CLAIMS

1. A method for establishing a cell line for recombinant protein production capable of stably expressing two or more foreign genes, comprising transferring a gene of a non-coding RNA suppressing the expression of NfkBia to a cell so that the gene is expressed.

2. The method according to claim 1, wherein the two or more foreign genes are genes encoding proteins that are difficult to express stably at the same time.

3. The method according to claim 2, wherein all of the proteins that are difficult to express stably at the same time belong to proteins having similar functions.

4. The method according to claim 3, wherein the proteins having similar functions are transporters or metabolic enzymes.

5. The method according to any of claims 1 to 4, wherein the method comprises transferring genes of two or more transporters to the cell.

6. The method according to any of claims 1 to 4, wherein the method comprises transferring genes of two or more metabolic enzymes to the cell.

7. The method according to any of claims 1 to 4, wherein the method comprises transferring genes of TauT and two metabolic enzymes to the cell.

8. A method for constructing a cell with a short doubling time, comprising transferring a DNA construct comprising a gene of a non-coding RNA suppressing the expression of NfkBia to a cell so that the non-coding RNA is expressed within the

cell.

9. A cell in which a gene of a non-coding RNA suppressing the expression of NfkBia is expressed by the transfer of the gene to the cell while two or more foreign genes are stably expressed.

10. The method according to any of claims 1 to 8 or the cell according to claim 9, wherein the non-coding RNA suppressing the expression of NfkBia is a small RNA up to 30 bases long comprising a consecutive sequence of 19 to 25 bases in length, the small RNA being capable of binding to a portion of NfkBia mRNA by base pairing.

11. The method according to any of claims 1 to 8 or the cell according to claim 9, wherein the non-coding RNA suppressing the expression of NfkBia is an mRNA-type non-coding RNA up to 561 bases long comprising a consecutive sequence of 19 to 25 bases in length, the mRNA-type non-coding RNA being capable of binding to a portion of NfkBia mRNA by base pairing.

12. The method according to any of claims 1 to 8 or the cell according to claim 9, wherein the non-coding RNA suppressing the expression of NfkBia is an mRNA-type non-coding RNA 561 to 1579 bases long comprising a consecutive sequence of 19 to 25 bases in length, the mRNA-type non-coding RNA being capable of binding to a portion of NfkBia mRNA by base pairing.

13. The method or the cell according to any of claims 10 to 12, wherein the consecutive sequence of 19 to 25 bases in length is an arbitrary partial sequence in the nucleotide sequence represented by SEQ ID NO: 2, or a complementary sequence thereof.

14. The method according to any of claims 1 to 8 or the cell according to claim 9, wherein the gene of a non-coding RNA suppressing the expression of NfkBia has a nucleotide sequence represented by any of SEQ ID NOs: 1 to 16 and 29.

15. The method according to any of claims 1 to 8 or the cell according to claim 9, wherein the gene of a non-coding RNA suppressing the expression of NfkBia is a DNA having the following nucleotide sequence or a complementary sequence thereof:

(a) a sequence capable of binding to a nucleotide sequence represented by any of SEQ ID NOs: 1 to 16 and 29 by base pairing;

(b) a nucleotide sequence identical to a nucleotide sequence represented by any of SEQ ID NOs: 1 to 16 and 29 except for 1 or several bases;

(c) a partial sequence of the 3'-untranslated region of the NfkBia gene, comprising a nucleotide sequence represented by any of SEQ ID NOs: 1 to 16 and 29; or

(d) a sequence consisting of a nucleotide sequence identical to the sequence (c) except for 1 or several bases.

16. The method according to any of claims 1 to 8 or the cell according to claim 9, wherein a gene of a desired protein is further transferred to the cell.

17. The method or the cell according to claim 16, wherein the desired protein is an antibody.

18. The method for establishing a cell line for recombinant protein production capable of stably expressing two or more foreign genes according to claim 1, wherein the method

comprises:

transferring a first foreign gene and the gene of a non-coding RNA suppressing the expression of NfkBia to the cell so that these genes are expressed; and

transferring a second foreign gene that is difficult to express stably at the same time with the first foreign gene, to the cell, wherein

the first and second foreign genes are protein-encoding genes, and

the established cell line for recombinant protein production expresses the gene of a non-coding RNA suppressing the expression of NfkBia, and the protein-encoding first and second foreign genes.

19. The method for establishing a cell line for recombinant protein production capable of stably expressing two or more foreign genes according to claim 1, wherein the method comprises:

transferring a first foreign gene and the gene of a non-coding RNA suppressing the expression of NfkBia to the cell so that these genes are expressed;

transferring a second foreign gene to the cell; and

transferring a third foreign gene that is difficult to express stably at the same time with the second foreign gene, to the cell, wherein

the first, second, and third foreign genes are protein-encoding genes, and

the established cell line for recombinant protein production expresses the gene of a non-coding RNA suppressing

the expression of NfkBia, and the protein-encoding first, second, and third foreign genes.

20. The method according to claim 18 or 19, wherein the first foreign gene is a taurine transporter (TAUT) gene.

21. The method according to any of claims 18 to 20, wherein a gene of a desired protein is further transferred to the cell to establish a host cell line for the production of the desired protein.

