

(19) **DANMARK**



Patent- og
Varemærkestyrelsen

(10) **DK/EP 3050961 T5**

(12) **Rettet oversættelse af
europæisk patentskrift**

-
- (51) Int.Cl.: **C 12 N 5/10 (2006.01)** **C 12 N 5/074 (2010.01)** **C 12 N 15/09 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2024-10-14**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2023-06-28**
- (86) Europæisk ansøgning nr.: **14847286.3**
- (86) Europæisk indleveringsdag: **2014-09-24**
- (87) Den europæiske ansøgnings publiceringsdag: **2016-08-03**
- (86) International ansøgning nr.: **JP2014075248**
- (87) Internationalt publikationsnr.: **WO2015046229**
- (30) Prioritet: **2013-09-24 JP 2013197308**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
- (73) Patenthaver: **ID Pharma Co., Ltd., 2-10-2 Fujimi , Chiyoda-ku, Tokyo 102-0071, Japan**
- (72) Opfinder: **BAN, Hiroshi, c/o DNAVEC CORPORATION, 6 Ohkubo, Tsukuba-shi, Ibaraki 300-2611, Japan**
IIDA, Akihiro, c/o DNAVEC CORPORATION, 6 Ohkubo, Tsukuba-shi, Ibaraki 300-2611, Japan
HASEGAWA, Mamoru, c/o DNAVEC CORPORATION, 6 Ohkubo, Tsukuba-shi, Ibaraki 300-2611, Japan
- (74) Fuldmægtig i Danmark: **Budde Schou A/S, Dronningens Tværgade 30, 1302 København K, Danmark**
- (54) Benævnelse: **Fremgangsmåde til forbedring af effektiviteten ved inducering af pluripotente stamceller**
- (56) Fremdragne publikationer:
EP-A1- 2 612 911
WO-A1-2010/008054
WO-A1-2012/029770
JP-A- 2013 519 371
OKITA K ET AL: "Generation of Mouse Induced Pluripotent Stem Cells Without Viral Vectors", SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, 9 October 2008 (2008-10-09), XP009107934, ISSN: 0036-8075, DOI: 10.1126/SCIENCE.1164270
LIN YE ET AL: "Blood Cell-Derived Induced Pluripotent Stem Cells Free of Reprogramming Factors Generated by Sendai Viral Vectors", STEM CELLS TRANSLATIONAL MEDICINE : SCTM, vol. 2, no. 8, 1 August 2013 (2013-08-01), pages 558-566, XP055338729, Durham ISSN: 2157-6564, DOI: 10.5966/sctm.2013-0006
YASUMITSU FUJIE ET AL: "New Type of Sendai Virus Vector Provides Transgene-Free iPS Cells Derived from Chimpanzee Blood", PLOS ONE, vol. 9, no. 12, 5 December 2014 (2014-12-05), page e113052, XP055338713, DOI: 10.1371/journal.pone.0113052
JUAN A. BERNAL: "RNA-Based Tools for Nuclear Reprogramming and Lineage-Conversion: Towards Clinical Applications", JOURNAL OF CARDIOVASCULAR TRANSLATIONAL RESEARCH, vol. 6, no. 6, 13 July 2013 (2013-07-13), pages 956-968, XP055338881, Boston ISSN: 1937-5387, DOI: 10.1007/s12265-013-9494-8
BAN, H. ET AL.: 'Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by

Fortsættes ...

temperature-sensitive Sendai virus vectors' PNAS vol. 108, no. 34, 2011, pages 14234 - 14239, XP002684868
JITSUTARO KAWAGUCHI ET AL.: 'Yori Shinpo shita Taisaibo Reprogramming-ho: Sendai Virus Vector (CytoTune-iPS) ni yoru Shinpo' THE CELL vol. 46, no. 5, May 2014, pages 29 - 30, XP008184899
LIN YE ET AL: "Blood Cell-Derived Induced Pluripotent Stem Cells Free of Reprogramming Factors Generated by Sendai Viral Vectors", STEM CELLS TRANSLATIONAL MEDICINE : SCTM, vol. 2, no. 8, 1 August 2013 (2013-08-01), Durham, pages 558 - 566, XP055338729, ISSN: 2157-6564, DOI: 10.5966/sctm.2013-0006
YASUMITSU FUJIE ET AL: "New Type of Sendai Virus Vector Provides Transgene-Free iPS Cells Derived from Chimpanzee Blood", PLOS ONE, vol. 9, no. 12, 5 December 2014 (2014-12-05), pages e113052, XP055338713, DOI: 10.1371/journal.pone.0113052
JUAN A. BERNAL: "RNA-Based Tools for Nuclear Reprogramming and Lineage-Conversion: Towards Clinical Applications", JOURNAL OF CARDIOVASCULAR TRANSLATIONAL RESEARCH, vol. 6, no. 6, 13 July 2013 (2013-07-13), Boston, pages 956 - 968, XP055338881, ISSN: 1937-5387, DOI: 10.1007/s12265-013-9494-8
BAN, H. ET AL.: "Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors", PNAS, vol. 108, no. 34, 2011, pages 14234 - 14239, XP002684868
JITSUTARO KAWAGUCHI ET AL.: "Yori Shinpo shita Taisaibo Reprogramming-ho: Sendai Virus Vector (CytoTune-iPS) ni yoru Shinpo", THE CELL, vol. 46, no. 5, May 2014 (2014-05-01), pages 29 - 30, XP008184899

DESCRIPTION

Technical Field

[0001] The present invention relates to methods for improving efficiency of the induction of pluripotent stem cells. Furthermore, the present invention relates to gene delivery vectors and gene delivery compositions for use in the induction of pluripotent stem cells, and uses thereof. Specifically, the present invention relates to techniques for enhancing the efficiency of inducing pluripotent stem cells, in the induction of pluripotent stem cells which contains the step of introducing a vector that contains the KLF gene, OCT gene and SOX gene in one vector in this order.

Background Art

[0002] Since the reported induction of pluripotent stem cells (induced pluripotent stem (iPS) cells; also called as "artificial pluripotent stem cells" or "induced pluripotent stem cells") from somatic cells (Non-Patent Document 1), iPS cells have been produced by introducing reprogramming factors into various mammalian cells including human and mouse cells (Non-Patent Documents 1 to 9). Many of them use retrovirus vectors to introduce reprogramming factors. However, since retrovirus vectors carry the risk of tumorigenesis as a result of integration into the host genome, their use is limited (Non-Patent Document 3). To solve this problem, attempts of inducing iPS cells using adenovirus vectors or plasmids have been made, but as long as DNA-type vectors are used, it is impossible to completely remove concerns over integration into the genome. Furthermore, the induction efficiency of iPS cells by these vectors is extremely low (Non-Patent Documents 10 to 13).

[0003] To solve these problems, the present inventors have previously developed a system for inducing iPS cells using a Sendai virus vector which is an RNA-type virus (Patent Documents 1 and 2). Induction efficiency of iPS cells using the Sendai virus vector was significantly higher than that in previous cases using other vectors. Since the Sendai virus vectors do not have a DNA phase during their lifecycle, and therefore there is no concern that they will become integrated into the host genome, they are excellent in terms of safety. Also, the vectors can be easily removed after induction of iPS cells. However, techniques of the present invention for further increasing the induction efficiency of iPS cells are not known.

Prior Art Documents

[Patent Documents]

[0004]

[Patent Document 1] International Publication No. WO 2010/008054

[Patent Document 2] International Publication No. WO 2012/029770

[Non-patent Documents]

[0005]

[Non-Patent Document 1] Takahashi, K. and Yamanaka, S. (2006) Cell 126, 663-676

[Non-Patent Document 2] Maherali, N. et al., (2007) Cell Stem Cell 1, 55-70

[Non-Patent Document 3] Okita, K. et al., (2007) Nature 448, 313-317

[Non-Patent Document 4] Wernig, M. et al., (2007) Nature 448, 318-324

[Non-Patent Document 5] Takahashi, K. et al., (2007) Cell 131, 861-872

[Non-Patent Document 6] Yu, J. et al., (2007) Science 318, 1917-1920

[Non-Patent Document 7] Lowry, W.E. et al., (2008) Proc. Natl. Acad. Sci. USA 105, 2883-2888

[Non-Patent Document 8] Park, I.H. et al., (2008) Nature 451, 141-146

[Non-Patent Document 9] Masaki, H. et al., (2008) Stem Cell Res. 1, 105-115

[Non-Patent Document 10] Stadtfeld, M. et al., (2008) Science 322, 945-949

[Non-Patent Document 11] Okita, K. et al., (2008) Science 322, 949-953

[Non-Patent Document 12] Yu, J. et al., (2009) Science 324, 797-801

[Non-Patent Document 13] Zhou, W. et al., (2009) stem cells 27, 2667-2674 EP2612911 discloses that the temperature sensitive K-O-S vector (KOS/TS12ΔF vector) was considerably inefficient at 37°C, and suggests performing a low-temperature cultivation step in view of said limited efficiency.

Summary of the Invention

[Problems to be Solved by the Invention]

[0006] The present invention relates to methods for improving efficiency of the induction of pluripotent stem cells. Furthermore, an objective of the present invention is to provide gene delivery vectors and gene delivery compositions for use in the induction of pluripotent stem cells, and uses thereof. Specifically, an objective of the present invention is to provide methods for enhancing the efficiency of producing pluripotent stem cells, and gene delivery vectors and compositions for use therein, in the induction of pluripotent stem cells which comprises the step of introducing a vector that comprises in one vector the KLF gene, OCT gene and SOX gene in this order.

[Means for Solving the Problems]

[0007] The present inventors searched for methods for further enhancing the efficiency of inducing pluripotent stem cells in the production of pluripotent stem cells which comprises the step of introducing a vector that comprises in one vector the KLF gene, OCT gene and SOX gene in this order (i.e., reprogramming factors). In particular, when a temperature-sensitive vector is used as this vector to induce pluripotent stem cells, although pluripotent stem cells are induced with a relatively high efficiency if cultured at a low temperature (36°C) after vector introduction, the induction efficiency of pluripotent stem cells may be significantly reduced if they are cultured at the standard cell culture temperature of 37°C. While the temperature-sensitive vector is superior in that the vector may be promptly removed after induction of pluripotent stem cells, the problem is that it is necessary to culture at a low temperature after vector introduction in order to efficiently induce such pluripotent stem cells.

[0008] The present inventors conducted dedicated research on methods that can induce pluripotent stem cells with high efficiency, by using a temperature-sensitive Sendai virus vector and not culturing at a low temperature after vector introduction. As a result, it was discovered that it is possible to significantly increase the efficiency of pluripotent stem cell induction in the introduction of a vector that comprises in one vector the KLF gene, OCT gene and SOX gene in this order without culturing at a low temperature, by further introducing a vector that comprises the KLF gene

but not the OCT gene and the SOX gene. Specifically, compared with when a vector comprising the KLF gene, OCT gene and SOX gene in one vector in this order is used in combination with only a vector expressing the MYC gene, iPS cells can be induced with remarkably high efficiency without low-temperature culturing by further combining these vectors with a vector that comprises the KLF gene but not the OCT gene and the SOX gene.

[0009] This allows high-efficiency induction of pluripotent stem cells without low-temperature cell culturing, and establishment of a system of rapid vector removal after induction of pluripotent stem cells.

