{CLAIMS}

{Claim 1}

A method of producing an induced pluripotent stem cell, comprising the step of introducing at least one kind of non-viral expression vector incorporating at least one gene that encodes a reprogramming factor into a somatic cell.

{Claim 2}

The method of claim 1, wherein the vector is a non-viral expression vector autonomously replicable outside a chromosome.

{Claim 3}

The method of claim 1 or 2, wherein the vector is a plasmid vector.

{Claim 4}

The method of any one of claims 1 to 3, wherein the gene that encodes a reprogramming factor is one or more selected from the genes testing positive as determined by a nuclear reprogramming factor screening method described in WO 2005/80598.

{Claim 5}

The method of any one of claims 1 to 4, wherein the gene that encodes a reprogramming factor is one or more kind of genes selected from the group consisting of an Oct family gene, a Klf family gene, a Sox family gene, a Myc family gene, a Lin family gene, and the Nanog gene.

{Claim 6}

The method of any one of claims 1 to 5, wherein the gene that encodes a reprogramming factor is one of the following combinations:

(a) a combination of two kinds of genes consisting of an Oct family gene and a Sox family gene;

(b) a combination of three kinds of genes consisting of an Oct family gene, a Klf family gene, and a Sox family gene;

(c) a combination of four kinds of genes consisting of an Oct family gene, a Klf family gene, a Sox family gene, and a Myc family gene;

(d) a combination of four kinds of genes consisting of an Oct family gene, a Sox family gene, a Lin family gene, and the Nanog gene; and

(e) a combination of six kinds of genes consisting of an Oct family gene, a Sox family gene, a Klf family gene, a Myc family gene, a Lin family gene, and the Nanog gene,

or any one of these combinations further comprising the TERT gene and/or the SV40 Large T antigen gene.

{Claim 7}

The method of any one of claims 1 to 6, wherein the gene that encodes a reprogramming factor is one of the following combinations:

(a) a combination of two kinds of genes consisting of Oct3/4 and Sox2;

(b) a combination of three kinds of genes consisting of Oct3/4, Klf4, and Sox2;

(c) a combination of four kinds of genes consisting of Oct3/4, Klf4, Sox2, and c-Myc;

(d) a combination of four kinds of genes consisting of Oct3/4, Sox2, Lin28, and Nanog; and

(e) a combination of six kinds of genes consisting of Oct3/4, Sox2, Klf4, c-Myc, Lin28, and Nanog,

or any one of these combinations further comprising the TERT gene and/or the SV40 Large T antigen gene.

{Claim 8}

The method of any one of claims 1 to 7, wherein the number of kinds of non-viral expression vectors introduced into the somatic cell is 1, 2, 3, or 4.

{Claim 9}

The method of claim 8, wherein the gene that encodes a reprogramming factor is a combination of three kinds of genes consisting of an Oct family gene, a Klf family gene, and a Sox family gene, or a combination of four kinds of genes consisting of an Oct family gene, a Klf family gene, a Sox family gene, and a Myc family gene, wherein the Oct family gene, the Klf

family gene, and the Sox family gene are incorporated in one kind of non-viral expression vector.

{Claim 10}

The method of claim 9, wherein the Oct family gene, the Klf family gene, and the Sox family gene are incorporated in one kind of non-viral expression vector in this order in the orientation from the 5′ to 3′ end.

{Claim 11}

The method of claim 9 or 10, wherein the Oct family gene, the Klf family gene, and the Sox family gene are incorporated in one kind of non-viral expression vector with an intervening sequence enabling polycistronic expression.

{Claim 12}

The method of any one of claims 9 to 11, wherein the Oct family gene is Oct3/4, the Klf family gene is Klf4, the Sox family gene is Sox2, and the Myc family gene is c-Myc.

{Claim 13}

The method of any one of claims 8 to 12, wherein a first non-viral expression vector and a second non-viral expression vector are concurrently introduced into a somatic cell.

{Claim 14}

The method of claim 13, wherein said introduction is repeatedly performed twice or more.

