prone to further improvements, e.g. in view of stability and productivity of a cargo or transgenes encoded thereby.

SUMMARY

[007] The present disclosure relates to novel transposase/transposon pairs, e.g. from *Acyrthosiphon pisum* (AP) or *Aphis craccivora* (AC). Each of the novel transposase/transposon pairs presents itself as an additional, highly efficient, orthogonal transposase/transposon pair, that is functionally active in eukaryotic cells and with a transposition efficacy that is at least comparable with the above mentioned commercially available transposase/transposon systems. The transposase according to the disclosure specifically recognizes the identified transposon ends comprising the inverted terminal repeats (ITRs), which are substantially different from any published transposase/transposon system, and transposition leads to the stable integration of any cargo polynucleotide (e.g., encoding a protein of interest, non-coding RNA, viral genome or gene(s)) into the host cell genome of interest.

[008] In one aspect the disclosure relates to a DNA transposon comprising a heterologous polynucleotide flanked by transposon flanking regions, wherein one transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 38 at one transposon end; and the other transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 39 at the other transposon end. The DNA transposon according to the disclosure is transposable by a transposase comprising the amino acid sequence of SEQ ID NO: 4.

[009] In certain examples, the one transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 40 at one transposon end; and the other transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 41 at the other transposon end. Preferably, the one and the other transposon flanking region each further comprises at least one inverted internal repeat (IIR) comprising (i) an internal repeat motif comprising the sequence tggtctac and (ii) its reverse complementary sequence (iii) separated by six to three, preferably four nucleotides. More preferably the at least one inverted internal repeat (IIR) motif has the nucleotide sequence of SEQ ID NO: 43. In a preferred example (a) the one transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 45 or 46 and further an inverted internal repeat motif having the nucleotide sequence of SEQ ID NO: 43 separated by about 50 to 200 nucleotides; and/or the other transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 47 or a sequence having at least 95% sequence identity with SEQ ID NO: 47, wherein the sequence comprises at least the nucleotide sequence of SEQ ID NO: 41 and the nucleotide sequence of SEQ ID NO: 43; or (b) the one and the other transposon flanking regions are inverted and comprise reverse complementary sequences of the sequences recited in (a).

[010] In certain examples (a) the one transposon flanking region comprises a sequence having at least 85% nucleotide sequence identity with SEQ ID NO: 2, 8, 10 or 58 and/or the other transposon flanking region comprises a sequence having at least 90% nucleotide sequence identity with SEQ ID NO: 3, 9, 11 or 59; or (b) the one and the other transposon flanking regions are inverted and comprise reverse complementary sequences of the sequences recited in (a). Preferably the one

transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 2, 8, 10 or 58 or the nucleotide sequence of SEQ ID NO: 29, 62, 63 or 64 and/or the other transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 3, 9, 11, 59, 48 or 49 or the nucleotide sequence of SEQ ID NO: 30, 65, 66, 67, 60 or 61. Alternatively the one and the other transposon flanking regions are inverted and the one transposon flanking region comprises the complementary sequences of SEQ ID NO: 3, 9, 11, 59, 48 or 49 or the complementary sequence of SEQ ID NO: 30, 65, 66, 67, 60 or 61 and/or the other transposon flanking region comprises the complementary sequence of SEQ ID NO: 2, 8, 10 or 58 or the complementary sequence of SEQ ID NO: 29, 62, 63 or 64, respectively.

[011] In preferred examples, the heterologous polynucleotide comprises at least one sequence selected from the group consisting of a sequence encoding a gene of interest, a complementary DNA (cDNA), a genome of interest and another genetic element.

[012] In another aspect, further provided is an expression vector comprising the DNA transposon according to the disclosure.

[013] The disclosure relates to a recombinant transposase comprising at least one heterologous nuclear localization signal (NLS) fused to the transposase, preferably at least a heterologous C-terminal and/or N-terminal NLS, wherein the transposase is an *Acyrthosiphon pisum* transposase or an *Aphis craccivora* transposase, and wherein the transposase comprises an amino acid sequence having at least 90% sequence identity with amino acids 10 to 585 of SEQ ID NO: 4.

[014] The disclosure relates to a recombinant transposase comprising an amino acid sequence having at least 90% sequence identity with amino acids 10 to 585 of SEQ ID NO: 4 and at least one heterologous NLS fused to the transposase. In a preferred example the transposase is an *Acyrthosiphon pisum* transposase or an *Aphis craccivora* transposase.

