CLAIMS

1. A method for transgene expression comprising:

(a) providing an eukaryotic, preferably a mammalian, host cell, wherein said host cell has been modified or treated to increase homologous recombination (HR), decrease non homologous end joining (NHEJ) and/or to enhance HR/NHEJ ratio in said cell, and

(b) transfecting said cell with at least one vector comprising said transgene, and with a matrix attachment region (MAR) element, wherein said MAR element is provided to said transgene in cis or trans.

2. A method according to Claim 1, wherein the transfection in (b) is a subsequent transfection and is preceded by an initial transfection with nucleic acid such as a vector or nucleic acid fragments.

3. A method according to Claim 2, wherein the said initial and subsequent transfection takes place at a time when more than 30% of the cells of the cell population are in the G1 phase.

4. A method according to any of Claims 2 or 3, wherein the nucleic acid of said initial transfection is at least one vector comprising a transgene and wherein at least one of said vectors of the initial transfection and at least one vector of said at least one subsequent transfection form one or more concatemeric structures prior and/or after integration into the genome of the cell.

5. A method according to any preceding claim, wherein the HR/NHEJ ratio of the cell is up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 times higher than a ratio found in the cell not comprising said transgenic sequence and not being mutated, respectively or wherein the NHEJ activity equals 0.

6. A method according to any preceding claim, wherein said at least one initial transfection is a single transfection.

7. The method according to any preceding claim, wherein the at least one subsequent transfection is a single transfection.

8. A method according to any preceding claim wherein the transgene is integrated into the genome of said cell as a concatemeric structure at a single locus.

9. A method according to any preceding claim, wherein the vector sequence of said vector of the at least one first transfection has 100% or at least 95%, 90%, 85% or 80% sequence identity with the vector sequence of at least the vector of a first of said subsequent transfection(s).

10. A method according to any of Claims 2 to 9, wherein the vector of the initial transfection comprises a MAR element and said MAR element has 100% or at least 95%, 90%, 85% or 80% sequence identity with the MAR element of at least the vector of a first of said subsequent transfection(s).

11. A method according to any of Claims 2 to 10, wherein the vector of the initial transfection comprises a transgene and the transgene has 100% or at least 95%, 90%, 85% or 80% sequence identity with the transgene of at least the vector of a first of said subsequent transfection(s).

12. A method according to any preceding claim, wherein the MAR element is located upstream of a promoter / enhancer sequence of said transgene.

13. A method according to any preceding claim, wherein the MAR sequence has at least 90% sequence identity with: SEQ ID NOs: 1-3 or is a variant thereof.

14. A recombinant eukaryotic, preferably mammalian, host cell, comprising

a transgenic sequence expressing a NHEJ suppressor, e.g. NU7026 and/or wortmannin,

a transgenic sequence expressing one or more HR enzymes, e.g. Rad 51, Rad 52, RecA, Rad 54, RuvC or BRCA2, or HR activators, e.g. RS-1,

a mutation inactivating or downregulating a NHEJ gene, e.g. mutation in a xrcc4 gene, RAD51 strand transferase gene, a DNA-dependent protein kinase gene, the Rad 52 gene, the RecA gene, the Rad 54 gene, the RuvC gene and/or the BRCA2 gene, and/or

a mutation enhancing expression or activity of an HR enzyme, an HR activator or a NHEJ suppressor, e.g. mutation in a xrcc4 gene, RAD51 strand transferase gene, a

DNA-dependent protein kinase gene, the Rad 52 gene, the RecA gene, the Rad 54 gene, the RuvC gene and/or the BRCA2 gene, and

a transgene integrated into the genome of said cell,

wherein the recombinant eukaryotic host cell

has an HR/NHEJ ratio more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 times higher than a ratio found in the cell not comprising said transgenic sequence of (a) and/or (b), and

comprises a matrix attachment region (MAR) element.

15. A cell according to Claim 14, wherein the transgene is integrated into one locus of the genome of the cell and forms a concatemeric structure, preferably a concatemeric structure that comprises at least 200, 300, 400, 500 or 600 copies of the transgene

16. A cell according to Claim 14 or 15, wherein the at least one MAR element has at least 90% sequence identity with SEQ ID NOs: 1-3 or is a variant of SEQ ID NOs: 1-3.

17. A cell according to Claim 14, 15 or 16, wherein the MAR element is located upstream of a promoter/ enhancer sequence of said transgene.

18. Use of any one of the recombinant eukaryotic host cells according to any of Claims 14 to 17 in the expression of said transgene.

19. A kit comprising

(a) in a first container, a vector comprising optionally a MAR element and restriction sites for integration of a transgene into said vector,

(b) in a second container, a recombinant eukaryotic host cell according to any of Claims 14 to 17, and

(c) instructions how to use said vector in transfecting said cell for transgene expression.