[0010] That is, the present invention relates to methods of enhancing the efficiency of producing pluripotent stem cells, and gene delivery vectors and compositions and such for use therein, in the induction of pluripotent stem cells which contains the step of introducing a vector that contains in one vector the KLF gene, OCT gene, and SOX gene in this order, and more specifically to inventions described in each of the claims. Inventions consisting of any combination of two or more inventions described in claims that cite the same claim are also inventions intended herein. More specifically, the present invention relates to the following:

1. [1] an in vitro method of producing pluripotent stem cells, wherein said method comprises, introducing a temperature-sensitive Sendai virus vector that comprises in one vector the KLF gene, OCT gene, and SOX gene in this order, and a Sendai virus vector that comprises the KLF gene but not the OCT gene and the SOX gene, and culturing in the range of 36.5 to 37.5°C, wherein the temperature-sensitive Sendai virus vector comprising in one vector the KLF gene, OCT gene, and SOX gene in this order is an F gene-deleted Sendai virus vector that comprises G69E, T116A, and A183S mutations in the M protein, A262T, G264R, and K461G mutations in the HN protein, D433A, R434A, K437A, and L511F mutations in the P protein, and N1197S and K1795E mutations in the L protein, and wherein the Sendai virus vector that comprises the KLF gene but not the OCT gene and the SOX gene is an F gene-deleted Sendai virus vector that comprises G69E, T116A, and A183S mutations in the M protein, A262T, G264R, and K461G mutations in the HN protein, L511F mutation in the P protein, and N1197S and K1795E mutations in the L protein;
2. [2] a composition for inducing pluripotent stem cells, wherein the composition comprises: (a) a temperature-sensitive Sendai virus vector that comprises in one vector the KLF gene, OCT gene, and SOX gene in this order, wherein said vector is a temperature-sensitive Sendai virus vector comprising in one vector the KLF gene, OCT gene and SOX gene in this order, which is an F gene-deleted Sendai virus vector that comprises G69E, T116A and A183S mutations in the M protein, A262T, G264R and K461G mutations in the HN protein, D433A, R434A, K437A and L511F mutations in the P protein, and N1197S and K1795E mutations in the L protein; and (b) a Sendai virus vector that comprises the KLF gene but not the OCT gene and the SOX gene, wherein said vector is a Sendai virus vector comprising the KLF gene but not the OCT gene and the SOX gene, which is an F gene-deleted Sendai virus vector that comprises G69E, T116A and A183S mutations in the M protein, A262T, G264R and K461G mutations in the HN protein, L511F mutation in the P protein, and N1197S and K1795E mutations in the L protein;
3. [3] the composition of [2], which further comprises a temperature-sensitive Sendai virus vector inserted with a MYC gene, preferably wherein the MYC gene-inserted temperature-sensitive Sendai virus vector is an F gene-deleted Sendai virus vector that comprises G69E, T116A, and A183S mutations in the M protein, A262T, G264R, and K461G mutations in the HN protein, D433A, R434A, K437A, and L511F mutations in the P protein, and L1361C, L15581, N1197S, and K1795E mutations in the L protein;
4. [4] an in vitro method for inducing pluripotent stem cells, comprising introducing the composition of [2] into cells, and culturing the cells in the range of 36.5 to 37.5°C;
5. [5] a kit for inducing pluripotent stem cells, wherein the kit comprises:
 1. (a) a temperature-sensitive Sendai virus vector that comprises in one vector the KLF gene, OCT gene, and SOX gene in this order, wherein said vector is a temperature-sensitive Sendai virus vector comprising in one vector the KLF gene, OCT gene, and SOX gene in this order, which is an F gene-deleted Sendai virus vector that comprises G69E, T116A, and A183S mutations in the M protein, A262T, G264R, and K461G mutations in the HN protein, D433A, R434A, K437A, and L511F mutations in the P protein, and N1197S and K1795E mutations in the L protein
 2. (b) a Sendai virus vector that comprises the KLF gene but not the OCT gene and the SOX gene, wherein said vector is a Sendai virus vector comprising the KLF gene but not the OCT gene and the SOX gene, which is an F gene-deleted Sendai virus vector that comprises G69E, T116A, and A183S mutations in the M protein, A262T, G264R, and K461G mutations in the HN protein, L511F mutation in the P protein,

and N1197S and K1795E mutations in the L protein; and
6. [6] the kit of [5], further comprising a temperature-sensitive Sendai virus vector inserted with a MYC gene.

[Effects of the Invention]

[0011] The present invention enables significant improvement of the efficiency of pluripotent stem cell production using a vector that contains the KLF gene, OCT gene, and SOX gene in this order. Specifically, it is useful that pluripotent stem cells can be induced with high efficiency without low-temperature culturing, and use of a temperature-sensitive vector enables prompt removal of the vector after the induction of pluripotent stem cells.

Brief Description of the Drawings

[0012]

Fig. 1 shows the induction efficiency of alkaline phosphatase-positive iPS-like cells. Alkaline phosphatase-staining images of the vector-infected human neonatal foreskin-derived fibroblasts (BJ) on day 28 are shown.

Fig. 2 shows the induction efficiency of alkaline phosphatase-positive iPS-like cells. All the conditions are the same as those in Fig. 3.

Fig. 3 shows alkaline phosphatase-stained colonies. The number of alkaline phosphatase-positive colonies that appeared under Conditions 1 and 2 was significantly high.

Fig. 4 shows the induction efficiency of alkaline phosphatase-positive iPS-like cells. The efficiency of inducing pluripotent stem cells from BJ cells is shown. All the conditions are the same as those in Fig. 3.

Fig. 5 shows the induction efficiency of alkaline phosphatase-positive iPS-like cells. It shows results of alkaline phosphatase-staining of the cells on day 28 after infection of human adult skin-derived fibroblasts (HDF) with the vector.

Fig. 6 shows the induction efficiency of alkaline phosphatase-positive iPS-like cells. The efficiency of inducing pluripotent stem cells from human adult skin-derived fibroblasts (HDF) is shown. All the conditions are the same as those in Fig. 5.

Fig. 7 shows the induction efficiency of alkaline phosphatase-positive iPS-like cells. It shows results of alkaline phosphatase-staining of the cells on day 28 after infection of human fetal lung cell-derived fibroblasts (MRC-5) with the vector.

Fig. 8 shows the induction efficiency of alkaline phosphatase-positive iPS-like cells. It shows the induction efficiency of alkaline phosphatase-positive colonies. All the conditions are the same as those in Fig. 7.

Fig. 9 shows the *in vivo* pluripotency of the induced artificial pluripotent stem cells.

Fig. 10 shows a karyotype analysis of the induced artificial pluripotent stem cells.

Fig. 11 shows induction of iPS cells from mouse fibroblasts (alkaline phosphatase-staining results).

Fig. 12 shows confirmation of vector removal by anti-Sendai virus antibody staining.

Fig. 13 shows confirmation of the marker gene expression by immunostaining.

Fig. 14 shows results of the assessment of mouse iPS cells (embryoid body formation).

Fig. 15 shows results of the assessment of mouse iPS cells (*in vitro* differentiation into endoderm).

Fig. 16 shows results of the assessment of mouse iPS cells (*in vitro* differentiation into mesoderm).

Fig. 17 shows results of the assessment of mouse iPS cells (*in vitro* differentiation into ectoderm).

Fig. 18 shows results of the assessment of mouse iPS cells (*in vitro* differentiation into ectoderm).

Fig. 19 shows induction of iPS cells from human monocytes.

Fig. 20 shows induction of iPS cells from human monocytes (alkaline phosphatase-staining images).

Fig. 21 shows induction of iPS cells from human monocytes (efficiency of iPS cell induction).

Fig. 22 shows induction of iPS cells from human peripheral blood mononuclear cells (appearance of cells of Donor 1).

Fig. 23 shows induction of iPS cells from human peripheral blood mononuclear cells (appearance of cells of Donor 2).

Fig. 24 shows induction of iPS cells from human peripheral blood mononuclear cells (alkaline phosphatase staining).

Fig. 25 shows induction of iPS cells from human peripheral blood mononuclear cells (induction efficiency).

Fig. 26 shows induction of iPS cells from human monocytes (appearance of cells of Donor 3).

Fig. 27 shows induction of iPS cells from human monocytes (appearance of cells of Donor 4).

Fig. 28 shows induction of iPS cells from human monocytes (alkaline phosphatase staining).

Fig. 29 shows induction of iPS cells from human monocytes (induction efficiency).

Mode for Carrying Out the Invention

[0013] Hereinafter, the mode for carrying out the present invention will be described in detail.

[0014] The present invention relates to methods for increasing the efficiency of pluripotent stem cell induction, which comprise the step of introducing a vector that contains the KLF gene but not the OCT gene and the SOX gene in pluripotent stem cell induction that comprises the step of introducing a vector containing in one vector the KLF gene, OCT gene, and SOX gene in this order. More specifically, the methods of the present invention are methods of improving the efficiency of pluripotent stem cell induction in methods that induce pluripotent stem cells by introducing a temperature-sensitive virus vector containing the KLF gene, OCT gene, and SOX gene in this order in one vector, and which comprise the step of further introducing a virus vector that contains the KLF gene but not the OCT gene and the SOX gene.

[0015] After vector introduction, it is not necessary to culture at a low temperature (for example, under 36.5°C and more specifically, for example, 36°C or lower). That is, with the use of a temperature-sensitive variant vector, the methods of the present invention are superior in that pluripotent stem cells can be induced with high efficiency without low-temperature culturing (for example, culturing at lower than 36.5°C) and the vector is rapidly eliminated after the induction of pluripotent stem cells. For example, the present invention relates to methods of improving the efficiency of pluripotent stem cell induction in methods that induce pluripotent stem cells without low-temperature culturing by introducing a temperature-sensitive Sendai virus vector containing in one vector the KLF gene, OCT gene, and SOX gene in this order, which include the step of further introducing a Sendai virus vector that contains the KLF gene but not the OCT gene and the SOX gene. The temperature for cell culture can be appropriately selected, and for example, it can be about 37°C, specifically 36.5°C to 37.5°C, preferably 36.6°C to 37.4°C, and more preferably 36.7°C to 37.3°C. In the present invention, pluripotent stem cell induction includes generation of pluripotent stem cells, and improvement of the efficiency of pluripotent stem cell induction includes improvement of the efficiency of pluripotent stem cell generation.

[0016] While introduction of the MYC gene is not essential for the induction of pluripotent stem cells, the efficiency of pluripotent stem cell induction can be increased by introducing the MYC gene. The MYC gene can be introduced, for

example, by using a temperature-sensitive virus vector, preferably a temperature-sensitive Sendai virus vector.

[0017] The present invention provides methods for reprogramming cells, which comprise the step of introducing into differentiated cells such as somatic cells or contacting them with a vector containing the KLF gene, OCT gene, and SOX gene in one vector in this order, and a vector that contains the KLF gene but not the OCT gene and the SOX gene. Moreover, the present invention provides methods for introducing the above reprogramming factor genes in cellular reprogramming, which comprise introducing these reprogramming factor genes using a vector containing the KLF gene, OCT gene, and SOX gene in one vector in this order and a vector that contains the KLF gene but not the OCT gene and the SOX gene, as well as compositions comprising the vectors for use in those methods.

[0018] In the present invention, "pluripotent stem cells" refer to stem cells produced from the inner cell mass of an embryo of an animal in the blastocyst stage or cells having phenotypes similar to those cells. Specifically, pluripotent stem cells induced in the present invention are cells that express alkaline phosphatase which is an indicator of ES-like cells. Here, "ES-like cells" indicate pluripotent stem cells having properties and/or morphologies that are similar to ES cells. Furthermore, preferably, when pluripotent stem cells are cultured, they form flat colonies containing cells with a higher proportion of nucleus volume than cytoplasm. Culturing may be carried out suitably with a feeder. Moreover, while cultured cells such as MEF stop proliferating in a few weeks, pluripotent stem cells can be passaged for a long period of time, and this can be confirmed based on their proliferative character that is not lost even when they are passaged, for example, 15 times or more, preferably 20 times or more, 25 times or more, 30 times or more, 35 times or more, or 40 times or more every three days. Furthermore, pluripotent stem cells preferably express endogenous OCT3/4 or Nanog, or more preferably, they express both of them. Furthermore, pluripotent stem cells preferably express TERT, and show telomerase activity (activity to synthesize telomeric repeat sequences). Moreover, pluripotent stem cells preferably have the ability to differentiate into three germ layers (the endoderm, mesoderm, and ectoderm) (this can be confirmed, for example, during teratoma formation and/or embryoid body formation). More preferably, pluripotent stem cells produce germline chimera when they are transplanted into blastocysts. Pluripotent stem cells capable of germline transmission are called germline-competent pluripotent stem cells. Confirmation of these phenotypes can be carried out by known methods (WO 2007/69666; Ichisaka T. et al., Nature 448 (7151): 313-7, 2007).

[0019] Furthermore, in the present invention, "differentiated" refers to that a differentiation stage of a cell is progressed more than before, and may refer to, for example, be more differentiated as compared to pluripotent stem cells, and includes states still possessing the ability to differentiate into multiple cell lineages (for example, somatic stem cells) and terminally differentiated states. Differentiated cells are cells (other than pluripotent stem cells) derived from pluripotent stem cells. Differentiated cells may be, for example, cells that do not have the ability to differentiate into the three germ layers (the endoderm, mesoderm, and ectoderm). Such cells will not have the ability to form the three germ layers unless they are reprogrammed. Furthermore, differentiated cells may be, for example, cells that cannot produce cells that are not of the germ layer type to which they belong. Differentiated cells may be somatic cells, and for example, they may be cells other than germ cells.

