CLAIMS

1. A method for producing a protein of interest, comprising introducing a protein expression vector which comprises a gene fragment comprising a DNA encoding a protein of interest and a selectable marker gene and transposon sequences at both terminals of the gene fragment, into a suspension mammalian cell which is capable of surviving and proliferating in a serum-free medium; integrating the gene fragment inserted between a pair of the transposon sequences into a chromosome of the mammalian cell to obtain a mammalian cell capable of expressing the protein of interest; and suspension-culturing the mammalian cell,

wherein the suspension mammalian cell is a suspension CHO cell, and

wherein the transposon sequences are nucleotide sequences derived from a pair of Tol1 transposons or nucleotide sequences derived from a pair of Tol2 transposons.

2. A method for producing a protein of interest, comprising the following steps (A) to (C):

(A) a step of simultaneously introducing the following expression vectors (a) and (b) into a suspension mammalian cell which is capable of surviving and proliferating in a serum-free medium:

(a) an expression vector which comprises a gene fragment comprising a DNA encoding a protein of interest and a selectable marker gene and transposon sequences at both terminals of the gene fragment,

(b) an expression vector which comprises a DNA encoding a transposase which recognizes the transposon sequences and has activity of transferring a gene fragment inserted between a pair of the transposon sequences into a chromosome,

(B) a step of expressing transiently the transposase from the expression vector introduced in the step (A) to integrate the gene fragment inserted between a pair of the transposon sequences into a chromosome of the mammalian cell to obtain a suspension mammalian cell capable of expressing the protein of interest, and

(C) a step of suspension-culturing the suspension mammalian cell capable of expressing the protein of interest obtained in the step (B) to produce the protein of interest,

wherein the suspension mammalian cell is a suspension CHO cell, and

wherein the transposon sequences are nucleotide sequences derived from a pair of Tol1 transposon or nucleotide sequences derived from a pair of Tol2 transposons.

3. A method for obtaining a suspension mammalian cell capable of expressing a protein of interest, comprising introducing a protein expression vector which comprises a gene fragment comprising a DNA encoding a protein of interest and

a selectable marker gene and transposon sequences at both terminals of the gene fragment into a suspension mammalian cell which is capable of surviving and proliferating in a serum-free medium; and integrating the gene fragment inserted between a pair of the transposon sequences, into a chromosome of the mammalian cell,

wherein the suspension mammalian cell is a suspension CHO cell, and

wherein the transposon sequences are nucleotide sequences derived from a pair of Tol1 transposons or nucleotide sequences derived from a pair of Tol2 transposons.

4. The method according to any one of claims 1 to 3, wherein the CHO cell is at least one selected from CHO-K1, CHO-K1SV, DUKXB11, CHO/DG44, Pro-3 and CHO-S.

5. The method according to any one of claims 1 to 3, wherein the selectable marker gene is a cycloheximide resistance gene.

6. The method according to claim 5, wherein the cycloheximide resistance gene is a gene encoding a mutant of human ribosomal protein L36a.

7. The method according to claim 6, wherein the mutant is a mutant in which proline at position 54 of the human ribosomal protein L36a is substituted with other amino acid.

8. The method according to claim 7, wherein the other amino acid is glutamine.

9. The method according to any one of claims 1 to 3, wherein the nucleotide sequences derived from a pair of Tol2 transposons are a nucleotide sequence comprising the nucleotide sequence shown in SEQ ID NO:2 and the nucleotide sequence shown in SEQ ID NO:3.

10. The method according to any one of claims 1 to 3, wherein the nucleotide sequences derived from a pair of Tol1 transposons are the nucleotide sequence shown in SEQ ID NO:14 and the nucleotide sequence shown in SEQ ID NO:15.

11. A suspension mammalian cell capable of producing a protein of interest, into which a protein expression vector comprising a gene fragment comprising a DNA encoding a protein of interest and a selectable marker gene and transposon sequences at both terminals of the gene fragment is introduced, to integrate the gene fragment inserted between a pair of the transposon sequences into a chromosome,

wherein the cell is a CHO cell capable of surviving and proliferating in a serum-free medium, and,

wherein the transposon sequences are nucleotide sequences derived from a pair of Tol1 transposons or nucleotide sequences derived from a pair of Tol2 transposons.

12. A suspension mammalian cell capable of producing a protein of interest, into which an expression vector (a) comprising a gene fragment comprising a DNA encoding a protein of interest and a selectable marker gene and transposon sequences at both terminals of the gene fragment, and an expression vector (b) comprising a DNA encoding a transposase (a transferase) which recognizes the transposon sequences and has activity of transferring the gene fragment inserted between a pair of the transposon sequences into a chromosome to integrate the gene fragment inserted between a pair of the transposon sequences into the chromosome,

wherein the cell is a CHO cell capable of surviving and proliferating in a serum-free medium, and,

wherein the transposon sequences are nucleotide sequences derived from a pair of Tol1 transposons or nucleotide sequences derived from a pair of Tol2 transposons.

13. The cell according to claim 11 or 12, wherein the CHO cell is at least one selected from CHO-K1, CHO-K1SV, DUKXB11, CHO/DG44, Pro-3 and CHO-S.

14. The cell according to claim 11 or 12, wherein the selectable marker gene is a cycloheximide resistance gene.

15. The cell according to claim 14, wherein the cycloheximide resistance gene is a gene encoding a mutant of human ribosomal protein L36a.

16. The cell according to claim 15, wherein the mutant is a mutant in which proline at position 54 of the human ribosomal protein L36a is substituted with other amino acid.

17. The cell according to claim 16, wherein the other amino acid is glutamine.

18. The cell according to claim 11 or 12, wherein the nucleotide sequences derived from a pair of the Tol2 transposons are the nucleotide sequence shown in SEQ ID NO:2 and the nucleotide sequence shown in SEQ ID NO:3.

19. The cell according to claim 11 or 12, wherein the nucleotide sequences derived from a pair of the Tol1 transposons are the nucleotide sequence shown in SEQ ID NO:14 and the nucleotide sequence shown in SEQ ID NO:15.

~~20. A protein expression vector for introducing into suspension mammalian cell, comprising a gene fragment comprising a DNA encoding a protein of interest and a selectable marker gene, and a pair of transposon sequences at both terminals of the gene fragment,~~

~~wherein a pair of the transposon sequences are nucleotide sequences derived from a pair of Tol1 transposons or nucleotide sequences derived from a pair of Tol2 transposons.~~

~~21. The protein expression vector for introducing into suspension mammalian cell according to claim 20, wherein the nucleotide sequences derived from a pair of the Tol2 transposons are the nucleotide sequence shown in SEQ ID NO:2 and the nucleotide sequence shown in SEQ ID NO:3.~~

~~22. The protein expression vector for introducing into suspension mammalian cell according to claim 20, wherein the nucleotide sequences derived from a pair of the Tol1 transposons are the nucleotide sequence shown in SEQ ID NO:14 and the nucleotide sequence shown in SEQ ID NO:15.~~