[014a] In another aspect, the present disclosure relates to a recombinant transposase comprising an amino acid sequence having at least 90% sequence identity with amino acids 10 to 585 of SEQ ID NO: 4 and at least one heterologous nuclear localization signal (NLS) fused to the transposase, preferably wherein the transposase is an *Acyrthosiphon pisum* transposase or an *Aphis craccivora* transposase.

[015] The disclosure relates to a recombinant transposase comprising an amino acid sequence having at least 90% sequence identity with amino acids 10 to 585 of SEQ ID NO: 4 and further comprises at least one mutation, preferably wherein at least one heterologous NLS is fused to the transposase. In a preferred example the recombinant transposase according to the disclosure is hyperactive compared to the transposase of SEQ ID NO: 4.

The at least one mutation may be an amino acid substitution selected from the group consisting of K87Y, Q273V, V212I/I215L, I363V/K365S, K87Y/Q273V, K87Y/A264S/Q273V, A264S/Q273V, S270P/Q273V, K87Y/A264S, L583F, K576I, S372E, S277N and any combination thereof, and/or the at least one mutation is a deletion of N584 and/or E585, wherein the indicated amino acid position of the substitution and/or deletion corresponds to the amino acid position in the amino acid sequence of SEQ ID NO: 4. Preferably the at least one mutation is an amino acid substitution selected from the group consisting of K87Y, Q273V, V212I/I215L, I363V/K365S, A264S/Q273V, K87Y/A264S, or a combination thereof, and/or the at least one mutation is a deletion of N584 and/or E585.

[015a] In another aspect, the present disclosure relates to a recombinant transposase comprising an amino acid sequence having at least 90% sequence identity with amino acids 10 to 585 of SEQ ID NO: 4, wherein the transposase comprises at least one mutation, preferably wherein the at least one mutation is an amino acid substitution selected from the group consisting of K87Y, Q273V, V212I/I215L, I363V/K365S, K87Y/Q273V, K87Y/A264S/Q273V, A264S/Q273V, S270P/Q273V, K87Y/A264S, L583F, K576I, S372E, S277N and any combination thereof, and/or the at least one mutation is a deletion of N584 and/or E585, wherein the indicated amino acid position of the substitution and/or deletion corresponds to the amino acid position in the sequence of SEQ ID NO: 4, preferably wherein at least one heterologous NLS is fused to the transposase.

[016] In yet another aspect, the disclosure relates to a polynucleotide encoding a transposase comprising an amino acid sequence having at least 90% sequence identity with amino acids 10 to 585 of SEQ ID NO: 4 operably linked to a eukaryotic promoter.

[017] In a related aspect, a polynucleotide encoding the recombinant transposase according to the disclosure is provided.

[018] The disclosure further relates to an expression vector encoding the recombinant transposase according to the disclosure or an expression vector comprising the polynucleotide encoding the transposase according to the disclosure.

[018a] In another aspect, the present disclosure relates to an expression vector comprising a polynucleotide encoding the recombinant transposase according to the disclosure or the polynucleotide according to the disclosure.

[019] In yet another aspect, the disclosure relates to an isolated mRNA encoding the recombinant transposase according to the disclosure.

[020] Also provided is an expression system or a kit comprising (a) a recombinant transposase source selected from the group consisting of (i) the expression vector encoding the recombinant transposase according to the disclosure; (ii) the isolated mRNA according to the disclosure and (iii) the recombinant transposase according to the disclosure; and (b) a DNA transposon according to the disclosure or an expression vector comprising said DNA transposon according to the disclosure.

[020a] In another aspect, the present disclosure relates to an expression system or a kit comprising

(a) a recombinant transposase source selected from the group consisting of

(i) the expression vector according to the disclosure;

(ii) the isolated mRNA according to the disclosure; and

(iii) the recombinant transposase according to the disclosure; and

(b) a DNA transposon according to the disclosure or an expression vector comprising the DNA transposon according to the disclosure.

[021] Also provided is a eukaryotic cell comprising the DNA transposon according to the disclosure. In certain examples, the eukaryotic cell is a yeast or a mammalian cell, preferably a mammalian cell, more preferably a rodent or human cell.

[021a] In another aspect, the present disclosure relates to a eukaryotic cell comprising the DNA transposon according to the disclosure, wherein the eukaryotic cell is a yeast or a mammalian cell, preferably a mammalian cell, more preferably a rodent or human cell.