[0020] In the present invention, reprogramming refers to converting the differentiation state of a particular cell to a less differentiated state, and includes for example, dedifferentiation of differentiated cells, such as inducing cells with differentiation pluripotency, for example pluripotent stem cells, from cells without differentiation pluripotency. Furthermore, in the present invention, dedifferentiation refers to conversion of a particular cell into a more premature (for example, undifferentiated) state. Dedifferentiation may be reversion of a cell to its initial state or intermediate state in its path of differentiation. Furthermore, dedifferentiation may be a change from a cell unable to produce cells that are not of the same germ layer type to which the cell belongs, into a cell that can differentiate into other germ layer type cells. Dedifferentiation also includes, for example, cells not having triploblastic differentiation ability acquiring this triploblastic differentiation ability. Additionally, dedifferentiation includes the production of pluripotent stem cells.

[0021] Furthermore, in the present invention, somatic cells are, for example, cells other than pluripotent stem cells and germ cells. Somatic cells include, for example, multicellular organism-constituting cells other than pluripotent stem cells, and cultured cells thereof. Somatic cells include for example, somatic stem cells and terminally differentiated cells.

[0022] The low-temperature culturing in the present invention refers to culturing at a temperature lower than 36.5°C. The low-temperature culturing refers to culturing at a low temperature of preferably under 36.4°C, more preferably, 36.3°C, 36.2°C, 36.1°C, 36°C, 35.9°C, 35.8°C, 35.7°C, 35.6°C, 35.5°C, 35.4°C, 35.3°C, 35.2°C or 35.1°C, and even more preferably lower than 35°C. The lower limit is, for example, 30°C, preferably 31°C, and more preferably 32°C, 33°C or 34°C. Further, about 37°C in the present invention refers to specifically, 36.5°C to 37.5°C, preferably 36.6°C to 37.4°C, and more preferably 36.7°C to 37.3°C.

[0023] Temperature sensitivity in the present invention means that the activity is significantly reduced at the standard temperature for cell culture (for example, 37°C to 38°C) compared to that at a low temperature (for example, 30°C to 36°C). More preferably, it means that the activity is significantly reduced at 37°C compared to that at 36°C. For example, in the case of an expression vector, the level of expression of the temperature-sensitive vector is significantly lower at the standard temperature for cell culture (for example, 37°C to 38°C) compared to the level of expression at a low temperature (for example, 30°C to 36°C). For example, the TS 7 (Y942H/L1361C/L1558I mutations in the L protein), TS 12 (D433A/R434A/K437A mutations in the P protein), TS 13 (D433A/R434A/K437A mutations in the P protein and L1558I mutation in the L protein), TS 14 (D433A/R434A/K437A mutations in the P protein and L1361C in the L protein), TS 15 (D433A/R434A/K437A mutations in the P protein and L1361C/L1558I in the L protein) mutations and such in Sendai virus are temperature-sensitive mutations.

[0024] Vectors in the present invention are preferably viral vectors. In the present invention, virus vectors are vectors having genomic nucleic acids derived from the virus, and that can express transgenes by incorporating the transgenes into the nucleic acids. Since Sendai virus vectors are chromosomally non-integrating virus vectors and are expressed in the cytoplasm, there is no risk that the introduced gene will become integrated into the chromosome (nucleus-derived chromosome) of the host. Therefore, the vectors are safe and can be removed after completion of reprogramming. In the present invention, Sendai virus vectors include infectious virus particles, as well as complexes of the viral core, viral genome, and viral proteins, and complexes comprising non-infectious viral particles and such, which are complexes having the ability to express loaded genes upon introduction into cells. For example, in Sendai viruses, ribonucleoproteins (the viral core portion) consisting of a Sendai virus genome and bound Sendai virus proteins (NP, P, and L proteins) can express transgenes in cells when they are introduced into cells (WO 00/70055). Introduction into cells can be appropriately carried out using transfection reagents and the like. Such ribonucleoproteins (RNPs) are also included in the Sendai virus vectors of the present invention.

[0025] Sendai virus is a Mononegavirales virus, belongs to Paramyxoviridae (including the genera Paramyxovirus, Morbillivirus, Rubulavirus, and Pneumovirus), and contains a single-stranded minus-strand (antisense strand of a viral protein-encoding sense strand) RNA as the genome. Minus-strand RNA is also called negative-strand RNA.

[0026] Mononegavirales includes viruses belonging to families such as Rhabdoviridae (including the genera Vesiculovirus, Lyssavirus, and Ephemerovirus), and Filoviridae, in addition to Paramyxovirus (Paramyxoviridae virus) (Virus, vol. 57, No. 1: pp29-36, 2007; Annu. Rev. Genet. 32, 123-162, 1998; Fields virology fourth edition, Philadelphia, Lippincott-Raven, 1305-1340, 2001; Microbiol. Immunol. 43, 613-624, 1999; Field Virology, Third edition pp.1205-1241, 1996). Other examples of Paramyxoviridae virus other than Sendai virus include Newcastle disease virus, mumps virus, measles virus, respiratory syncytial virus (RS virus), rinderpest virus, distemper virus, simian parainfluenza virus (SV5), and human parainfluenza viruses I, II, and III; influenza virus belonging to the Orthomyxoviridae family; and the vesicular stomatitis virus and Rabies virus belonging to the Rhabdoviridae family. Further examples include Sendai virus (SeV), human parainfluenza virus-1 (HPIV-1), human parainfluenza virus-3 (HPIV-3), phocine distemper virus (PDV), canine distemper virus (CDV), dolphin morbillivirus (DMV), peste-des-petits-ruminants virus (PDPR), measles virus (MV), rinderpest virus (RPV), Hendra virus (Hendra), Nipah virus (Nipah), human parainfluenza virus-2 (HPIV-2), simian parainfluenza virus 5 (SV5), human parainfluenza virus-4a (HPIV-4a), human parainfluenza virus-4b (HPIV-4b), mumps virus (Mumps), and Newcastle disease virus (NDV). More preferably, examples include viruses selected from the group consisting of Sendai virus (SeV), human parainfluenza virus-1 (HPIV-1), human parainfluenza virus-3 (HPIV-3), phocine distemper virus (PDV), canine distemper virus (CDV), dolphin morbillivirus (DMV), peste-des-petits-ruminants virus (PDPR), measles virus (MV), rinderpest virus (RPV), Hendra virus (Hendra), and Nipah virus (Nipah).

[0027] For examples of accession numbers in the database for the nucleotide sequences of Sendai virus genes, see

M29343, M30202, M30203, M30204, M51331, M55565, M69046, and X17218 for the NP gene; M30202, M30203, M30204, M55565, M69046, X00583, X17007, and X17008 for the P gene; D11446, K02742, M30202, M30203, M30204, M69046, U31956, X00584, and X53056 for the M gene; D00152, D11446, D17334, D17335, M30202, M30203, M30204, M69046, X00152, and X02131 for the F gene; D26475, M12397, M30202, M30203, M30204, M69046, X00586, X02808, and X56131 for the HN gene; and D00053, M30202, M30203, M30204, M69040, X00587, and X58886 for the L gene. Examples of viral genes encoded by other viruses include CDV, AF014953; DMV, X75961; HPIV-1, D01070; HPIV-2, M55320; HPIV-3, D10025; Mapuera, X85128; Mumps, D86172; MV, K01711; NDV, AF064091; PDPR, X74443; PDV, X75717; RPV, X68311; SeV, X00087; SV5, M81442; and Tupaia, AF079780 for the NP gene (also referred to as the N gene); CDV, X51869; DMV, Z47758; HPIV-1, M74081; HPIV-3, X04721; HPIV-4a, M55975; HPIV-4b, M55976; Mumps, D86173; MV, M89920; NDV, M20302; PDV, X75960; RPV, X68311; SeV, M30202; SV5, AF052755; and Tupaia, AF079780 for the P gene; CDV, AF014953; DMV, Z47758; HPIV-1, M74081; HPIV-3, D00047; MV, ABO16162; RPV, X68311; SeV, AB005796; and Tupaia, AF079780 for the C gene; CDV, M12669; DMV Z30087; HPIV-1, S38067; HPIV-2, M62734; HPIV-3, D00130; HPIV-4a, D10241; HPIV-4b, D10242; Mumps, D86171; MV, ABO12948; NDV, AF089819; PDPR, Z47977; PDV, X75717; RPV, M34018; SeV, U31956; and SV5, M32248 for the M gene; CDV, M21849; DMV, AJ224704; HPN-1, M22347; HPIV-2, M60182; HPIV-3, X05303, HPIV-4a, D49821; HPIV-4b, D49822; Mumps, D86169; MV, AB003178; NDV, AF048763; PDPR, Z37017; PDV, AJ224706; RPV, M21514; SeV, D17334; and SV5, AB021962 for the F gene; and CDV, AF112189; DMV, AJ224705; HPIV-1, U709498; HPIV-2, D000865; HPIV-3, AB012132; HPIV-4A, M34033; HPIV-4B, AB006954; Mumps, X99040; MV, K01711; NDV, AF204872; PDPR, Z81358; PDV, Z36979; RPV, AF132934; SeV, U06433; and SV-5, S76876 for the HN (H or G) gene. However, multiple strains are known for each of the viruses, and genes consisting of a sequence other than those exemplified above may exist due to strain differences. Sendai virus vectors carrying viral genes derived from any of these genes are useful as vectors of the present invention. For example, Sendai virus vectors of the present invention comprise a nucleotide sequence having 90% or higher, preferably 95% or higher, 96% or higher, 97% or higher, 98% or higher, or 99% or higher identity to the coding sequence of any of the above-mentioned viral genes. Furthermore, the Sendai virus vectors of the present invention comprise, for example, a nucleotide sequence encoding an amino acid sequence having 90% or higher, preferably 95% or higher, 96% or higher, 97% or higher, 98% or higher, or 99% or higher identity to an amino acid sequence encoded by the coding sequence of any one of the above-mentioned viral genes. Furthermore, Sendai virus vectors of the present invention comprise, for example, a nucleotide sequence encoding an amino acid sequence with ten or less, preferably nine or less, eight or less, seven or less, six or less, five or less, four or less, three or less amino acid substitutions, in an amino acid sequence encoded by the coding sequence of any one of the above-mentioned viral genes.

[0028] The sequences referenced by the database accession numbers such as the nucleotide sequences and amino acid sequences described herein refer to sequences on, for example, the filing date and priority date of this application, and can be identified as sequences at the time of either the filing date or priority date of the present application, and are preferably identified as sequences on the filing date of this application. The sequences at the respective time points can be identified by referring to the revision history of the database.

[0029] The Sendai virus vectors used in the present invention may be derivatives, and the derivatives comprise viruses with modified viral genes, and chemically modified viruses, and such, so that the ability of the virus to introduce genes is not impaired.

[0030] Furthermore, Sendai viruses may be derived from natural strains, wild-type strains, mutant strains, laboratory-passaged strains, and artificially constructed strains and such. An example is the Z strain (Medical Journal of Osaka University Vol. 6, No. 1, March 1955 p1-15). That is, these viruses may be virus vectors having similar structures as viruses isolated from nature, or viruses artificially modified by genetic recombination, as long as the desired reprogramming can be induced. For example, they may have mutations or deletions in any of the genes of the wild-type virus. Furthermore, incomplete viruses such as DI particles (J. Virol. 68: 8413-8417, 1994) may also be used. For example, viruses having a mutation or deletion in at least one gene encoding a viral envelope protein or a coat protein can be preferably used. Such virus vectors are, for example, virus vectors that can replicate the genome in infected cells but cannot form infectious virus particles. Since there is no worry of spreading the infection to the surroundings, such transmission-defective virus vectors are highly safe. For example, virus vectors that do not contain at least one gene encoding an envelope protein such as F and/or HN or a spike protein, or a combination thereof may be used (WO 00/70055 and WO 00/70070; Li, H.-O. et al., J. Virol. 74(14): 6564-6569 (2000)). If proteins necessary for genome replication (for example, NP, P, and L proteins) are encoded in the genomic RNA, the

genome can be amplified in infected cells. To produce defective type of viruses, for example, the defective gene product or a protein that can complement it is supplied in trans in the virus-producing cell (WO 00/70055 and WO 00/70070; Li, H.-O. et al., *J. Virol.* 74(14): 6564-6569 (2000)). Furthermore, a method of collecting virus vectors as noninfective virus particles (VLP) without completely complementing the defective viral protein is also known (WO 00/70070). Furthermore, when virus vectors are collected as RNPs (for example, RNPs containing the N, L, and P proteins and genomic RNA), vectors can be produced without complementing the envelope proteins.

