CLAIMS

1. A recombinant nucleic acid molecule comprising:

(a) a 5’ and a 3’ transposon-specific inverted terminal repeat (ITR),

(b) at least one nucleic acid sequence encoding a transgene expression processing (TEP) protein or a TEP functional RNA, located between the 5’ and 3’ ITRs and which is under the control of a promoter, and

(c) at least one MAR (matrix attachment region) element;

in which the molecule does not comprise a selection gene which, in use, is used during expression of a gene of interest.

2. A recombinant nucleic acid molecule according to Claim 1, in which there is provided at least one transgene also located between the 5’ and 3’ ITRs and which is under the control of a transgene promoter.

3. A recombinant nucleic acid molecule according to Claim 1 or 2, in which the MAR element is located between the 5’ and a 3’ ITRs.

4. A recombinant nucleic acid molecule according to any preceding claim, in which the TEP protein or TEP functional RNA is a protein or a functional RNA that is, directly or indirectly, involved in integration of nucleic acid sequences into a genome, processing or translation of the transgene RNA product or is involved in ER translocation, secretion, processing, folding, ER-Golgi-plasma membrane transport, glycosylation and/or another post-translational modification of proteins such as transgene expression products.

5. A recombinant nucleic acid molecule according to any preceding claim, in which the TEP protein is a protein of the protein secretion pathway, a protein of the recombination pathway, a protein processing or metabolic protein including chaperones such as BiP, or a combination thereof.

6. A recombinant nucleic acid molecule according to any preceding claim, in which the TEP protein is one or more of the following proteins of the protein secretion pathway:

(a) one or more of the following proteins of the protein secretion pathway: hSRP14, hSec61α1, hSec61β, hSec61γ, hSRP54, hSRP9, hSRPRα, hSRPβ, and hCANX; or

(b) one or more of the following amino acid sequences of proteins of the protein secretion pathway: hSRP14 having SEQ ID NO: 13, hSec61α1 having SEQ ID NO: 15, hSec61β having SEQ ID NO: 17, hSec61γ having SEQ ID NO: 19, hSRP54 having SEQ ID NO: 21, hSRP9 having SEQ ID NO: 23, hSRPRα having SEQ ID NO: 25, hSRPβ having SEQ ID NO: 27, and hCANX having SEQ ID NO: 29 and amino acid sequences having more than 80%, 90%, 95% or 98% sequence identity with the specified sequences.

7. A recombinant nucleic acid molecule according to any preceding claim, in which the TEP protein is one or more of the following protein processing or metabolic proteins: hUCP4, hCMPSAT, rST6Gal1, hCOMSC, hT-Synthase, hP4HA1, hP4HB, hGILZ, hCyPB, hNRF2, hHK1, hPDI, hPIN1, hSEPW1, hCALR, hDDOST, hHSP40, hATP5A1, hSERCA2, hPDIA4, hHSC70 /HSPA8, hHYOU1, hCMP-SAS, hBeclin-1, hERdj3, CHO-AGE, hWip1, hRTP4, hREEP2, hDPM1 and hDRiP78.

8. A recombinant nucleic acid molecule according to any preceding claim, in which the TEP protein is a synthetic BIP protein derivative (DroBiP) having 80%, 90%, 95% or 100% sequence identity with the SEQ ID NO: 60.

9. A recombinant nucleic acid molecule according to any preceding claim, in which the MAR element is selected from SEQ ID NOs: 1 (MAR 1-68), 2 (MAR 1_6), 3 (MAR X_S29), 4 (MAR S4), 5 (chicken lysozyme MAR), is a engineered, in particular rearranged counterpart and/or has at least 80%, 90%, 95%, 98%, 99% or 100% sequence identity with any of SEQ ID NOs: 1 to 5.

10. A recombinant nucleic acid molecule according to any preceding claim, in which the TEP functional RNA within the cell comprises/consists of nucleic acid sequences encoding a functional RNA, preferably a miRNA or a shRNA, that interferes with the expression of at least one protein of a recombination pathway, such as, but not limited to, Rad51, Rad51B, Rad51C, Rad51D, Xrcc2, Xrcc3, Rad52, Rad54, Brca1, Brca2, Cyclin D1, Ercc, MDC1, Bard1, Ligase 1, Mre 11 and/or 53BP1.

11. A recombinant nucleic acid molecule according to any preceding claim, in which the nucleic acid molecule is at least 5000, 6000, 7000, 8000, 90000 or 10000 bps long.

12. A recombinant nucleic acid molecule according to any preceding claim, in which the 5' and a 3' ITRs are 5' and 3' ITRs of the Sleeping Beauty or preferably PiggyBac Transposon.