[022] Also provided is a non-human transgenic animal comprising the DNA transposon according to the disclosure.

[023] Also provided is a method for preparing a cell comprising a stably integrated heterologous polynucleotide, comprising (a) introducing a DNA molecule comprising the DNA transposon according to the disclosure or the expression vector comprising the DNA transposon according to the disclosure into a eukaryotic cell,

wherein the heterologous polynucleotide comprises a sequence encoding a gene of interest, a complementary DNA (cDNA), a genome of interest or another genetic element, and wherein the heterologous polynucleotide further comprises a sequence encoding a selectable marker; (b) introducing a recombinant transposase source into said eukaryotic cell, wherein the recombinant transposase source is selected from the group consisting of: (i) the expression vector encoding the recombinant transposase according to the disclosure; (ii) the isolated mRNA according to the disclosure, and (iii) the recombinant transposase according to the disclosure; and (c) culturing the eukaryotic cell in a medium under conditions to select for the selectable marker, wherein the DNA transposon is stably integrated into the genome of the eukaryotic cell.

[023a] In another aspect, the present disclosure relates to a method for preparing a cell comprising a stably integrated heterologous polynucleotide, comprising

(a) introducing a DNA molecule comprising the DNA transposon according to the disclosure or an expression vector comprising the DNA transposon according to the disclosure into a eukaryotic cell, wherein the heterologous polynucleotide comprises a sequence encoding a gene of interest, a complementary DNA (cDNA), a genome of interest or another genetic element, and wherein the heterologous polynucleotide further comprises a sequence encoding a selectable marker;

(b) introducing a recombinant transposase source into said eukaryotic cell, wherein the recombinant transposase source is selected from the group consisting of:

(i) the expression vector according to the disclosure;

(ii) the isolated mRNA according to the disclosure; and

(iii) the recombinant transposase according to the disclosure; and

(c) culturing the eukaryotic cell in a medium under conditions to select for the selectable marker, wherein the DNA transposon is stably integrated into the genome of the eukaryotic cell.

[024] Also provided is a method for preparing a protein of interest, comprising (a) introducing a DNA molecule comprising the DNA transposon according to the disclosure or the expression vector comprising the DNA transposon according to the disclosure into a eukaryotic cell, wherein the heterologous polynucleotide comprises a sequence encoding at least one protein of interest and further a sequence encoding a selectable marker; (b) introducing a recombinant transposase source into said eukaryotic cell, wherein the recombinant transposase source is selected from the group consisting of: (i) the expression vector encoding the recombinant transposase according to the disclosure; (ii) the isolated mRNA according to the disclosure, and (iii) the recombinant transposase according to the disclosure; and (c) culturing the eukaryotic cell in a medium under conditions to select for the selectable marker, wherein the heterologous polynucleotide comprising a sequence encoding

the at least one protein of interest and a sequence encoding a selectable marker is integrated into the genome of the eukaryotic cell, (d) optionally isolating a single clone for clonal expansion to prepare a monoclonal cell line; (e) culturing the eukaryotic cell under conditions to produce the protein of interest; and (f) harvesting and optionally purifying the protein of interest.

[024a] In another aspect, the present disclosure relates to a method for preparing a protein of interest, comprising

(a) introducing a DNA molecule comprising the DNA transposon according to the disclosure or an expression vector comprising the DNA transposon according to the disclosure into a eukaryotic cell, wherein the heterologous polynucleotide comprises a sequence encoding at least one protein of interest and further a sequence encoding a selectable marker;

(b) introducing a recombinant transposase source into said eukaryotic cell, wherein the recombinant transposase source is selected from the group consisting of:

(i) the expression vector according to the disclosure;

(ii) the isolated mRNA according to the disclosure; and

(iii) the recombinant transposase according to the disclosure; and

(c) culturing the eukaryotic cell in a medium under conditions to select for the selectable marker, wherein the heterologous polynucleotide comprising a sequence encoding the at least one protein of interest and a sequence encoding a selectable marker is integrated into the genome of the eukaryotic cell;

(d) optionally isolating a single clone for clonal expansion to prepare a monoclonal cell line;

(e) culturing the eukaryotic cell under conditions to produce the protein of interest; and

(f) harvesting and optionally purifying the protein of interest.