[0031] Disclosed are methods of gene delivery in reprogramming and methods for producing reprogrammed cells using Sendai virus vectors having mutations and/or deletions in the viral gene, and compositions and kits therefor. For example, in the envelope protein and coat proteins, many mutations including attenuation mutations and temperature-sensitive mutations are known. Sendai viruses having these mutant protein genes can be used favorably in the present invention. In the present invention, vectors with lowered cytotoxicity are desirably used. Cytotoxicity can be measured, for example by quantifying the release of lactic acid dehydrogenase (LDH) from cells. For example, vectors with significantly lowered cytotoxicity compared to the wild type can be used. Regarding the degree of lowering of cytotoxicity, for example, vectors showing a significant decrease of, for example 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, or 50% or more in the LDH release level compared to the wild-type in a culture medium of human-derived HeLa cell (ATCC CCL-2) or simian-derived CV-1 cell (ATCC CCL 70) infected at MOI (multiplicity of infection) 3 and cultured for three days can be used. Furthermore, mutations that decrease cytotoxicity also include temperature-sensitive mutations. Temperature-sensitive mutations refer to mutations which significantly decrease the activity at the viral host's ordinary temperature (for example, 37°C to 38°C) when compared to that at a low temperature (30°C to 36°C, for example, 30°C to 32°C). Such proteins with temperature-sensitive mutations are useful since the viruses can be produced under permissive temperatures (low temperatures). When infected at 37°C, the virus vectors having useful temperature-sensitive mutations in the present invention show, a growth rate or gene expression level of, for example, 1/2 or less, preferably 1/3 or less, more preferably 1/5 or less, more preferably 1/10 or less, and more preferably 1/20 or less compared to when cultured cells are infected at 32°C.

[0032] A Sendai virus vector used in the present invention may be a wild type as long as it does not inhibit reprogramming, can make reprogramming factors be expressed, and can induce or support induction of reprogramming by the reprogramming factors, and it has deletions or mutations in preferably at least one, more preferably at least 2, 3, 4, 5, or more viral genes. Deletions and mutations may be arbitrarily combined and introduced to each of the genes. Herein, a mutation may be a function-impairing mutation or a temperature-sensitive mutation, and is preferably a mutation that decreases the virus particle forming ability (or the nontransmissible virus-like particle (NTVLP) formation ability in the case of nontransmissible viruses (Inoue et al., *J. Virol.* 77:3238-3246, 2003)), the proliferation rate, and/or the expression level of any of the carried gene to preferably 1/2 or less, more preferably 1/3 or less, more preferably 1/5 or less, more preferably 1/10 or less, and more preferably 1/20 or less at least at 37°C as compared to the wild type or to a virus not having the mutation. The use of such modified virus vectors can be useful particularly for the induction of pluripotent stem cells. For example, Sendai virus vectors used favorably in the present invention have at least two deleted or mutated viral genes. Such viruses include those with deletions of at least two viral genes, those with mutations in at least two viral genes, and those with a mutation in at least one viral gene and a deletion of at least one viral gene. The at least two mutated or deleted viral genes are preferably genes encoding envelope-constituting proteins. For example, vectors with deletion of the F gene with further deletion of the M and/or the HN gene or further mutation (for example, NTVLP formation-suppressing mutation and/or temperature-sensitive mutation) in the M and/or the HN gene are used favorably in the present invention. Furthermore, for example, vectors with deletion of the F gene with further deletion of the M or the HN gene and further mutation in the remaining M and/or HN gene (for example, NTVLP formation-suppressing mutation and/or temperature-sensitive mutation) are also used favorably in the present invention. Vectors used in the present invention have at least three mutated viral genes encoding envelope-constituting proteins; F, HN, and M.

[0033] The mutations of the M gene of Sendai virus include amino acid substitutions of a site selected from the group consisting of position 69 (G69), position 116 (T116), and position 183 (A183) of the M protein (Inoue, M. et al., *J. Virol.* 2003, 77: 3238-3246). Specifically, G69, T116, and A183 of the Sendai virus M protein are substituted to Glu (E), Ala (A), and Ser (S), respectively. Mutations can be introduced according to known mutation methods, for example, using oligonucleotides and such.

[0034] Furthermore, mutations in the HN gene include amino acid substitution of a site selected from the group consisting of position 262 (A262), position 264 (G264), and position 461 (K461) of the HN protein of a Sendai virus (Inoue, M. et al., *J. Virol.* 2003, 77: 3238-3246). A262, G264, and K461 of the Sendai virus HN protein are substituted to Thr (T), Arg (R), and Gly (G), respectively. Furthermore, for example, using the temperature-sensitive vaccine strain Urabe AM9 of the mumps virus as a reference, amino acids at positions 464 and 468 of the HN protein can be mutated (Wright, K. E. et al., *Virus Res.* 2000, 67: 49-57).

[0035] Furthermore, Sendai viruses have mutations in the P gene and/or the L gene. Such mutations are specifically, mutation of Glu at position 86 (E86) in the SeV P protein, and substitution of Leu at position 511 (L511) in the SeV P protein to other amino acids. As mentioned above, preferred amino acid substitutions are substitutions to other amino acids with a side chain having different chemical properties. Specific examples include substitution of the amino acid at position 86 to Lys, and substitution of the amino acid at position 511 to Phe. The L protein includes substitutions of Asn at position 1197 (N1197) and Lys at position 1795 (K1795) in the SeV L protein to other amino acids, and are substitutions of the amino acid at position 1197 to Ser, and substitution of the amino acid at position 1795 to Glu. Mutations of the P gene and L gene can significantly increase the effects of sustained infectivity, suppression of release of secondary particles, or suppression of cytotoxicity. Further combination with mutations and/or deletions of envelope protein genes can dramatically increase these effects. Furthermore, examples for the L gene include substitution of Tyr at position 1214 (Y1214) and/or substitution of Met at position 1602 (M1602) of the SeV L protein to other amino acids, and similarly as above, preferred amino acid substitutions are substitutions to other amino acids with a side chain having different chemical properties. Specific examples are substitution of the amino acid at position 1214 to Phe, and substitution of the amino acid at position 1602 to Leu. The above-mentioned mutations can be arbitrarily combined.

[0036] For example, Sendai virus vectors in which at least G at position 69, T at position 116, and A at position 183 of the SeV M protein, at least A of position 262, G of position 264, and K of position 461 of the SeV HN protein, at least L of position 511 of the SeV P protein, and at least N of position 1197 and K of position 1795 of the SeV L protein are each substituted as above, and in which the F gene is also deficient or deleted; and F-gene-deleted or -deficient Sendai virus vectors whose cytotoxicity is similar to or lower than those mentioned above and/or whose suppression of NTVLP formation at 37°C is similar to or higher than those mentioned above are particularly preferred for the expression of nuclear reprogramming factors in the present invention.

In addition to the above-mentioned mutations, each of the viral proteins may have mutations on other amino acids (for example, on ten or less, five or less, four or less, three or less, two or less, or one amino acid). Since vectors comprising the above-mentioned mutations show a high temperature sensitivity, after completion of reprogramming, the vectors can be removed easily by culturing the cells at a normal temperature (for example, about 37°C, specifically 36.5°C to 37.5°C, preferably 36.6°C to 37.4°C, and more preferably 36.7°C to 37.3°C). For vector removal, culturing may also be carried out at a slightly high temperature (for example, 37.5°C to 39°C, preferably 38°C to 39°C, or 38.5°C to 39°C).

[0037] Specific vectors may be, for example, an F gene-deleted Sendai virus vector (for example, Z strain) in which the M protein has G69E, T116A, and A183S mutations; the HN protein has A262T, G264R, and K461G mutations; the P protein has L511F mutation; and the L protein has N1197S and K1795E mutations; and vectors produced by further introducing a TS 7, TS 12, TS 13, TS 14, or TS 15 mutation into this vector are more preferred. Specifically, examples include SeV18+/TSAF (WO 2010/008054 and WO 2003/025570) and SeV(PM)/TSAF, and vectors produced by further introducing an aforementioned TS 7, TS 12, TS 13, TS 14, or TS 15 mutation into these vectors, but are not limited thereto.

[0038] "TSAF" means carrying G69E, T116A, and A183S mutations in the M protein, A262T, G264R, and K461G mutations in the HN protein, L511F mutation in the P protein, and N1197S and K1795E mutations in the L protein, and deletion of the F gene.

[0039] The temperature-sensitive Sendai virus vector comprising in one vector the KLF gene, OCT gene and SOX gene in this order is an F gene-deleted Sendai virus vector that carries specifically G69E, T116A and A183S mutations in the M protein, A262T, G264R and K461G mutations in the HN protein, L511F mutation in the P protein, and N1197S and K1795E mutations in the L protein, and preferably it further has the TS 7, TS 12, TS 13, TS 14 or TS 15 mutations described above. More preferably, it is an F gene-deleted Sendai virus vector that carries G69E,

T116A and A183S mutations in the M protein, A262T, G264R and K461G mutations in the HN protein, L511F mutation in the P protein, and N1197S and K1795E mutations in the L protein, and further carries the TS 12 mutations. In the Sendai virus containing the KLF gene, OCT gene and SOX gene, preferably the KLF gene, OCT gene and SOX gene are incorporated in this order immediately after the P gene of Sendai virus, i.e., immediately downstream of the P gene (right to the 5' side of the minus-strand RNA genome).

[0040] Without limitations thereto, in particular, SeV(PM)KOS/TS7ΔF, SeV(PM)KOS/TS12ΔF or such is a preferable vector, and SeV(PM)KOS/TS12ΔF is more preferable.

[0041] At this time, it is combined with a Sendai virus vector expressing the KLF gene (a vector carrying the KLF gene but not the OCT gene and the SOX gene). The Sendai virus vector encoding the KLF gene (i.e. carrying the KLF gene but not the OCT gene and the SOX gene) can be either temperature sensitive or not, and preferably the vector has a mutation that would suppress NTVLP formation at 37°C. NTVLP formation can be measured according to the reference of Inoue et al., J. Virol. 77:3238-3246, 2003. A vector is an F gene-deleted Sendai virus vector that carries G69E, T116A and A183S mutations in the M protein, A262T, G264R and K461G mutations in the HN protein, L511F mutation in the P protein, and N1197S and K1795E mutations in the L protein. The Sendai virus vector carrying the KLF gene but not the OCT gene and the SOX gene can express the loaded gene (KLF gene) at 37°C. For the vector, a vector that has a higher efficiency in expressing the loaded gene at 37°C than the Sendai virus vector containing the KLF gene, OCT gene and SOX gene in this order in one vector is used. Specifically, it is preferably a vector that has lower temperature sensitivity (high resistance and relatively high expression level of the loaded gene at 37°C) than the temperature sensitive Sendai virus vector containing the KLF gene, OCT gene and SOX gene in this order in one vector. The KLF gene can be inserted, for example, upstream of the N gene of the Sendai virus vector (3' side of the N gene in the genome), and in particular, SeV18+KLF4/TSΔF is a preferable vector.

[0042] At this time, it is preferable to combine it with a temperature-sensitive virus vector encoding the MYC gene. A temperature-sensitive virus vector encoding the MYC gene is, for example, a temperature-sensitive Sendai virus vector that encodes the MYC gene. More specifically, it is an F gene-deleted Sendai virus vector that carries G69E, T116A and A183S mutations in the M protein, A262T, G264R and K461G mutations in the HN protein, L511F mutation in the P protein, and N1197S and K1795E mutations in the L protein, and preferably it is a vector that further has the TS 7, TS 12, TS 13, TS 14 or TS 15 mutations described above. More preferably, it is an F gene-deleted Sendai virus vector that carries G69E, T116A and A183S mutations in the M protein, A262T, G264R and K461G mutations in the HN protein, L511F mutation in the P protein, and N1 197S and K1795E mutations in the L protein, and further carries the TS 15 mutations. The MYC gene is incorporated, for example, immediately after the HN gene, i.e., immediately downstream of the HN gene (right to the 5' side of the minus-strand RNA genome), for example, between the HN gene and the L gene. More specifically, it can be SeV(HNL)c-rMYC/TS12ΔF, SeV(HNL)c-rMYC/TS13ΔF, or SeV(HNL)c-rMYC/TS15ΔF, and in particular SeV(HNL)c-rMYC/TS15ΔF is preferred. Favorable results can be obtained by combining, in particular, SeV(PM)KOS/TS12ΔF, SeV(HNL)c-rMYC/TS15ΔF, and SeV18+KLF4/TSΔF.

