at least one polynucleotide of interest is selected from the group consisting of a polynucleotide encoding a polypeptide, a non-coding polynucleotide, a polynucleotide comprising a promoter sequence, a polynucleotide encoding a mRNA, a polynucleotide encoding a tag, and a viral polynucleotide.~~

~~68. The targeting system of any one of claims 61 to 67, wherein the transposase of (ii) is selected from the group consisting of a wild-type piggyBac transposase, a hyperactive piggyBac transposase, a wild-type piggyBac-like transposase, and a hyperactive piggyBac-like transposase.~~

~~69. The targeting system of claim 68, wherein the PiggyBac-like transposase is selected from the group consisting of piggyBat transposase, piggyBac-like transposase from *Xenopus tropicalis*, and piggyBac-like transposase from *Bombyx mori*.~~