[024b] In another aspect, the present disclosure relates to a method for preparing a virus or virus like particle of interest, comprising

(a) introducing a DNA molecule comprising the DNA transposon according to the disclosure or an expression vector comprising the DNA transposon according to the disclosure into a eukaryotic cell, wherein the heterologous polynucleotide comprises a sequence encoding at least one virus or virus like particle of interest and further a sequence encoding a selectable marker;

(b) introducing a recombinant transposase source into said eukaryotic cell, wherein the recombinant transposase source is selected from the group consisting of:

(i) the expression vector according to the disclosure;

(ii) the isolated mRNA according to the disclosure; and

(iii) the recombinant transposase according to the disclosure; and

(c) culturing the eukaryotic cell in a medium under conditions to select for the selectable marker, wherein the heterologous polynucleotide comprising a sequence encoding the at least one virus or virus like particle of interest and a sequence encoding a selectable marker is integrated into the genome of the eukaryotic cell;

(d) optionally isolating a single clone for clonal expansion to prepare a monoclonal cell line;

(e) culturing the eukaryotic cell under conditions to produce the virus or virus like particle of interest; and

(f) harvesting and optionally purifying the virus or virus like particle.

DESCRIPTION OF THE FIGURES

[025] FIGURE 1: Plasmid maps. Top: Exemplary plasmid encoding the AP transposase with a heterologous nuclear localization signal (NLS) under the control of a CMV promoter. Bottom: Exemplary transposon vector encoding a heavy and light chain gene of a therapeutic antibody under the control of CMV promoter. A metabolic selection marker (Glutamine Synthetase) is expressed under the control of a SV40 promoter. The transposon cargo is defined with flanking sequences SeqID2 left (Seq ID NO: 2) and SeqID3 right (Seq ID NO: 3). Both Flanking sequences harbour ITR sequences of Seq ID NO:1.

[026] FIGURE 2: Selection experiment applying *Acyrthosiphon pisum* transposase and transposon in CHO (CHO K1 $GS^{-/-}$) cell deficient of an endogenous glutamine synthetase (GS), with a transposon providing the GS selection marker. As control CHO K1 $GS^{-/-}$ were transfected with the same transposon plasmid, but without the transposase plasmid. Viability is monitored during the selection process of the pool establishment of recombinant CHO cells. Shown is % viability versus day [d] post-transfection.

[027] FIGURE 3: Productivity of stable CHO pools transfected with AP transposase and transposon expressing a therapeutic antibody. Control cells were as described for Figure 2. Shown is the titer [mg/L] versus day [d] post-transfection.

[028] FIGURE 4: Copy number determination of genomically integrated heavy chain (HC) and light chain (LC) genes via ddPCR of stable CHO pools transfected with AP transposase and transposon.

[029] FIGURE 5: Selection experiment after transfection of various *Acyrthosiphon pisum* (AP) transposase constructs with and without N- and/or C-terminal heterologous nuclear localization signals (NLS) as indicated and a transposon into CHO-K1 GS-/- cells. Viability was monitored during the selection process of the transfected CHO pools. Shown is % viability versus day [d] post-transfection. AP: wild-type AP transposase (SEQ ID NO: 4), NLS_AP: wild-type AP transposase with heterologous N-terminal NLS (SEQ ID NO: 6); and NLS_AP_NLS: wild-type AP transposase with heterologous N- and C-terminal NLS (SEQ ID NO: 7).

[030] FIGURE 6: Antibody titer determination of stable CHO pools 23 days post transfection with various *Acyrthosiphon pisum* transposase constructs with and without N- and/or C-terminal heterologous nuclear localization signals (NLS) as indicated. Shown is the titer [mg/L] for the different constructs. AP: wild-type AP transposase (SEQ ID NO: 4), NLS_AP: wild-type AP transposase with heterologous N-terminal NLS (SEQ ID NO: 6); and NLS_AP_NLS: wild-type AP transposase with heterologous N- and C-terminal NLS (with flag; SEQ ID NO: 7).

[031] FIGURE 7: Selection experiment after transfection of *Acyrthosiphon pisum* transposase constructs with (NLS_AP_NLS with flag; SEQ ID NO: 7) and without a N-terminal flag-tag

CLAIMS

1. A DNA transposon comprising a heterologous polynucleotide flanked by transposon flanking regions, wherein one transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 38 at one transposon end; and the other transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 39 at the other transposon end.

