TRANSPOSASE WITH ENHANCED INSERTION SITE SELECTION PROPERTIES

The present invention relates to a polypeptide comprising a transposase and at least one heterologous chromatin reader element (CRE). Further, the present invention relates to a polynucleotide encoding the polypeptide. Furthermore, the present invention relates to a vector comprising the polynucleotide. In addition, the present invention relates to a kit comprising a transposase and at least one heterologous chromatin reader element (CRE).

BACKGROUND OF THE INVENTION

Transposons have recently been developed as potent, non-viral gene delivery tools. In particular, the performance of a generated producer cell line can be improved, when the integration of plasmid DNA is supported using a transposon. For instance, a transposon allows the integration of a greater size of heterologous DNA and the integration of a higher number of heterologous DNA copies into each genome. Furthermore, integration via a transposon provides an efficient method for the reduction of plasmid backbone integration and/or the reduction of concatemers.

Transposable elements or transposons are DNA-sections, which can move from one locus to another part of the genome. Two classes of transposable elements are distinguished: retrotransposons, which replicate through an RNA intermediate (class 1), and “cut-and-paste” DNA transposons (class 2). Class 2 transposons are characterised by short inverted terminal repeats (ITRs) and element-encoded transposases, enzymes with excision and insertion activity. 23 superfamilies of DNA transposons are currently described [Bao et al., 2015 [doi:10.1186/s13100-015-0041-9.]]. In the natural configuration, the transposase gene is located between the inverted repeats. A number of class 2 transposons have been shown to facilitate insertion of heterologous DNA into the genome of eukaryotes, for example, a transposon from the moth *Trichoplusia ni* (PiggyBac), a transposon from the bat *Myotis lucifugus* (PiggyBat), a reconstructed transposon from salmon species (Sleeping Beauty), or a transposon from the medaka *Oryzias latipes* (Tol2). These transposons have many applications in genetic manipulation of a host genome, including transgene delivery and insertional mutagenesis. For instance, the PiggyBac (PB) DNA transposon (previously described as IFP2) is used technologically and commercially in genetic engineering by virtue of its property to efficiently transpose between vectors and chromosomes [US 6218185 B1]. For these applications the DNA to be integrated is flanked by two PB ITRs in a PB vector. By co-delivery of PB transposase the flanked DNA is excised precisely form the PB vector and integrated into the target genome at TTAA specific sites.

CLAIMS

1. A polypeptide comprising a transposase or a fragment or a derivative thereof having transposase function and at least one heterologous chromatin reader element (CRE).

2. The polypeptide of claim 1, wherein the at least one heterologous CRE is connected to the transposase, preferably via a linker.

3. The polypeptide of claim 1 or 2, wherein the at least one heterologous CRE is connected to the N-terminus and/or C-terminus of the transposase, preferably via a linker.

4. The polypeptide of any one of claims 1 to 3, wherein the at least one heterologous CRE is a chromatin reader domain (CRD).

5. The polypeptide of claim 4, wherein the at least one heterologous CRD is a naturally occurring CRD recognising histone methylation degree and/or acetylation state of histones.

6. The polypeptide of claim 5, wherein
the naturally occurring CRD recognising histone methylation degree is a plant homeodomain (PHD) type zinc finger, or
the naturally occurring CRD recognising the acetylation state of histones is a bromodomain.

7. The polypeptide of claim 6, wherein
the PHD type zinc finger is a transcription initiation factor TFIID subunit 3 PHD, or
the bromodomain is a histone acetyltransferase KAT2A domain.

8. The polypeptide of claim 7, wherein
the transcription initiation factor TFIID subunit 3 PHD has an amino acid sequence according to SEQ ID NO: 20, or
the histone acetyltransferase KAT2A domain has an amino acid sequence according to SEQ ID NO: 21.

9. The polypeptide of any one of claims 1 to 8, wherein the CRE is an artificial CRE recognising histone tails with specific methylated and/or acetylated sites.

10. The polypeptide of claim 9, wherein the artificial CRE is selected from the group consisting of a micro antibody, a single chain antibody, an antibody fragment, an affibody, an affilin, an anticalin, an atrimer, a DARPin, a FN2 scaffold, a fynomer, and a Kunitz domain.

11. The polypeptide of any one of claims 1 to 10, wherein the transposase is selected from the group consisting of a wild-type PiggyBac transposase, a hyperactive PiggyBac transposase, a wild-type PiggyBac-like transposase, a hyperactive PiggyBac-like transposase, a sleeping beauty transposase, and a Tol2 transposase, and the PiggyBac-like transposase is preferably selected from the group consisting of PiggyBat, PiggyBac-like transposase from *Xenopus tropicalis*, and PiggyBac-like transposase from *Bombyx mori*.

12. The polypeptide of any one of claims 1 to 11, wherein the polypeptide further comprises at least one heterologous DNA binding domain and/or a heterologous nuclear localization signal (NLS).

13. A polynucleotide encoding the polypeptide of any one of claims 1 to 12.

14. A vector comprising the polynucleotide of claim 13.

15. A method for producing a transgenic cell comprising the steps of:
    (i) providing a cell, and
    (ii) introducing
    a transposable element comprising at least one polynucleotide of interest, and
    a polypeptide of any one of claims 1 to 12,
    a polynucleotide of claim 13, or
    a vector of claim 14
    into the cell, thereby producing the transgenic cell.

16. The method of claim 15, wherein the introduction takes place via electroporation, transfection, injection, lipofection, and/or (viral) infection.

17. The method of claim 15 or 16, wherein the transposable element comprises terminal repeats (TRs).

18. The method of claim 17, wherein the at least one polynucleotide of interest is flanked by TRs.

19. The method of any one of claims 15 to 18, wherein the transposable element is
a DNA transposable element, or
a retrotransposable element.

20. The method of claim 19, wherein
the DNA transposable element comprises inverted terminal repeats (ITRs), or
the retrotransposable element is a long terminal repeat (LTR) retrotransposable element.

21. The method of any one of claims 15 to 20, wherein the cell is eukaryotic cell.

22. The method of claim 21, wherein the eukaryotic cell is a vertebrate, yeast, fungus, insect cell.

23. The method of any one of claims 15 to 22, 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.

24. A transgenic cell obtainable by the method of any one of claims 15 to 23.

25. Use of a transgenic cell of claim 24 for the production of a protein or virus.

26. A kit comprising
(i) a transposable element comprising a cloning site for inserting at least one polynucleotide of interest, and
(ii) a polypeptide of any one of claims 1 to 12,
a polynucleotide of claim 13,
a vector of claim 14, or
at least one heterologous CRE and a polypeptide comprising a transposase or a fragment or a derivative thereof having transposase function.