[0043] The cytotoxicity of vectors can be measured, for example, by quantifying the release of lactate dehydrogenase (LDH) from cells. Specifically, for example, HeLa (ATCC CCL-2) or simian CV-1 (ATCC CCL70) is infected at MOI 3, and the amount of LDH released into the culture solution after three days of culture is measured. The lower the amount of LDH released, the lower the cytotoxicity. Furthermore, temperature sensitivity can be determined by measuring the speed of viral proliferation or the expression level of the loaded gene at the viral host's ordinary temperature (for example, 37°C to 38°C). If the speed of viral proliferation and/or expression level of the loaded gene are decreased as compared to those without mutations, the mutation is judged to be a temperature-sensitive mutation, and the lower the proliferation speed and/or expression level, the higher the temperature sensitivity is judged to be.

[0044] Furthermore, when using an envelope virus, a virus containing a protein in the envelope that is different from the envelope protein originally carried by the virus may be used. For example, by expressing a desired exogenous envelope protein in a virus-producing cell when producing the virus, a virus containing this protein can be produced. Such proteins are not particularly limited and desired proteins, such as adhesion factors, ligands, and receptors that

confer an ability to infect to mammalian cells can be used. Specific examples include the G protein of vesicular stomatitis virus (VSV) (VSV-G). The VSV-G protein may be derived from any VSV strain, and for example, VSV-G protein derived from the Indiana serotype strain (J. Virology 39: 519-528 (1981)) may be used, but it is not limited thereto. Sendai virus vector used in the present invention can include arbitrary combinations of other virus-derived envelope proteins.

[0045] Reconstitution of recombinant Sendai viruses carrying nuclear reprogramming factors can be carried out using known methods. As specific procedures, typically, Sendai virus can be produced by the steps of (a) transcribing a cDNA encoding the Sendai virus genomic RNA (minus strand) or a complementary strand thereof (plus strand) in a cell that expresses viral proteins (NP, P, and L) necessary for virus particle formation, and (b) collecting a culture supernatant containing the produced viruses. Viral proteins necessary for particle formation may be expressed from the transcribed viral genomic RNA, or they may be provided in trans from sources other than genomic RNA. For example, they can be provided by introducing expression plasmids encoding the NP, P, and L proteins into cells. When viral genes necessary for particle formation are lacking in the genomic RNA, those viral genes may be separately expressed in virus-producing cells to complement particle formation. To express the viral proteins or the RNA genome in cells, vectors having a DNA encoding such proteins or genomic RNA linked downstream of a suitable promoter that functions in a host cell is introduced into the host cell. The transcribed genomic RNA is replicated in the presence of viral proteins, and infectious virus particles are formed. When producing a defective type of virus lacking genes such as those of the envelope proteins, the missing protein, other viral proteins that can complement the function of those proteins, or such may be expressed in the virus-producing cells.

[0046] For example, production of Sendai virus can be carried out by using the following known methods (WO 97/16539; WO 97/16538; WO 00/70055; WO 00/70070; WO 01/18223; WO 03/025570; WO 2005/071092; WO 2006/137517; WO 2007/083644; WO 2008/007581; Hasan, M. K. et al., J. Gen. Virol. 78: 2813-2820, 1997; Kato, A. et al., 1997, EMBO J. 16: 578-587; and Yu, D. et al., 1997, Genes Cells 2: 457-466; Durbin, A. P. et al., 1997, Virology 235: 323-332; Whelan, S. P. et al., 1995, Proc. Natl. Acad. Sci. USA 92: 8388-8392; Schnell, M. J. et al., 1994, EMBO J. 13: 4195-4203; Radecke, F. et al., 1995, EMBO J. 14: 5773-5784; Lawson, N. D. et al., Proc. Natl. Acad. Sci. USA 92: 4477-4481; Garcin, D. et al., 1995, EMBO J. 14: 6087-6094; Kato, A. et al., 1996, Genes Cells 1: 569-579; Baron, M. D. and Barrett, T., 1997, J. Virol. 71: 1265-1271; Bridgen, A. and Elliott, R. M., 1996, Proc. Natl. Acad. Sci. USA 93: 15400-15404; Tokusumi, T. et al., Virus Res. 2002, 86: 33-38; Li, H.-O. et al., J. Virol. 2000, 74: 6564-6569). Regarding methods for proliferation of viruses and methods for producing recombinant viruses, see also "Uirusu-gaku Jikken-gaku Kakuron (Detailed Virology Experiments)", second revised edition (National Institute of Infectious Diseases Students Institute edition, Maruzen, 1982).

[0047] Into the above-mentioned Sendai virus, the KLF gene, OCT gene, and SOX gene are incorporated in this order. Into another Sendai virus, the KLF gene is incorporated (the OCT gene and SOX gene are not incorporated into this vector). Generally, these foreign genes can be inserted immediately before (3' side on the genome) or immediately after (5' side on the genome of) any of the viral genes (NP, P, M, F, HN, or L).

[0048] Regarding the Sendai virus containing the KLF gene, OCT gene, and SOX gene, preferably, the KLF gene, OCT gene, and SOX gene are incorporated in this order immediately after the Sendai virus P gene, *i.e.*, immediately downstream of the P gene (immediately 5' side on the minus-strand RNA genome). For example, a transcription initiation signal (S sequence), a transcription termination signal (E sequence), and a spacer sequence (intervening sequence (I sequence) and such) may be included between the P gene and the KLF gene; however other transcription units (for example, transcription units encoding protein-coding genes) are not included. Since the Sendai virus carries a minus-strand RNA as the genome, opposite to the usual, the 3' side corresponds to the upstream and the 5' side corresponds to the downstream of the genome. When the KLF gene, OCT gene, and SOX gene are positioned in this order in the Sendai virus genome, among the three genes the KLF gene is positioned closest to the 3' side, and the SOX gene is positioned closest to the 5' side. Since the minus-strand RNA encodes genes in the antisense orientation, the KLF gene, OCT gene, and SOX gene are encoded as an antisense strand on the genome and not as a protein-coding strand (sense strand). When the Sendai virus genome enters a cell, it produces an antigenomic RNA using this genome as template. Antigenome is a plus strand, and the KLF gene, OCT gene and SOX gene are loaded as a protein-coding strand (sense strand). In antigenomic RNA, the KLF gene, OCT gene, and SOX gene are positioned so that among the three genes, the KLF gene is positioned closest to the 5' side and the SOX gene is positioned closest to the 3' side.

[0049] The three genes are preferably adjacent to each other (*i.e.*, with no other genes interposed among the three genes). Each of the genes may be appropriately sandwiched between the Sendai virus S (Start) sequence and the E (End) sequence. The S sequence is a signal sequence that initiates transcription, and the E sequence terminates the transcription. The region between the S sequence and the E sequence becomes a single transcription unit. Where appropriate, a sequence that serves as a spacer (intervening sequence) can be inserted between the E sequence of a certain gene and the S sequence of the next gene. For example, a Sendai virus vector that contains a nucleic acid having the constitution of S-KLF gene-E-I-S-OCT gene-E-I-S-SOX gene-E (S, I, and E refer to the S sequence, the I (intervening) sequence, and the E sequence, respectively) in its genome can be used favorably. The P gene and KLF gene on the Sendai virus genome will be linked in the following manner: P gene-E-I-S-KLF gene-, and such.

[0050] When the OCT gene, SOX gene, and KLF gene are positioned in this order, for example, a Sendai virus vector that contains a nucleic acid having the constitution of S-OCT gene-E-I-S-SOX gene-E-I-S-KLF gene-E (S, I, and E refer to the S sequence, the I (intervening) sequence, and the E sequence, respectively) in its genome can be used favorably. The P gene and SOX gene on the Sendai virus genome will be linked in the following manner: P gene-E-I-S-SOX gene-, and so on.

[0051] While a desired S sequence of Sendai virus may be used as the S sequence, for example, the 3'-UCCCWUUWC-5' (W = A or U; V = A, C, or G) sequence (SEQ ID NO: 1) may be used favorably. In particular, 3'-UCCCAGUUUC-5' (SEQ ID NO: 2), 3'-UCCCACUUAC-5' (SEQ ID NO: 3), and 3'-UCCCACUUUC-5' (SEQ ID NO: 4) are preferred. When these sequences are presented as DNA sequences encoding the plus strand, they are 5'-AGGGTCAAAG-3' (SEQ ID NO: 5), 5'-AGGGTGAATG-3' (SEQ ID NO: 6), and 5'-AGGGTGAAAG-3' (SEQ ID NO: 7), respectively. An E sequence of the Sendai virus vector is preferably, for example, 3'-AUUCUUUUU-5' (the plus strand-encoding DNA is 5'-TAAGAAAAA-3'). The I sequence may be, for example, any three bases, and specifically, 3'-GAA-5' (5'-CTT-3' in the plus strand DNA) may be used.

[0052] The genome of wild-type Sendai virus includes a short 3' leader region followed by a nucleocapsid (NP) gene, a phospho (P) gene, a matrix (M) gene, a fusion (F) gene, a hemagglutinin-neuraminidase (HN) gene, and a large (L) gene, and then a short 5' trailer region, in this order. Viral genes can be positioned in this order in Sendai virus vectors as well. Production of recombinant vectors comparable to wild-type viruses, and various mutant vectors are already known. Furthermore, it has been shown that gene delivery is possible using the RNP alone without its envelope (WO 00/70055). Therefore, reprogramming can be carried out using Sendai virus RNP as a virus vector.

[0053] Sendai viruses are sufficiently functional as vectors if they carry the NP gene, P gene and L gene; and they can replicate genome in cells and express the loaded foreign genes (KLF, OCT, SOX, and such). In a Sendai virus carrying the three genes, NP gene, P gene and L gene as viral genes, the set of KLF, OCT, and SOX genes is inserted, for example, between the P gene and the L gene. In a vector containing the M gene, the set of KLF, OCT, and SOX genes is inserted, for example, between the P gene and the M gene (WO 97/16539). When the F gene is included in an M gene-deleted Sendai virus vector, the set of KLF, OCT, and SOX genes is inserted between the P gene and the F gene (WO 00/70070). For the M and F gene-deleted Sendai virus vector, the position of insertion is between the P gene and the HN gene; and for the F, M, and HN gene-deleted Sendai virus vector, the position of insertion is between the P gene and the L gene (WO 2003/025570, WO 2006/137517). Viral gene-deleted vectors are preferable since they are very safe. In the present invention, a vector having at least the F gene deletion can be preferably used.

[0054] Moreover, for a vector containing the KLF gene but not containing the OCT gene and the SOX gene, the KLF gene is inserted for example upstream of the NP gene (between the 3' leader sequence and the NP gene).

[0055] The above-mentioned Sendai virus vector containing the KLF, OCT, and SOX genes and the Sendai virus vector containing the KLF gene but not containing the OCT gene and the SOX gene can be appropriately used by the two of them for gene delivery in reprogramming, but they are more preferably used in reprogramming that further involves introduction of the MYC gene. A vector expressing the Glis1 gene (Maekawa et al., Nature, 474:225-229, 2011) instead of the MYC gene may also be combined. The MYC gene or Glis1 gene can be inserted into the above-mentioned Sendai virus vector containing the KLF, OCT and SOX genes or the Sendai virus vector containing the

KLF gene but not the OCT gene and the SOX gene, but it may also be used after insertion into a different vector. When inserting the MYC gene or Glis1 gene into a different vector, desired vectors such as plasmids, virus vectors, and non-virus vectors (for example, liposomes) can be used. Examples of virus vectors include Adenovirus vectors and retrovirus vectors, but are not limited thereto. More preferably, the MYC gene or Glis1 gene is inserted into a Sendai virus vector. In the present invention, the above-mentioned Sendai virus vector containing the KLF, OCT, and SOX genes and the Sendai virus vector containing the KLF gene but not the OCT gene and the SOX gene are preferably used in combination with a different Sendai virus vector inserted with the MYC gene or Glis1 gene. The above-mentioned Sendai virus vector can be used as the starting Sendai virus vector for insertion of the MYC gene or Glis1 gene.