2. The DNA transposon of claim 1, wherein
   (a) the one transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 40 at one transposon end; and the other transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 41 at the other transposon end;
   (b) the one and the other transposon flanking region each further comprises at least one inverted internal repeat (IIR) comprising (i) an internal repeat motif comprising the nucleotide sequence tggtctac and (ii) its reverse complementary sequence (iii) separated by six to three, preferably four nucleotides, wherein preferably the at least one inverted internal repeat (IIR) motif has the nucleotide sequence of SEQ ID NO: 43; and/or
   (c) (i) the one transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 45 or 46 and further an inverted internal repeat motif having the nucleotide sequence of SEQ ID NO: 43 separated by about 50 to 200 nucleotides; and/or the other transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 47 or a nucleotide sequence having at least 95% sequence identity with SEQ ID NO: 47, wherein the sequence comprises at least the nucleotide sequence of SEQ ID NO: 41 and the nucleotide sequence of SEQ ID NO: 43; or (ii) wherein the one and the other transposon flanking regions are inverted and comprise reverse complementary sequences of the sequences recited in (i).

3. The DNA transposon of claim 1, wherein
   (a) (i) the one transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 45 or 46 and further an inverted internal repeat motif having the nucleotide sequence of SEQ ID NO: 43 separated by about 50 to 200 nucleotides and wherein the one transposon flanking region comprises a nucleotide sequence having 85% sequence identity with SEQ ID NO: 10 or 63, or with SEQ ID NO: 2 or 29; and/or the other transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 47 or a nucleotide sequence having at least 95% sequence identity with SEQ ID NO: 47, wherein the sequence comprises at least the nucleotide sequence of SEQ ID NO: 41 and the nucleotide sequence of SEQ ID NO: 43; or (ii) wherein the one and the other transposon flanking regions are inverted and comprise reverse complementary sequences of the sequences recited in (i); and/or
   (b) (i) the one transposon flanking region comprises a nucleotide sequence having at least 85% nucleotide sequence identity with SEQ ID NO: 2, 8, 10, or 58 and/or the other

transposon flanking region comprises a nucleotide sequence having at least 90% nucleotide sequence identity with SEQ ID NO: 3, 9, 11 or 59; or (ii) wherein the one and the other transposon flanking regions are inverted and comprise reverse complementary sequences of the sequences recited in (i); and/or

(c) (i) the one transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 2, 8, 10 or 58 or the sequence of SEQ ID NO: 29, 62, 63 or 64 and/or the other transposon flanking region comprises the nucleotide sequence of SEQ ID NO: 3, 9, 11, 59, 48 or 49 or the nucleotide sequence of SEQ ID NO: 30, 65, 66, 67, 60 or 61; or (ii) wherein the one and the other transposon flanking regions are inverted and comprise reverse complementary sequences of the sequences recited in (i); or

(d) (i) the one transposon flanking region comprises a nucleotide sequence having at least 95% sequence identity with SEQ ID NO: 29, 62, 63 or 64 and/or the other transposon flanking region comprises a nucleotide sequence having at least 97% sequence identity with SEQ ID NO: 30, 65, 66, 67, 60 or 61; or (ii) wherein the one and the other transposon flanking regions are inverted and comprise reverse complementary sequences of the sequences recited in (i).

4. The DNA transposon of claim 1, wherein the heterologous polynucleotide comprises at least one sequence selected from the group consisting of a sequence encoding a gene of interest, a complementary DNA (cDNA), a genome of interest and another genetic element.

5. The DNA transposon of claim 1, wherein the transposon is transposable by a transposase comprising the amino acid sequence of SEQ ID NO: 4.

6. An expression vector comprising the DNA transposon of any one of claims 1 to 5.

7. A recombinant transposase comprising an amino acid sequence having at least 90% sequence identity with amino acids 10 to 585 of SEQ ID NO: 4 and at least one heterologous nuclear localization signal (NLS) fused to the transposase, preferably wherein the transposase is an *Acyrthosiphon pisum* transposase or an *Aphis craccivora* transposase.

8. A recombinant transposase comprising an amino acid sequence having at least 90% sequence identity with amino acids 10 to 585 of SEQ ID NO: 4, wherein the transposase comprises at least one mutation, preferably wherein the at least one mutation is an amino acid substitution selected from the group consisting of K87Y, Q273V, V212I/I215L, I363V/K365S, K87Y/Q273V, K87Y/A264S/Q273V, A264S/Q273V, S270P/Q273V, K87Y/A264S, L583F, K576I, S372E, S277N and any combination thereof, and/or the at least one mutation is a deletion of N584 and/or E585, wherein the indicated amino acid position of the substitution and/or deletion corresponds to the amino acid position in the sequence of SEQ ID NO: 4, preferably wherein at least one heterologous NLS is fused to the transposase.