13. A recombinant nucleic acid molecule according to any preceding claim, in which the recombinant nucleic acid molecule is part of a vector.

14. A recombinant nucleic acid molecule according to Claim 13, in which the vector comprises a singular MAR element, two or more MAR elements, in which the element(s) are located between the 5' and 3' ITRs.

15. A recombinant nucleic acid according to Claim 13, in which the vector comprises two MAR elements, in which a first MAR element is positioned upstream of the TEP or TEP functional RNA and a second MAR element is positioned downstream of the TEP or TEP functional RNA, in which the first MAR element comprises a MAR 1_6 element and/or an element that has at least 80%, 90%, 95%, 98%, 99% or 100% sequence identity with SEQ ID NO: 2, in particular a rearranged MARs based on MAR 1-6, more in particular elements that have at least 80%, 90%, 95%, 98%, 99% or 100% sequence identity with SEQ ID NO: 8 (MARs 1_6R2) and the second MAR element comprises a MAR 1-68 element and/or an element that has at least 80%, 90%, 95%, 98%, 99% or 100% sequence identity with SEQ ID NO: 1.

16. A recombinant nucleic acid molecule according to Claim 13, in which the vector comprises the singular MAR element, in which the singular MAR element is positioned downstream of the TEP or TEP functional RNA, in which the singular MAR element is a MAR 1-68 or a MAR X-29 element and/or an element that has at least 80%, 90%, 95%, 98%, 99% or 100% sequence identity with SEQ ID NOs. 1 or 3, in particular a rearranged MAR based on MAR 1-68 or a MAR X-29, in particular an element that has at least 80%, 90%, 95%, 98%, 99% or 100% sequence identity with SEQ ID NOs: 6, 7 or 10 (MARs 1_68R, 1_68R2 or X_29R3) or 9, and is preferably a MAR X-29 element and/or an element that has at least 80%, 90%, 95%, 98%, 99% or 100% sequence identity with SEQ ID NO: 3.

17. A recombinant nucleic acid molecule according to any of Claims 13 to 16, in which the TEP or TEP functional RNA is under the control of an EF1 alpha promoter and is optionally followed by a BGH polyA signal.

18. A recombinant nucleic acid molecule according to any of Claims 13 to 17, in which the vector comprises promoter(s) and/or enhancer(s) or fusions thereof such as GAPDH, CGAPDH, SV40p, CMVp, CHO Ef1 alpha, CHO Actb and/or CHO Hspa5.

19. An *in vitro* method for expressing a TEP or TEP functional RNA comprising providing a recombinant mammalian cell comprising a transgene, and the vector is a vector according to any of Claims 13 to 18 which expresses the TEP or TEP functional RNA, in which the TEP or TEP functional RNA expressed via the vector optionally increases an expression of a transgene in the mammalian cell by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70%.

20. An *in vitro* method according to Claim 19, in which the vector comprises a singular MAR X-29 element and/or a nucleic acid sequence having at least 80%, 90%, 95%, 98%, 99% or 100% sequence identity with SEQ ID NO: 3 and in which, after more than 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 weeks of cultivation, the TEP or TEP functional RNA expressed via the vector increases an expression of the gene of interest by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60% or at least 70%.

21. A recombinant mammalian cell comprising not more than 20, 15, 10 or 5 copies of a recombinant nucleic acid molecule according to any of Claims 1 to 18.

22. A recombinant mammalian cell according to Claim 21 in which recombinant nucleic acid molecule is integrated into the genome of the cell as single copies.

23. A recombinant mammalian cell according to Claim 21 or 22, in which the nucleic acid sequence(s) encoding a TEP protein within the cell comprise(s)/consist(s) of nucleic acid sequences encoding one or more of the proteins according to any of Claims 7, 8 and/or 9.

24. A recombinant mammalian cell according to Claim 23, in which the nucleic acid sequence(s) encoding the TEP functional RNA within the cell comprise(s)/consist(s) of nucleic acid sequence(s) encoding a functional RNA, preferably a miRNA or a shRNA, that

interferes with the expression of at least one recombination protein, preferably an HR gene, such as, but not limited to, Rad51, Rad51B, Rad51C, Rad51D, Xrcc2, Xrcc3, Rad52, Rad54, Brca1, Brca2, Cyclin D1, Ercc1, MDC1, Bard1, Ligase 1, Mre 11 and/or 53BP1.

25. A recombinant mammalian cell according to any of Claims 21 to 24, in which the cell is a primary stem cell, a hamster, a CHO (Chinese hamster ovary) cell, a human cell, or HEK293 cell.

