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

~~1.~~ 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

~~3.~~ 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 suspension mammalian cell is a cell capable of surviving and proliferating in a serum-free medium.~~

~~5. The method according to any one of claims 1 to 4, wherein the suspension mammalian cell is at least one selected from a suspension CHO cell in which a CHO cell is adapted to suspension culture, a PER.C6 cell, a rat myeloma cell YB2/3HL.P2.G11.16Ag.20 (or also called YB2/0) and a suspension mouse myeloma cell NS0 adapted to suspension culture.~~

~~6.~~4. The method according to any one of claims 1 to 3 ~~claim 5~~, wherein the CHO cell is at least one selected from CHO-K1, CHO-K1SV, DUKXB11, CHO/DG44, Pro-3 and CHO-S.

~~7.~~5. The method according to any one of claims 1 to 3~~6~~, wherein the selectable marker gene is a cycloheximide resistance gene.

~~8.~~6. The method according to claim 5~~7~~, wherein the cycloheximide resistance gene is a gene encoding a mutant of human ribosomal protein L36a.

~~9.~~7. The method according to claim 6~~8~~, wherein the mutant is a mutant in which proline at position 54 of the human ribosomal protein L36a is substituted with other amino acid.

~~10.~~8. The method according to claim 7~~9~~, wherein the other amino acid is glutamine.

~~11. The method according to any one of claims 1 to 10, wherein a pair of the transposon sequences are nucleotide sequences derived from a pair of DNA-type transposons which function in a mammalian cell.~~

~~12. The method according to claim 11, wherein the nucleotide sequences derived from a pair of DNA-type transposons are nucleotide sequences derived from a~~

~~pair of Tol1 transposons or nucleotide sequences derived from a pair of Tol2 transposons.~~

~~13.~~9. The method according to any one of claims 1 to 3 ~~claim 12~~, 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.

~~14.~~10. The method according to any one of claims 1 to 3 ~~claim 12~~, 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.

~~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.

~~16.~~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.

~~17. The cell according to claim 15 or 16, wherein the cell is a cell capable of surviving and proliferating in a serum-free medium.~~

~~18. The cell according to any one of claims 15 to 17, wherein the cell is at least one suspension mammalian cell selected from a suspension CHO cell in which a CHO cell is adapted to suspension culture, a PER.C6 cell, a rat myeloma cell YB2/3HL.P2.G11.16Ag.20 (or also called YB2/0) and a suspension mouse myeloma cell NS0 adapted to suspension culture.~~

~~19.~~13. The cell according to claim 11 or 12~~18~~, wherein the CHO cell is at least one selected from CHO-K1, CHO-K1SV, DUKXB11, CHO/DG44, Pro-3 and CHO-S.

~~20.~~14. The cell according to claim 11 or 12 ~~any one of claims 15 to 19~~, wherein the selectable marker gene is a cycloheximide resistance gene.

~~21.~~15. The cell according to claim 14~~20~~, wherein the cycloheximide resistance gene is a gene encoding a mutant of human ribosomal protein L36a.

~~22.~~16. The cell according to claim 15~~21~~, wherein the mutant is a mutant in which proline at position 54 of the human ribosomal protein L36a is substituted with other amino acid.

~~23.~~17. The cell according to claim 16~~22~~, wherein the other amino acid is glutamine.

~~24. The cell according to any one of claims 15 to 23, wherein a pair of the transposon sequences are nucleotide sequences derived from a pair of DNA-type transposons which function in a mammalian cell.~~

~~25. The cell according to claim 24, wherein the nucleotide sequences derived from a pair of the DNA-type transposons are nucleotide sequences derived from a pair of Tol1 transposons or nucleotide sequences derived from a pair of Tol2 transposons.~~

~~26.~~18. The cell according to claim 11 or 12~~25~~, 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.

~~27.~~19. The cell according to claim 11 or 12~~25~~, 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.

~~28.~~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.

29. The protein expression vector according to claim 28, 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.

30.21. The protein expression vector for introducing into suspension mammalian cell according to claim 2029, 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.

31.22. The protein expression vector for introducing into suspension mammalian cell according to claim 2029, 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.