9. A polynucleotide encoding a transposase comprising an amino acid sequence having at least 90% sequence identity with amino acids 10 to 585 of SEQ ID NO: 4 operably linked to a eukaryotic promoter.

10. A polynucleotide encoding the recombinant transposase of claim 7 or 8.

11. An expression vector comprising a polynucleotide encoding the recombinant transposase of claim 7 or 8 or the polynucleotide of claim 9 or 10.

12. An isolated mRNA encoding the recombinant transposase of claim 7 or 8.

13. An expression system or a kit comprising

(a) a recombinant transposase source selected from the group consisting of

(i) the expression vector of claim 11;

(ii) the isolated mRNA of claim 12; and

(iii) the recombinant transposase of claim 7 or 8; and

(b) a DNA transposon of any one of claims 1 to 5 or an expression vector comprising the DNA transposon of any one of claims 1 to 5.

14. A eukaryotic cell comprising the DNA transposon of any one of claims 1 to 5, wherein the eukaryotic cell is a yeast or a mammalian cell, preferably a mammalian cell, more preferably a rodent or human cell.

15. A method for preparing a cell comprising a stably integrated heterologous polynucleotide, comprising

(a) introducing a DNA molecule comprising the DNA transposon according to any one of claims 1 to 5 or an expression vector comprising the DNA transposon of any one of claims 1 to 5 into a eukaryotic cell, wherein the heterologous polynucleotide comprises a sequence encoding a gene of interest, a complementary DNA (cDNA), a genome of interest or another genetic element, and wherein the heterologous polynucleotide further comprises a sequence encoding a selectable marker;

(b) introducing a recombinant transposase source into said eukaryotic cell, wherein the recombinant transposase source is selected from the group consisting of:

(i) the expression vector of claim 11;

(ii) the isolated mRNA of claim 12; and

(iii) the recombinant transposase of claim 7 or 8; and

(c) culturing the eukaryotic cell in a medium under conditions to select for the selectable marker, wherein the DNA transposon is stably integrated into the genome of the eukaryotic cell.

16. A method for preparing a protein of interest, comprising

(a) introducing a DNA molecule comprising the DNA transposon according to any one of claims 1 to 5 or an expression vector comprising the DNA transposon of any one of claims 1 to 5 into a eukaryotic cell, wherein the heterologous polynucleotide comprises a sequence encoding at least one protein of interest and further a sequence encoding a selectable marker;

(b) introducing a recombinant transposase source into said eukaryotic cell, wherein the recombinant transposase source is selected from the group consisting of:

(i) an expression vector of claim 11;

(ii) the isolated mRNA of claim 12; and

(iii) the recombinant transposase of claim 7 or 8;

(c) culturing the eukaryotic cell in a medium under conditions to select for the selectable marker, wherein the heterologous polynucleotide comprising a sequence encoding the at least one protein of interest and a sequence encoding a selectable marker is integrated into the genome of the eukaryotic cell;

(d) optionally isolating a single clone for clonal expansion to prepare a monoclonal cell line;

(e) culturing the eukaryotic cell under conditions to produce the protein of interest; and

(f) harvesting and optionally purifying the protein of interest.

17. A method for preparing a virus or virus like particle of interest, comprising

(a) introducing a DNA molecule comprising the DNA transposon according to any one of claims 1 to 5 or an expression vector comprising the DNA transposon of any one of claims 1 to 5 into a eukaryotic cell, wherein the heterologous polynucleotide comprises a sequence encoding at least one virus or virus like particle of interest and further a sequence encoding a selectable marker;

(b) introducing a recombinant transposase source into said eukaryotic cell, wherein the recombinant transposase source is selected from the group consisting of:

(i) an expression vector of claim 11;

(ii) the isolated mRNA of claim 12; and

(iii) the recombinant transposase of claim 7 or 8;

(c) culturing the eukaryotic cell in a medium under conditions to select for the selectable marker, wherein the heterologous polynucleotide comprising a sequence encoding the at least one virus or virus like particle of interest and a sequence encoding a selectable marker is integrated into the genome of the eukaryotic cell;

(d) optionally isolating a single clone for clonal expansion to prepare a monoclonal cell line;

(e) culturing the eukaryotic cell under conditions to produce the virus or virus like particle of interest; and

(f) harvesting and optionally purifying the virus or virus like particle.