{Claim 15}

The method of any one of claims 1 to 14, wherein the somatic cell is a somatic cell of a mammal, including a human.

{Claim 16}

An induced pluripotent stem cell that can be obtained by the method of any one of claims 1 to 15.

{Claim 17}

The induced pluripotent stem cell of claim 16, wherein whole or part of the at least one non-viral expression vector introduced is substantially not integrated in the chromosome.

{Claim 18}

A non-viral expression vector incorporating a gene that

encodes at least one reprogramming factor.

{Claim 19}

The vector of claim 18, wherein the vector is a plasmid vector.

{Claim 20}

The vector of claim 18 or 19, wherein an Oct family gene, a Klf family gene, and a Sox family gene are incorporated.

{Claim 21}

The vector of claim 20, wherein the Oct family gene, the Klf family gene, and the Sox family gene are incorporated in this order in the orientation from the 5′ to 3′ end.

{Claim 22}

The vector of claim 21, wherein the Oct family gene, the Klf family gene, and the Sox family gene are incorporated via a sequence enabling polycistronic expression.

{Claim 23}

The vector of any one of claims 20 to 22, wherein the Oct family gene is Oct3/4, the Klf family gene is Klf4, and the Sox family gene is Sox2.

{Claim 24}

An induced pluripotent stem cell wherein one or more transgenes encoding reprogramming factor are integrated into the cellular genome in the form of plasmid.

{Claim 25}

An induced pluripotent stem cell wherein no exogenous genes induced is integrated into the cellular genome.

{Claim 26}

A use of the 2A sequence derived from foot-and-mouth disease virus for preparing a non-viral expression vector for the induction of an induced pluripotent stem cell, harboring two or more kinds of reprogramming factors.

Claims

1. A method of producing an induced pluripotent stem cell, comprising the step of introducing genes that encode reprogramming factors concurrently into a somatic cell by one or more kinds of plasmid vector,
wherein the genes that encode reprogramming factors comprise an Oct 3/4 gene, a Klf family gene, and Sox family gene, and
wherein whole or part of the genes introduced is not integrated in the chromosome.

2. The method of claim 1, wherein the plasmid vector is autonomously replicable outside a chromosome.

3. The method of claim 1 or 2, the genes that encode reprogramming factors are expressed for at least 6 days.

4. The method of any one of claims 1 to 3, wherein the number of kinds of plasmid vectors introduced into the somatic cell is 1 or 2.

5. The method of any one of claims 1 to 4, wherein the first kind of plasmid vector incorporates at least two kinds of genes selected from the genes that encode reprogramming factors ligated via 2A sequence derived from foot-and-mouth disease virus.

6. The method of any one of claims 1 to 5, wherein the Oct 3/4 gene, the KIf family gene, and the Sox family gene are incorporated in the first kind of plasmid vector.

7. The method of any one of claims 1 to 6, wherein the Sox family gene is selected from the group consisting of Sox1 gene,Sox2 gene, Sox3 gene, Sox15 gene, Sox17 gene, and Sox18 gene, and
wherein the Klf family gene is selected from the group consisting of Klf1 gene, Klf2 gene, Klf4 gene and Klf5 gene.

8. The method of any one of claims 1 to 6, wherein the KIf family gene is KIf4 gene, and the Sox family gene is Sox2 gene.

9. The method of any one of claims 1 to 8, wherein the genes that encode reprogramming factors further comprises Myc family gene.

10. The method of claim 9, wherein the Myc family gene is selected from the group consisting of c-Myc gene, N-Myc gene, L-Myc gene and T58A gene.

11. The method of claim 10, wherein the Myc family gene is c-Myc gene.

12. The method any one of claims 9 to 11, wherein Myc family gene is introduced into the somatic cell by the second kind of plasmid vector.

13. The method any one of claims 1 to 12, wherein said introduction is repeatedly performed twice or more.

14. The method of any one of claims 1 to 13, wherein the somatic cell is a somatic cell of a mammal, including a human.