[0056] When inserting the MYC gene or Glis1 gene into a Sendai virus genome, a desirable site can be selected for the position of gene insertion in the vector. For example, the MYC gene or Glis1 gene is preferably positioned toward the rear of the minus-strand RNA genome (5' side), for example, more towards the 5' end than to the center of the minus-strand RNA virus genome (the position is further on the 5' end than that of the gene in the middle). That is, among the multiple protein-coding sequences positioned on the genome, it is preferably positioned at a site closer to the 5' end than the 3' end (see the Examples). The MYC gene or Glis1 gene can be positioned, for example, at the very end of the 5' side (*i.e.*, first position from the 5' end), or at the second or third position from the 5' end. The MYC gene or Glis1 gene may be positioned at the second position from the 5' end of the genome, specifically, between the HN gene and the L gene (between HN-L) when the L gene is at the very end of the 5' side of the genome with the HN gene next to it. In particular, the MYC gene or Glis1 gene is preferably inserted immediately upstream (3' side, for example, between the HN gene and the L gene), or immediately downstream (5' side, for example, between the L gene and the 5' trailer sequence) of the L gene in the Sendai virus genome. A Sendai virus vector in which the MYC gene has been inserted between the HN gene and L gene is most preferred. The Sendai virus vector may be, for example, an F gene-deleted Sendai virus vector (for example, the Z strain) in which the M protein has G69E, T116A, and A183S mutations; the HN protein has A262T, G264R, and K461G mutations; the P protein has L511F mutation; and the L protein has N1197S and K1795E mutations; and vectors produced by further introducing a TS 7, TS 12, TS 13, TS 14, or TS 15 mutation into this vector are more preferable.

[0057] MYC genes including not only wild type c-MYC but also the T58A mutant, N-MYC, and L-MYC can induce pluripotent stem cells (WO 2007/69666; Blalock R. et al., Cell Stem Cell, 1: 245-247, 2007). As such, since the family genes can be selected in various ways and used, reprogramming can be induced by appropriately selecting the MYC family genes. Furthermore, a continuous A or T nucleotide sequence of the MYC gene can be substituted by appropriately introducing silent mutations such that the encoded amino acid sequence is not changed.

[0058] For example, the amount of wild-type c-MYC expressed by an RNA virus vector such as a Sendai virus vector was found to be small. However, by introducing one or more, preferably two or more, three or more, or four or more mutations selected from a378g, t1122c, t1125c, a1191g, and a1194g, or all five of these mutations into the wild-type c-MYC, the gene can be highly expressed in a stable manner from a vector. In the present invention, for example, the modified c-MYC gene shown in SEQ ID NO: 8 (SEQ ID NO: 9 for the amino acid sequence) (referred to as "c-rMYC") can be used suitably. Specifically, examples include SeV(HNL)c-rMYC/TSΔF, SeV(L)c-rMYC/TSΔF, SeV(HNL)c-rMYC/TS15ΔF (WO 2010/008054; Fusaki et al., Proc Jpn Acad Ser B Phys Biol Sci. Vol. 85, p348-362 (2009)), and preferably SeV(HNL)c-rMYC/TS15ΔF in particular, but are not limited thereto.

[0059] When appropriate, genes for cellular reprogramming and such can be loaded additionally into the above-mentioned Sendai virus vector carrying the KLF, OCT, and SOX genes, the Sendai virus vector containing the KLF gene but not the OCT gene and the SOX gene, and/or the Sendai virus vector containing the MYC gene. Furthermore, other vectors loaded with genes for cellular reprogramming and such may also be combined with the above-mentioned Sendai virus vectors. The genes to be loaded may be desired genes involved in the induction and such of various stem cells such as pluripotent stem cells from differentiated cells. For example, such genes that increase the efficiency of reprogramming can be loaded.

[0060] The present invention provides uses of the Sendai virus vectors of the present invention for introducing genes in cellular reprogramming, and uses of these vectors for expressing reprogramming factors in cells to induce reprogramming of those cells. Furthermore, the present invention provides agents containing the Sendai virus vectors of the present invention for introducing genes in cellular reprogramming (transfer agents, gene transfer

agents) and agents containing these vectors for expressing reprogramming factors in cells. Furthermore, the present invention relates to agents containing the Sendai virus vectors of the present invention for expressing reprogramming factors in cells to induce reprogramming of the cells. Furthermore, when carrying out nuclear reprogramming of cells, the vectors of the present invention are also useful for expressing desired genes in these cells. Sendai virus vectors of the present invention can be utilized for cellular reprogramming according to the present invention. The induction of reprogramming may be specifically an induction of pluripotent stem cells. The present invention can be used for medical uses and for non-medical uses, and is useful in medical and non-medical embodiments. For example, the present invention can be used for therapeutic, surgical, and/or diagnostic, or non-therapeutic, non-surgical, and/or non-diagnostic purposes.

[0061] In the present invention, a nuclear reprogramming factor refers to a gene used, by itself or together with a number of factors, for inducing a differentiated state of a certain cell to change to a more undifferentiated state, or a product thereof, and includes for example, a gene used for inducing dedifferentiation of differentiated cells, or a product thereof. The nuclear reprogramming factors in the present invention include factors essential for nuclear reprogramming and accessory factors (auxiliary factors) which increase the efficiency of nuclear reprogramming. In the present invention, desired genes to be used for nuclear reprogramming can be loaded into a vector. For example, genes to be used for the production of pluripotent stem cells can further be loaded. Specifically, as the nuclear reprogramming factors for induction of pluripotent stem cells, for example, genes that are expressed in ES cells or early embryo but are not expressed or whose expression is decreased in many differentiated somatic cells (ES cell-specific genes and such) can be used. Such genes are preferably genes that encode transcription factors, nucleoproteins, or such. Methods for identifying nuclear reprogramming factors are already known (WO 2005/80598), and in fact, genes identified using this method have been shown to be useful in reprogramming into pluripotent stem cells (WO 2007/69666).

[0062] Examples of such genes include DPPA5 (developmental pluripotency associated 5, ES cell specific gene 1 (ESG1); accession numbers NM_001025290, NM_025274, XM_236761), F-box protein 15 (Fbx15, NM_152676, NM_015798), Nanog (NM_024865, AB093574), ECAT1 (ES cell associated transcript 1; AB211062, AB211060), ERAS (ES cell expressed Ras; NM_181532, NM_181548), DNMT3L (DNA (cytosine-5-)-methyltransferase 3-like; NM_013369, NM_019448), ECAT8 (AB211063, AB211061), GDF3 (growth differentiation factor 3; NM_020634, NM_008108), SOX15 (SRY (sex determining region Y)-box 15; NM_006942, NM_009235), DPPA4 (developmental pluripotency associated 4; NM_018189, NM_028610), DPPA2 (NM_138815, NM_028615), FTHL17 (ferritin, heavy polypeptide-like 17; NM_031894, NM_031261), SALL4 (sal-like 4; NM_020436, NM_175303), OCT3/4 (also called POU5F1; NM_002701, NM_203289, NM_013633, NM_001009178), SOX2 (NM_003106, NM_011443, XM_574919), Rex-1 (ZFP42 (zinc finger protein 42 homolog); NM_174900, NM_009556), Utf1 (undifferentiated embryonic cell transcription factor 1; NM_003577, NM_009482), TCL1A (T-cell leukemia/lymphoma 1A; NM_021966, NM_009337), DPPA3 (also called Stella, NM_199286, NM_139218, XM_216263), KLF4 (Kruppel-like factor 4; NM_004235, NM_010637), catenin β 1 (cadherin-associated protein beta 1; NM_001904, NM_007614; including the S33Y mutant), c-MYC (NM_002467, NM_010849; including the T58A mutant), STAT3 (signal transducer and activator of transcription 3; NM_139276, NM_213659), GRB2 (growth factor receptor-bound protein 2; NM_002086, NM_008163), Glis1 gene (Maekawa et al., Nature, 474: 225-229, 2011; NM_147193, NM_147221), and other genes which are members of the families to which these genes belong. These genes induce pluripotent stem cells upon introduction into cells (WO 2007/69666). Therefore, from among the above-mentioned genes, a gene that is not yet loaded into the vector can be loaded additionally into a Sendai virus vector containing the KLF, OCT, and SOX genes, a Sendai virus vector containing the KLF gene but not containing the OCT gene and SOX gene, and/or a Sendai virus vector containing the MYC gene; alternatively, other vectors equipped with these genes can be used in combination with these Sendai virus vectors. These genes may be incorporated individually into separate vectors, or multiple genes can be incorporated into a single vector together. Furthermore, the individual genes can be incorporated into a single type of vector, or different types of vectors (including chromosome-integrating viral vector and/or non-viral vector) can be used in combination with the above-mentioned Sendai virus vector of the present invention containing the KLF gene, OCT gene, and SOX gene, or the Sendai virus vector containing the KLF gene but not containing the OCT gene and SOX gene. In addition, individual virus vectors are packaged separately, and can be used by combining them at the time of use. Alternatively, multiple virus vectors carrying different genes can be combined in advance as a kit, or they may be mixed to produce a composition. Furthermore, one or more non-integrating virus vectors containing any combination (or all) of these genes, and kits or compositions further containing these vectors

can be used favorably for cellular reprogramming, particularly in the production of pluripotent stem cells. In the case of compositions, the vectors may be appropriately combined with a pharmaceutically acceptable carrier and/or a medium, and for example, sterilized water, pH buffers, physiological saline solutions, culture solutions, and such may be mixed in. In these systems, some or most of the nuclear reprogramming genes can be substituted with their expression products that are proteins. More specifically, the compositions and kits of the present invention may include other vectors (chromosome-integrating virus vectors and/or non-viral vectors) that express reprogramming factors and/or compounds, proteins, or such that induce reprogramming as long as they include the above-mentioned Sendai virus vector containing at least the KLF gene, OCT gene, and SOX gene and the Sendai virus vector containing the KLF gene but not containing the OCT gene and SOX gene. All of the factors necessary for reprogramming are preferably expressed from Sendai virus vectors; however, some of them may be expressed from Sendai virus vectors, and the remaining factors may be provided from other vectors and/or compounds (for example, proteins or low-molecular-weight compounds). Furthermore, the methods of the present invention for producing reprogrammed cells are not limited to methods that carry out all gene deliveries using Sendai virus vectors. More specifically, the methods of the present invention only need to use the above-mentioned Sendai virus vector containing at least the KLF gene, OCT gene, and SOX gene and the Sendai virus vector containing the KLF gene but not containing the OCT gene and SOX gene, and include combined use of other vectors (chromosome-integrating virus vectors and/or non-virus vectors) expressing reprogramming factors and/or compounds and such that induce reprogramming. Preferably, they are used by further combining with the above-mentioned Sendai virus vector containing the MYC gene or Glis1 gene.

[0063] In the invention described in the present specification, it is preferable to combine a vector in which specifically the KLF gene, OCT gene, and SOX gene are inserted in this order into one Sendai virus vector (preferably SeV(PM)KOS/TS12ΔF) with a Sendai virus vector that contains the KLF4 gene but not the OCT gene and the SOX gene (preferably SeV18+KLF4/TSΔF). At this time, it is more preferable to combine it further with a Sendai virus vector expressing the MYC gene (preferably SeV(HNL)c-rMYC/TS15ΔF).

[0064] The factors that are introduced (the KLF gene, OCT gene, SOX gene, MYC gene, and the like) may be selected appropriately according to the origin of the cells to be reprogrammed, and they may be derived from humans or other mammals such as mice, rats, rabbits, pigs, or primates such as monkeys. Furthermore, the genetic and protein sequences do not necessarily have to be wild-type sequences, and as long as they can induce reprogramming, they may have mutations. Examples of producing pluripotent stem cells using mutant genes are known (WO 2007/69666). For example, a gene encoding an amino acid sequence with one or a small number of (for example, a few, not more than three, not more than five, not more than ten, not more than 15, not more than 20, or not more than 25) amino acid additions, deletions, substitutions, and/or insertions, and which can induce reprogramming may be used in the present invention. Furthermore, as long as biological activity (ability to induce reprogramming) is maintained, for example, polypeptides with deletions or additions of one to several residues (for example, 2, 3, 4, 5, 6, 10, 15, or 20 residues) of amino acids of the N terminus and/or the C terminus, polypeptides with substitution of one to several residues (for example, 2, 3, 4, 5, 6, 10, 15, or 20 residues) of amino acids, and such may be used. Variants which may be used include for example, fragments, analogs, and derivatives of natural proteins, and fusion proteins of natural proteins with other polypeptides (for example, those with addition of heterologous signal peptides or antibody fragments). Specifically, polypeptides comprising a sequence with one or more amino acid substitutions, deletions, and/or additions in the wild-type amino acid sequence, and having a biological activity (for example, activity to induce reprogramming) equivalent to that of wild-type proteins are included. When using a fragment of a wild-type protein, normally, the fragment contains a continuous region of 70% or more, preferably 80% or more, 85% or more, more preferably 90% or more, 95% or more, or 98% or more of the wild-type polypeptide (a mature form in the case of a secretory protein).