Claims

[Claim 1]

A method for establishing a cell line for recombinant protein production capable of stably expressing two or more transporters or two or more metabolic enzymes, comprising transferring a gene of a non-coding RNA suppressing the expression of NfkBia to a cell so that the gene is expressed.

[Claim 2]

The method according to claim 1, wherein the method comprises transferring genes of two or more transporters to the cell.

[Claim 3]

The method according to claim 1, wherein the method comprises transferring genes of two or more metabolic enzymes to the cell.

[Claim 4]

The method according to claim 1, wherein the method comprises transferring genes of TauT and two metabolic enzymes to the cell.

[Claim 5]

A method for constructing a cell with a short doubling time, comprising transferring a DNA construct comprising a gene of a non-coding RNA suppressing the expression of NfkBia to a cell so that the non-coding RNA is expressed within the cell.

[Claim 6]

A cell in which a gene of a non-coding RNA suppressing the expression of NfkBia is expressed by the transfer of the gene to the cell while two or more foreign genes are stably expressed, wherein said foreign genes are genes of two or more transporters or genes of two or more metabolic enzymes.

[Claim 7]

The method according to any of claims 1 to 5 or the cell according to claim 6, wherein the non-coding RNA suppressing the expression of NfkBia is a small RNA up to 30 bases long comprising a consecutive sequence of 19 to 25 bases in length, the small RNA being capable of binding to a portion of NfkBia mRNA by base pairing.

[Claim 8]

The method according to any of claims 1 to 5 or the cell according to claim 6, wherein the non-coding RNA suppressing the expression of NfkBia is an mRNA-type non-coding RNA up to 561 bases long comprising a consecutive sequence of 19 to 25 bases in length, the mRNA-type non-coding RNA being capable of binding to a portion of NfkBia mRNA by base pairing.

[Claim 9]

The method according to any of claims 1 to 5 or the cell according to claim 6, wherein the non-coding RNA suppressing the expression of NfkBia is an mRNA-type non-coding RNA 561 to 1579 bases long comprising a consecutive sequence of 19 to 25 bases in length, the mRNA-type non-coding RNA being capable of binding to a portion of NfkBia mRNA by base pairing.

[Claim 10]

The method or the cell according to any of claims 7 to 9, wherein the consecutive sequence of 19 to 25 bases in length is an arbitrary partial sequence in the nucleotide sequence represented by SEQ ID NO: 2, or a complementary sequence thereof.

[Claim 11]

The method according to any of claims 1 to 5 or the cell according to claim 6, wherein the gene of a non-coding RNA suppressing the expression of NfkBia has a nucleotide sequence represented by any of SEQ ID NOs: 1 to 16 and 29.

[Claim 12]

The method according to any of claims 1 to 5 or the cell according to claim 6, wherein the gene of a non-coding RNA suppressing the expression of NfkBia is a DNA having the following nucleotide sequence or a complementary sequence thereof:

(a) a sequence capable of binding to a nucleotide sequence represented by any of SEQ ID NOs: 1 to 16 and 29 by base pairing;

(b) a nucleotide sequence identical to a nucleotide sequence represented by any of SEQ ID NOs: 1 to 16 and 29 except for 1 base;

(c) a partial sequence of the 3'-untranslated region of the NfkBia gene, comprising a nucleotide sequence represented by any of SEQ ID NOs: 1 to 16 and 29; or

(d) a sequence consisting of a nucleotide sequence identical to the sequence (c) except for 1 base.

[Claim 13]

The method according to any of claims 1 to 5 or the cell according to claim 6, wherein a gene of a desired protein is further transferred to the cell.

[Claim 14]

The method or the cell according to claim 13, wherein the desired protein is an antibody.

[Claim 15]

The method for establishing a cell line for recombinant protein production capable of stably expressing two or more foreign genes according to claim 1, wherein the method comprises:

transferring a first foreign gene encoding a protein, and the gene of a non-coding RNA suppressing the expression of NfkBia to the cell so that these genes are expressed; and

transferring a second foreign gene encoding a protein to the cell, wherein the first and second foreign genes encode transporters, and

the established cell line for recombinant protein production expresses the gene of a

non-coding RNA suppressing the expression of NfkBia, and the protein-encoding first and second foreign genes.

[Claim 16]

The method for establishing a cell line for recombinant protein production capable of stably expressing two or more foreign genes according to claim 1, wherein the method comprises:

transferring a first foreign gene encoding a protein and the gene of a non-coding RNA suppressing the expression of NfkBia to the cell so that these genes are expressed;

transferring a second foreign gene encoding a protein to the cell; and

transferring a third foreign gene encoding a protein to the cell, wherein

the second and third foreign genes encode metabolic enzymes, and

the established cell line for recombinant protein production expresses the gene of a non-coding RNA suppressing the expression of NfkBia, and the protein-encoding first, second, and third foreign genes.

[Claim 17]

The method according to claim 15 or 16, wherein the first foreign gene is a taurine transporter (TAUT) gene.

[Claim 18]

The method according to any of claims 15 to 17, wherein a gene of a desired protein is further transferred to the cell to establish a host cell line for the production of the desired protein.