CLAIMS

1. A method for transgene expression comprising:

(a) providing an eukaryotic, preferably a mammalian, host cell, wherein said host cell has been modified or treated to increase homologous recombination (HR), decrease non homologous end joining (NHEJ) and/or to enhance HR/NHEJ ratio in said cell, and

(b) transfecting said cell with at least one vector comprising said transgene, and with a matrix attachment region (MAR) element, wherein said MAR element is provided to said transgene in cis or trans.

2. A method according to Claim 1, wherein the transfection in (b) is a subsequent transfection and is preceded by an initial transfection with nucleic acid such as a vector or nucleic acid fragments.

3. A method according to Claim 2, wherein the said initial and subsequent transfection takes place at a time when more than 30% of the cells of the cell population are in the G1 phase.

4. A method according to any of Claims 2 or 3, wherein the nucleic acid of said initial transfection is at least one vector comprising a transgene and wherein at least one of said vectors of the initial transfection and at least one vector of said at least one subsequent transfection form one or more concatemeric structures prior and/or after integration into the genome of the cell.

5. A method according to any preceding claim, wherein the HR/NHEJ ratio of the cell is up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 times higher than a ratio found in the cell not comprising said transgenic sequence and not being mutated, respectively or wherein the NHEJ activity equals 0.

6. A method according to any preceding claim, wherein said at least one initial transfection is a single transfection.

7. The method according to any preceding claim, wherein the at least one subsequent transfection is a single transfection.

8. A method according to any preceding claim wherein the transgene is integrated into the genome of said cell as a concatemeric structure at a single locus.

9. A method according to any preceding claim, wherein the vector sequence of said vector of the at least one first transfection has 100% or at least 95%, 90%, 85% or 80% sequence identity with the vector sequence of at least the vector of a first of said subsequent transfection(s).

10. A method according to any of Claims 2 to 9, wherein the vector of the initial transfection comprises a MAR element and said MAR element has 100% or at least 95%, 90%, 85% or 80% sequence identity with the MAR element of at least the vector of a first of said subsequent transfection(s).

11. A method according to any of Claims 2 to 10, wherein the vector of the initial transfection comprises a transgene and the transgene has 100% or at least 95%, 90%, 85% or 80% sequence identity with the transgene of at least the vector of a first of said subsequent transfection(s).

12. A method according to any preceding claim, wherein the MAR element is located upstream of a promoter / enhancer sequence of said transgene.

13. A method according to any preceding claim, wherein the MAR sequence has at least 90% sequence identity with: SEQ ID NOs: 1-3 or is a variant thereof.

14. A recombinant eukaryotic, preferably mammalian, host cell, comprising

(a) wortmannin and/or a transgenic sequence expressing a NHEJ suppressor, e.g. NU7026,

(b) a transgenic sequence expressing one or more HR enzymes, e.g. Rad 51, Rad 52, RecA, Rad 54, RuvC or BRCA2, or HR activators, e.g. RS-1,

(c) a mutation inactivating or downregulating a NHEJ gene, e.g. mutation in a xrcc4 gene, RAD51 strand transferase gene, a DNA-dependent protein kinase gene, the Rad 52 gene, the RecA gene, the Rad 54 gene, the RuvC gene and/or the BRCA2 gene, and/or

(d) a mutation enhancing expression or activity of an HR enzyme, an HR activator or a NHEJ suppressor, e.g. mutation in a xrcc4 gene, RAD51 strand transferase gene, a

DNA-dependent protein kinase gene, the Rad 52 gene, the RecA gene, the Rad 54 gene, the RuvC gene and/or the BRCA2 gene, and

(e) a transgene integrated into the genome of said cell,

wherein the recombinant eukaryotic host cell

has an HR/NHEJ ratio more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 times higher than a ratio found in the cell not comprising said transgenic sequence of (a) and/or (b), and

comprises a matrix attachment region (MAR) element.

15. A cell according to Claim 14, wherein the transgene is integrated into one locus of the genome of the cell and forms a concatemeric structure, preferably a concatemeric structure that comprises at least 200, 300, 400, 500 or 600 copies of the transgene

16. A cell according to Claim 14 or 15, wherein the at least one MAR element has at least 90% sequence identity with SEQ ID NOs: 1-3 or is a variant of SEQ ID NOs: 1-3.

17. A cell according to Claim 14, 15 or 16, wherein the MAR element is located upstream of a promoter/ enhancer sequence of said transgene.

18. Use of any one of the recombinant eukaryotic host cells according to any of Claims 14 to 17 in the expression of said transgene.

19. A kit comprising

(a) in a first container, a vector comprising optionally a MAR element and restriction sites for integration of a transgene into said vector,

(b) in a second container, a recombinant eukaryotic host cell according to any of Claims 14 to 17, and

(c) instructions how to use said vector in transfecting said cell for transgene expression.