[0065] Variants of amino acid sequences can be prepared, for example, by introducing mutations to the DNAs encoding the natural polypeptide (Walker and Gaastra, eds. *Techniques in Molecular Biology* (MacMillan Publishing Company, New York, 1983); Kunkel, *Proc. Natl. Acad. Sci. USA* 82: 488-492, 1985; Kunkel et al., *Methods Enzymol.* 154: 367-382, 1987; Sambrook et al., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Plainview, N.Y.), 1989; U.S. Pat. No. 4,873,192). An example of guidance for substituting amino acids without affecting biological activity includes the report by Dayhoff *et al.* (Dayhoff et al., in *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), 1978).

[0066] The number of amino acids that are modified is not particularly limited, but for example, it is 30% or less, preferably 25% or less, more preferably 20% or less, more preferably 15% or less, more preferably 10% or less, 5% or less, or 3% or less of all amino acids of the natural mature polypeptide, and is, for example, 15 amino acids or less, preferably ten amino acids or less, more preferably eight amino acids or less, more preferably five amino acids or less, or more preferably three amino acids or less. When substituting amino acids, activities of the protein can be expected to be maintained by substitution to an amino acid with similar side chain properties. Such substitutions are called conservative substitutions in the present invention. Examples of conservative substitutions include substitution and such among amino acids within each of the groups such as basic amino acids (such as lysine, arginine, and histidine), acidic amino acids (for example, aspartic acid and glutamic acid), uncharged polar amino acids (for example, glycine, asparagine, glutamine, serine, threonine, tyrosine, and cysteine), nonpolar amino acids (for example, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, and tryptophan), β -branched amino acids (for example, threonine, valine, isoleucine), and aromatic amino acids (for example, tyrosine, phenylalanine, tryptophan, and histidine). Furthermore, examples include substitution among amino acids whose relationship in the BLOSUM62 substitution matrix (S. Henikoff and J.G. Henikoff, Proc. Acad. Natl. Sci. USA 89: 10915-10919, 1992) is positive.

[0067] The modified proteins exhibit a high homology to the amino acid sequence of the wild-type protein. High homology refers to amino acid sequences having, for example, 70% or higher, 75% or higher, 80% or higher, 85% or higher, 90% or higher, 93% or higher, 95% or higher, or 96% or higher identity. Amino acid sequence identity can be determined using, for example, the BLASTP program (Altschul, S. F. et al., J. Mol. Biol. 215: 403-410, 1990). A search can be carried out using default parameters in the Web page of BLAST at NCBI (National Center for Biotechnology Information) (Altschul S.F. et al., Nature Genet. 3: 266-272, 1993; Madden, T.L. et al., Meth. Enzymol. 266: 131-141, 1996; Altschul S.F. et al., Nucleic Acids Res. 25: 3389-3402, 1997; Zhang J. & Madden T.L., Genome Res. 7: 649-656, 1997). Alignment of two sequences can be produced, for example, by the Blast 2 sequences program which compares two sequences (Tatiana A et al., FEMS Microbiol Lett. 174: 247-250, 1999) and the identity of the sequences can be determined. Gaps and mismatches are treated similarly, and for example, a value of identity with respect to the entire amino acid sequence of a naturally-derived cytokine (mature form after secretion) is calculated. Specifically, the proportion of the number of matching amino acids in the total number of amino acids of the wild-type protein (mature form in the case of a secreted protein) is calculated.

[0068] Furthermore, genes can be introduced with a silent mutation such that the encoded amino acid sequence is not changed. Particularly, in AT rich genes, by substituting five or more consecutive A or T nucleotides with G or C such that the encoded amino acid sequence is not changed, high expression of genes can be stably obtained.

[0069] Examples of modified proteins or proteins used for reprogramming are proteins encoded by nucleic acids that hybridize under stringent conditions with a part or all of the coding region of a gene encoding the wild-type protein and having an activity (activity to induce reprogramming) equivalent to that of the wild-type protein. In hybridization, for example, a probe is prepared either from a nucleic acid comprising a sequence of the coding region of the wild-type protein gene or a complementary sequence thereof or from a nucleic acid which is the object of hybridization, and identification can be carried out by detecting whether or not the probe hybridizes to the other nucleic acid. Stringent hybridization conditions are, for example, conditions of performing hybridization in a solution containing 5x SSC, 7% (W/V) SDS, 100 μ g/mL denatured salmon sperm DNA, 5x Denhardt's solution (1x Denhardt's solution includes 0.2% polyvinyl pyrrolidone, 0.2% bovine serum albumin, and 0.2% Ficoll) at 60°C, preferably 65°C, and more preferably 68°C, and then washing by shaking for two hours in 2x SSC, preferably in 1x SSC, more preferably in 0.5x SSC, and more preferably in 0.1x SSC at the same temperature as hybridization.

[0070] Examples of genes particularly preferable for inducing cellular reprogramming typically include F-box protein 15 (Fbx15, NM_152676, NM_015798), Nanog (NM_024865, AB093574), ERAS (ES cell expressed Ras; NM_181532, NM_181548), DPPA2 (NM_138815, NM_028615), OCT3/4 (also called POU5F1; NM_002701, NM_203289, NM_013633, NM_001009178), SOX2 (NM_003106, NM_011443, XM_574919), TCL1A (T-cell leukemia/lymphoma 1A; NM_021966, NM_009337), KLF4 (Kruppel-like factor 4; NM_004235, NM_010637), catenin β 1 (cadherin-associated protein beta 1; NM_001904, NM_007614; including the S33Y mutant), c-MYC (NM_002467, NM_010849; including the T58A mutant), and Glis1 gene (Maekawa et al., Nature, 474: 225-229, 2011; NM_147193, NM_147221), as well as other genes which are members of the families to which these genes belong. When these

genes are introduced, the proportion of colonies showing the morphology of pluripotent stem cells has been reported to be higher than when the four types of genes (OCT3/4, SOX2, KLF4, and c-MYC) are introduced (WO 2007/69666). Therefore, further use of Sendai virus vectors further carrying any one of the above-mentioned genes or a combination thereof in addition to the KLF gene, OCT gene, and SOX gene, Sendai virus vectors carrying a gene other than the OCT gene and SOX gene in addition to the KLF gene, or Sendai virus vectors carrying any one of the above-mentioned genes or a combination thereof in addition to the Sendai virus vector of the present invention carrying the KLF gene, OCT gene, and SOX gene and the vector containing the KLF gene but not the OCT gene and SOX gene, or a combination of these vectors, is useful for use in the induction of cellular reprogramming in the present invention, and in particular, they can be used favorably for the induction of pluripotent stem cells. Individual virus vectors can be used by combining them at the time of use. Furthermore, they can be combined in advance as a kit, or they may be mixed to form a composition. Furthermore, methods for further introducing one or more Sendai virus vectors containing any combination of any (or all) of the above-mentioned genes in addition to the vector containing in one vector the KLF gene, OCT gene, and SOX gene in this order and the vector containing the KLF gene but not containing the OCT gene and the SOX gene, and kits or compositions containing these vectors are also included in the present invention.

[0071] It is preferable to combine the vectors of the present invention for expressing the KLF gene, OCT gene, and SOX gene and the vectors containing the KLF gene but not containing the OCT gene and SOX gene particularly with vectors for expressing the MYC gene and/or the Glis1 gene (Takahashi, K. and Yamanaka S., *Cell* 126: 663-676, 2006; Lowry WE et al., *Proc Natl Acad Sci USA*, 105(8): 2883-8, 2008; Masaki, H. et al., *Stem Cell Res.* 1: 105-115, 2008; Maekawa et al., *Nature*, 474: 225-229, 2011; WO 2007/69666). Herein, the SOX protein, the KLF protein, the MYC protein, and the OCT protein, and their genes refer to proteins and genes which are members belonging to the SOX family, the KLF family, the MYC family, and the OCT family, respectively. There are reports that by making adjustments so that one or more members from each of these four families are expressed, pluripotent stem cells can be induced from various differentiated cells. For example, regarding the SOX family genes, the use of any of the SOX1, SOX2, SOX3, SOX15, and SOX17 genes has been reported to be able to induce pluripotent stem cells (WO 2007/69666). Regarding the KLF family as well, pluripotent stem cells could be induced with KLF4 or KLF2 (WO 2007/69666). Regarding the MYC family as well, not only the wild-type c-MYC but the T58A mutant, N-MYC, and L-MYC could also induce pluripotent stem cells (WO 2007/69666; Bliloch R. et al., *Cell Stem Cell*, 1: 245-247, 2007). This way, since genes of the families can be selected in various ways and then used, reprogramming can be induced by appropriately selecting genes from the four families mentioned above.

[0072] Specifically, the KLF family includes KLF1 (NM_006563, NM_010635), KLF2 (NM_016270, NM_008452), KLF4 (NM_004235, NM_010637), and KLF5 (NM_001730, NM_009769); the MYC family includes c-MYC (NM_002467, NM_010849, including the T58A mutant), N-MYC (NM_005378, NM_008709), and L-MYC (NM_005376, NM_005806); the OCT family includes OCT1A (NM_002697, NM_198934), OCT3/4 (NM_002701, NM_203289, NM_013633, NM_001009178), and OCT6 (NM_002699, NM_011141); and the SOX family includes SOX1 (NM_005986, NM_009233), SOX2 (NM_003106, NM_011443, XM_574919), SOX3 (NM_005634, NM_009237), SOX7 (NM_031439, NM_011446), SOX15 (NM_006942, NM_009235), SOX17 (NM_022454, NM_011441), and SOX18 (NM_018419, NM_009236). Sendai virus vectors carrying any one of these genes according to the order of the present invention are useful for use in inducing dedifferentiation of cells in the present invention, and can be used favorably for induction of pluripotent stem cells in particular. The most preferred KLF gene, OCT gene, and SOX gene are the OCT3/4 gene, SOX2 gene, and KLF4 gene, respectively.

[0073] MYC family genes are not essential for induction of pluripotent stem cells, and pluripotent stem cells can be induced using only the genes of the three families excluding the MYC family genes (Nakagawa M. et al., *Nat Biotechnol.* 26(1): 101-6, 2008; Wering M. et al., *Cell Stem Cell* 2(1): 10-2, 2008). When the MYC gene is not expressed, for example, p53 siRNA and UTF1 can be used to significantly increase the induction efficiency of pluripotent stem cells (Y. Zhao et al., *Cell Stem Cell*, 3 (5): 475-479, 2008; N. Maherali, and K. Hochedfinger, *Cell Stem Cell*, 3 (6): 595-605, 2008). Furthermore, induction of pluripotent stem cells has been also reported to be possible using only the genes of the three families excluding the KLF family genes (Park IH et al., *Nature*, 451(7175): 141-6, 2008). In addition, by combined use of the G9a histone methyltransferase inhibitor (BIX-01294; Kubicek, S. et al., *Mol. Cell* 25: 473-481, 2007), induction of pluripotent stem cells has been reported to be possible from fetal NPC using only three genes, *i.e.*, the KLF gene, SOX gene, and MYC gene (Shi Y et al., *Cell Stem Cell*, 2(6): 525-8, 2008). Virus vectors that encode the respective genes can be separately prepared individually. They can be used by

combining them at the time of use. Any combination or all of them may be combined to form a kit or mixed to form a composition. In addition to the Sendai virus vector of the present invention carrying the KLF gene, OCT gene, and SOX gene and the Sendai virus vector containing the KLF gene but not containing the OCT gene and SOX gene, the present invention also relates to kits or compositions containing any combination (or all) of genes other than these three genes and compounds. The genes may be suitably loaded onto recombinant vectors. Furthermore, a portion of the recombinant vectors included in this kit can be substituted with proteins, synthetic compounds, or such having corresponding functions.

