Claims

[Claim 1]

A method for producing a protein of interest, comprising introducing at least one expression vector which comprises a gene fragment comprising a DNA encoding a protein of interest and also comprises a pair of transposon sequences at both terminals of the gene fragment, into a suspension mammalian cell; integrating the gene fragment comprising the DNA encoding the protein of interest inserted between a pair of the transposon sequences into a chromosome of the mammalian cell to obtain a mammalian cell which expresses the protein of interest; and suspension-culturing the mammalian cell,

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

wherein the pair of the transposon sequences are nucleotide sequences derived from a pair of Tol2 transposons which function in a mammalian cell.

[Claim 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:

(a) at least one expression vector which comprises a gene fragment comprising a DNA encoding a protein of interest and also comprises a pair of 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 obtaining a suspension mammalian cell which expresses the protein of interest by expressing transiently the transposase from the expression vector which is introduced into the suspension mammalian cell in the step (A) to integrate the gene fragment inserted between a pair of the transposon sequences into a chromosome of the mammalian cell, and

(C) a step of suspension-culturing the suspension mammalian cell which expresses 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 which is capable of surviving and proliferating in a serum-free medium,

wherein the pair of the transposon sequences are nucleotide sequences derived from a pair of Tol2 transposons which function in a mammalian cell.

[Claim 3]

A method for obtaining a suspension mammalian cell which expresses a protein of interest, comprising introducing at least one expression vector which comprises a gene fragment comprising a DNA encoding a protein of interest and also comprises transposon sequences at both terminals of the gene fragment into a suspension mammalian cell, 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 which is capable of surviving and proliferating in a serum-free medium,

wherein the pair of the transposon sequences are nucleotide sequences derived from a pair of Tol2 transposons which function in a mammalian cell.

[Claim 4]

The method according to any one of claims 1 to 3, wherein at least one of the expression vectors which comprises a gene fragment comprising a DNA encoding the protein of interest and also comprises a pair of transposon sequences at both terminals of the gene fragment is an expression vector which comprises a gene fragment comprising a DNA encoding the protein of interest and a selectable marker gene and also comprises a pair of transposon sequences at both terminals of the gene fragment.

[Claim 5]

The method according to any one of claims 1 to 4, comprising introducing an expression vector which comprises a gene fragment comprising a selectable marker and comprises a pair of transposon sequences at both terminals of the gene fragment into a mammalian cell in addition to the expression vector which comprises a gene fragment comprising a DNA encoding the protein of interest and also comprises a pair of transposon sequences at both terminals of the gene fragment.

[Claim 6]

The method according to any one of claims 1 to 5, wherein the DNA encoding the protein of interest is a DNA encoding an antibody.

[Claim 7]

The method according to any one of claim 6, wherein the DNA encoding an antibody is at least one of a DNA encoding a H chain of the antibody and a DNA encoding a L chain of the antibody.

[Claim 8]

The method according to any one of claims 4 to 7, wherein an expression vector selected from the following (a) to (d) is introduced into a suspension mammalian cell:

(a) an expression vector which comprises a gene fragment comprising a DNA encoding a H chain of an antibody and also comprises a pair of transposon sequences at both terminals of the gene fragment, an expression vector which comprises a gene fragment comprising a DNA encoding a L chain of an antibody and also comprises a

pair of transposon sequences at both terminals of the gene fragment and an expression vector which comprises a gene fragment comprising a selectable marker gene and also comprises a pair of transposon sequences at both terminals of the gene fragment

(b) an expression vector which comprises a gene fragment comprising a DNA encoding a H chain of an antibody and a selectable marker gene and also comprises a pair of transposon sequences at both terminals of the gene fragment and an expression vector which comprises a gene fragment comprising a DNA encoding a L chain of an antibody and also comprises a pair of transposon sequences at both terminals of the gene fragment,

(c) an expression vector which comprises a gene fragment comprising a DNA encoding a L chain of an antibody and a selectable marker gene and also comprises a pair of transposon sequences at both terminals of the gene fragment and an expression vector which comprises a gene fragment comprising a DNA encoding a H chain of an antibody and also comprises a pair of transposon sequences at both terminals of the gene fragment, and

(d) an expression vector which comprises a gene fragment comprising a DNA encoding a H chain and a L chain of an antibody and a selectable marker gene and also comprises a pair of transposon sequences at both terminals of the gene fragment.

~~[Claim 9]~~

~~The method according to any one of claims 1 to 8, 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.~~

[Claim 9~~10~~]

The method according to claims 1-8~~9~~, wherein the CHO cell is at least one selected from CHO-K1, CHO-K1SV, DUKXB 11, CHO/DG44, Pro-3 and CHO-S.

[Claim 10~~11~~]

The method according to any one of claims 4 to 9~~10~~, wherein the selectable marker gene is a cycloheximide resistance gene.

[Claim 11~~12~~]

The method according to any one of claim 10~~11~~, wherein the cycloheximide resistance gene is a ribosome protein.

[Claim 12~~13~~]

The method according to claims 1-11, 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.