26. An *in vitro* method for transfecting mammalian cells, in particular hamster cells, comprising transfecting the mammalian cells with

(i) at least one, at least two, at least three or at least four of the recombinant nucleic acids molecules according to any of Claims 1 to 12 and/or

(ii) at least one, at least two, at least three or at least four isolated TEP functional RNA and at least one transgene.

27. An *in vitro* method according to Claim 26, in which the transgene is part of a recombinant nucleic acid molecule.

28. An *in vitro* method according to Claim 26 or 27, in which the transgene can be transfected in a second, subsequent transfection.

29. An *in vitro* method according to Claim 28, in which the second, subsequent transfection can also include transfection together with an isolated nucleic acid or mRNA expressing a transposase which recognizes the 5' and the 3' ITR.

30. An *in vitro* method according to any of Claims 26 to 29, in which the cell is transfected with more than one, including at least two, at least three or at least four of the recombinant nucleic acid molecules encoding one, two or three of the TEP proteins specified in Claim 6.

31. An *in vitro* method according to any of Claims 26 to 30, in which each of the nucleic acid molecules is part of a vector and the vectors are co-transfected.

32. An *in vitro* method according to Claim 31, in which the co-transfection, preferably a co-transfection of recombinant nucleic acid molecules encoding proteins SRP14, SRP9 and

SRP54 increases transgene integration and/or expression in the cell relative to a cell not subject to such co-transfection.

33. An *in vitro* method according to any of Claims 26 to 32, in which a number of the mammalian cells that stably express the TEP protein or TEP functional RNA is obtained to obtain recombinant mammalian cells and in which the number of recombinant mammalian cells is independent from the presence of the MAR element.

34. An *in vitro* method according to any of Claims 26 to 33, in which the mammalian cells are transfected a second time.

35. An *in vitro* method according to any of Claims 26 to 34, in which at least 30%, 40% or 45% of the mammalian cells become recombinant mammalian cells and express the transgene.

36. An *in vitro* method according to any of Claims 26 to 35, in which the mammalian cell is transfected with the at least one isolated TEP functional RNA and a vector comprising the at least one transgene.

37. An *in vitro* method according to Claim 36, in which a 3' ITR and 5' ITR flanks the at least one transgene.

38. An *in vitro* method according to Claim 37, in which there is also provided an isolated nucleic acid or mRNA expressing a transposase which recognizes the 5' and the 3' ITR.

39. An *in vitro* method according to any of Claims 26 to 38, in which the transgene is a therapeutic protein such as an immunoglobulin, hormone, cytokine or growth factor.

40. An *in vitro* method according to Claim 26, in which the TEP functional RNA is encoded by a recombinant nucleic acid sequences encoding a shRNA or comprises/consists of a siRNA that interferes with the expression of at least one HR gene, such as, but not limited to, Rad51, Rad51B, Rad51C, Rad51D, Xrcc2, Xrcc3, Rad52, Rad54, Brca1, Brca2, Cyclin D1, Ercc1, MDC1, Bard1, Ligase 1, Mre 11 and/or 53BP1.

41. An *in vitro* method according to any of Claims 26 to 42, in which transgene integration and/or expression is increased in such a cell relative to a cell not transfected with the isolated nucleic acid molecules and/or the at least one of the isolated TEP functional RNAs.

42. A kit comprising in one container at least one vector comprising a recombinant nucleic acid molecule according to any of Claims 1 to 12.

43. A kit according to Claim 42 comprising a second container comprising a vector encoding a compatible transposase and in a further container instruction of how to use the vector or vectors.

44. A kit according to Claim 42, in which more than one vector is provided in one or more containers and in which the TEP proteins are at least two of the following: a chaperone, SRP14, SRP9, or SRP54.

45. A kit according to Claim 42, in which the TEP functional RNA(s) within the vector(s) comprise(s)/consist(s) of nucleic acid sequences encoding a miRNA, siRNA or a shRNA that interferes with the expression of at least one HR gene, such as, but not limited to, Rad51, Rad51B, Rad51C, Rad51D, Xrcc2, Xrcc3, Rad52, Rad54, Brca1, Brca2 Cyclin D1, Ercc1, MDC1, Bard1, Ligase 1, Mre 11 and/or 53BP1, and optionally in a further container siRNA(s) that interferes with the expression of at least one HR gene, such as, but not limited to, Rad51, Rad51B, Rad51C, Rad51D, Xrcc2, Xrcc3, Rad52, Rad54, Brca1, Brca2, Cyclin D1, Ercc1, MDC1, Bard1, Ligase 1, Mre 11 and/or 53BP1.

46. *In vitro* use of a recombinant nucleic acid according to any of Claims 1 to 12 and/or a recombinant mammalian cell according to any of Claims 21 to 24 for increasing transgene integration and/or expression.