[0074] Other genes can be further combined to the above-described combination of genes to increase the efficiency of induction of reprogramming. Examples of such genes include TERT (NM_198253, NM_009354) and/or SV40 large T antigen (NC_001669.1, Fiers, W. (05-11-1978) *Nature* 273(5658): 113-120) (Park IH. et al., *Nature*, 451 (7175): 141-6, 2008). One or more genes selected from the group consisting of HPV16 E6, HPV16 E7, and Bmi1 (NM_005180, NM_007552) may also be further combined. Furthermore, one or any combination of genes selected from the group consisting of Fbx15 (*Mol Cell Biol.* 23(8): 2699-708, 2003), Nanog (*Cell* 113: 631-642, 2003), ERas (*Nature* 423: 541-545, 2003), DPPA2 (*Development* 130: 1673-1680, 2003), TCL1A (*Development* 130: 1673-1680, 2003), and β -Catenin (*Nat Med* 10(1): 55-63, 2004) may be expressed. In addition, one or more genes selected from the group consisting of ECAT1 (AB211062, AB211060), DPPA5 (NM_001025290, NM_025274, XM_236761), DNMT3L (NM_013369, NM_019448), ECAT8 (AB211063, AB211061), GDF3 (NM_020634, NM_008108), SOX15 (NM_006942, NM_009235), DPPA4 (NM_018189, NM_028610), FTHL17 (NM_031894, NM_031261), SALL4 (NM_020436, NM_175303), Rex-1 (NM_174900, NM_009556), Utf1 (NM_003577, NM_009482), DPPA3 (NM_199286, NM_139218, XM_216263), STAT3 (NM_139276, NM_213659), and GRB2 (NM_002086, NM_008163) may be combined. Combinations comprising each NANOG gene (NM_024865, AB093574) and LIN28 gene (NM_024674) in addition to the KLF gene, the OCT gene, and the SOX gene are useful for inducing pluripotent stem cells (Yu J. et al., *Science*, 318(5858): 1917-20, 2007). In addition, further combining with MYC gene is preferred (Liao J et al., *Cell Res.* 18(5): 600-3, 2008). By additionally expressing these genes, induction of pluripotent stem cells may be promoted (WO 2007/69666). When mature B cells are the subjects, for example, the myelocytic transcription factor C/EBP α (CCAAT/enhancer-binding-protein α) (NM_004364) can be ectopically expressed, or expression of the B cell transcription factor Pax5 (paired box 5; NM_016734) can be suppressed to promote reprogramming (Hanna J, *Cell.* 133(2): 250-64, 2008). These factors can also be expressed using the Sendai virus vectors of the present invention. Furthermore, a portion of the recombinant vectors included in this kit can be substituted with proteins, synthetic compounds, and such which have corresponding functions.

[0075] Furthermore, besides expressing the above-mentioned factors, for example, by combining the addition of compounds, the efficiency of reprogramming can be increased. For example, bFGF (basic fibroblast growth factor) and/or SCF (stem cell factor) can promote the induction of pluripotent stem cells, and moreover can replace the function of c-MYC in the induction of pluripotent stem cells (WO 2007/69666). Furthermore, MAP kinase inhibitor (PD98056) is also useful for establishing pluripotent stem cells that are closer to ES cells, and such (WO 2007/69666). Furthermore, DNA methylase (Dnmt) inhibitors and/or histone deacetylase (HDAC) inhibitors are reported to improve the efficiency of induction of pluripotent stem cells (Huangfu D et al., *Nat Biotechnol.* (Published online: 22 June 2008, doi:10.1038/nbt1418); *Nat. Biotechnol.* 26: 795-797 (2008)). For example, combined use of an HDAC inhibitor (VPA) enables induction of pluripotent stem cells by introduction of only two genes, OCT4 and SOX2 (Huangfu, D. et al., *Nat Biotechnol.* 2008 26(11): 1269-75). Vectors of the present invention are useful as agents used in combination with administration of these compounds. As Dnmt inhibitors, for example, 5-azacytidine and such are useful, and as HDAC inhibitors, for example, suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA), valproic acid (VPA) and such are useful. Furthermore, when using 5-azacytidine, combined use of glucocorticoid (dexamethasone) can increase the efficiency.

[0076] To reprogram cells, for example, (1) a Sendai virus vector of the present invention containing the KLF gene, OCT gene, and SOX gene in this order and a Sendai virus vector containing the KLF gene but not containing the OCT gene and SOX gene, (2) a combination of the vectors of (1) with a Sendai virus vector for expressing the MYC gene or Glis1 gene, (3) a combination produced by further adding to (1) and/or (2) a Sendai virus vector for expressing the NANOG gene and a Sendai virus vector for expressing LIN28, or (4) a combination of the vector of any one of (1) to (4) with vectors carrying other desired reprogramming factors mentioned above, and/or desired reprogramming-inducing compounds, and such may be introduced into cells. When a number of vectors and/or

compounds are combined and introduced, the introduction is preferably carried out simultaneously, and specifically, it is preferable to complete the addition of all vectors encoding the reprogramming factors and/or compounds within 48 hours or less, preferably 36 hours or less, more preferably 24 hours or less, 18 hours or less, twelve hours or less, ten hours or less, eight hours or less, six hours or less, three hours or less, two hours or less, or one hour or less of the addition of the first vector, compound, or such. The dose of the vectors can be prepared appropriately and the MOI is, for example, 0.1 or more, preferably 0.3 or more, 0.5 or more, 1 or more, 2 or more, 3 or more, 4 or more, or 5 or more, and 100 or less, preferably 90 or less, 80 or less, 70 or less, 60 or less, 50 or less, 40 or less, 30 or less, 20 or less, 10 or less, or 5 or less. Preferably, infection is carried out, for example, at MOI of 0.3 to 100, more preferably at MOI of 0.5 to 50, more preferably at MOI of 1 to 40, more preferably at MOI of 1 to 30, more preferably at MOI of 2 to 30, and for example at MOI of 3 to 30. The induced pluripotent stem cells form flat colonies very similar to those of ES cells, and express alkaline phosphatase. Furthermore, the induced pluripotent stem cells may express the undifferentiated-cell markers Nanog, OCT4, and/or SOX2, and the like. The induced pluripotent stem cells preferably express TERT and/or show telomerase activity. The present invention also relates to methods for producing cells that express alkaline phosphatase and preferably further express Nanog and/or TERT which are undifferentiated-cell markers, and to a use of the above-mentioned Sendai virus vectors in the production of these cells and in the production of pharmaceutical agents for inducing these cells.

[0077] According to the present invention, pluripotent stem cells can be produced from desired cells including adult skin cells and neonatal foreskin cells. According to the present invention, colonies of pluripotent stem cells can be induced from at least certain types of cells (for example adult skin cells and/or neonatal foreskin cells) for example at an incidence rate of 0.03×10^{-5} or more, 0.1×10^{-5} or more, 0.3×10^{-5} or more, 0.5×10^{-5} or more, 0.8×10^{-5} or more, or 1×10^{-5} or more (for example, 1×10^{-5} to 1×10^{-3}), and preferably at an incidence rate of 1.5×10^{-5} or more, 1.7×10^{-5} or more, 2.0×10^{-5} or more, 2.5×10^{-5} or more, 3×10^{-5} or more, 4×10^{-5} or more, 5×10^{-5} or more, 8×10^{-5} or more, 1×10^{-4} or more, 2×10^{-4} or more, 3×10^{-4} or more, 5×10^{-4} or more, 8×10^{-4} or more, 1×10^{-3} or more, 1.5×10^{-3} or more, 2×10^{-3} or more, 5×10^{-3} , 1×10^{-2} or more, 1.5×10^{-2} or more, 2×10^{-2} or more, 2.5×10^{-2} or more, 3×10^{-2} or more, 4×10^{-2} or more, or 5×10^{-2} or more. For example, by the present invention, pluripotent stem cells can be induced from human peripheral blood mononuclear cells with an efficiency of at least 1×10^{-2} (0.01 %), preferably at least 3×10^{-2} , 5×10^{-2} , 8×10^{-2} , 1×10^{-1} , 2×10^{-1} , 3×10^{-1} , 4×10^{-1} , or 5×10^{-1} ; and pluripotent stem cells can be induced from human peripheral blood monocytes with an efficiency of at least 1×10^{-3} (0.001 %), preferably at least 3×10^{-3} , 5×10^{-3} , 8×10^{-3} , 1×10^{-2} , 2×10^{-2} , 3×10^{-2} , 4×10^{-2} , or 5×10^{-2} . The MOI may be appropriately established as described above, and for example, it can be performed at 5, 10, or 30. The efficiency of appearance of pluripotent stem cell colonies by the vector of the present invention containing the KLF gene, OCT gene, and SOX gene in this order and a Sendai virus vector that contains the KLF gene but not the OCT gene and the SOX gene is, for example, 1.2 times or more, preferably 1.3 times or more, 1.5 times or more, 1.7 times or more, twice or more, 2.5 times or more, three times or more, 3.5 times or more, four times or more, 4.5 times or more, or five times or more for at least given cells compared to the situation in which the Sendai virus vector of the present invention expressing the KLF gene, OCT gene, and SOX gene is used, but the Sendai virus vector that contains the KLF gene but not the OCT gene and the SOX gene is not used. The methods of the present invention exert remarkable effects compared to conventional methods in that the introduction efficiency of pluripotent stem cells when cultured at 37°C after vector introduction is significantly high and the vector is rapidly removed after induction of pluripotent stem cells.

[0078] Differentiated cells which become the object of induction of reprogramming are not particularly limited, and desired somatic cells and such may be used. Production of pluripotent stem cells from somatic cells has been shown to be possible not only from cells derived from fetal mice but also from differentiated cells collected from the tail portion of adult mice, and from liver cells, and gastric mucosal cells, and this suggests that the production is not dependent on the cell type or the state of differentiation (WO 2007/069666; Aoi T. et al., Science [Published Online February 14, 2008]; Science. 2008; 321(5889): 699-702). Induction of pluripotent stem cells has been confirmed to be possible in humans as well, from various cells such as adult facial skin-derived fibroblasts, adult synoviocytes, neonatal foreskin-derived fibroblasts, adult mesenchymal stem cells, skin cells from the palm of an adult, and embryonic cells (Takahashi K et al. (2007) Cell 131: 861-872; Park IH et al., Nature, 451(7175): 141-6, 2008). Furthermore, induction of pluripotent stem cells has been reported similarly from terminally differentiated cells such

as pancreatic β cells and B lymphocytes as well (Stadtfeld M et al., Curr Biol. 2008 May 21. [PubMed, PMID: 18501604]; Curr Biol. 2008, 18(12): 890-4; Hanna J. et al., Cell. 133(2): 250-64, 2008). These findings suggest that induction of pluripotent stem cells do not depend on the cells serving as the origin. Methods of the present invention can be applied in the induction of pluripotent stem cells from these desired somatic cells. Specifically, differentiated cells which are the object of reprogramming include fibroblasts, synoviocytes, mucosal cells of the oral cavity, stomach, or such, liver cells, bone marrow cells, tooth germ cells, blood cells (for example, lymphocytes and leukocytes), and other desired cells. Blood cells may be peripheral blood cells. Fibroblasts, monocytes, mononuclear cells, and the like are also preferred. Furthermore, cells may be derived, for example, from cells of embryos, fetuses, newborns, children, adults, or the aged. The origin of the animals is not particularly limited, and includes mammals such as humans and non-human primates (monkeys and such), rodents such as mice and rats, and non-rodents such as bovine, pigs, and goats.

[0079] From the colonies of cells which have completed reprogramming, cells from which the vectors have been removed can be selected appropriately. For example, cells from which the vectors have been naturally removed may be selected. To this end, for example, negative selection can be carried out using antibodies specific to the virus vectors (for example, anti-HN antibodies). Furthermore, when using temperature-sensitive vectors, the vectors can be removed easily by culturing at normal temperatures (for example, about 37°C, specifically 36.5°C to 37.5°C, preferably 36.6°C to 37.4°C, and more preferably 36.7°C to 37.3°C), or by culturing at slightly high temperatures (for example, 37.5°C to 39°C, preferably 38°C to 39°C, or 38.5°C to 39°C). When SeV(PM)/TS Δ F, or vectors with further introduction of mutations such as TS 7, TS 12, TS 13, TS 14, or TS 15 into SeV(PM)/TS Δ F are used, passaging leads to natural loss of the vector. Examples of preferred vectors include the combination of SeV(PM)KOS/TS12 Δ F and SeV18+KLF4/TS Δ F, but are not limited thereto. Furthermore, in this case, the MYC gene can be introduced using SeV(HNL)c-rMYC/TS12 Δ F, SeV(HNL)c-rMYC/TS13 Δ F, SeV(HNL)c-rMYC/TS15 Δ F, or such. The combination of SeV(PM)KOS/TS12 Δ F, SeV18+KLF4/TS Δ F, and SeV(HNL)c-rMYC/TS15 Δ F is particularly preferable. Furthermore, not loading the MYC gene into the Sendai virus vectors carrying the KLF gene, OCT gene, and SOX gene has the advantage that reprogramming can be done without use of the carcinogenesis-related MYC gene. This is also advantageous because it is possible to freely select the fourth factor for use from c-MYC, L-MYC, Glis1, or such in addition to the KLF gene, OCT gene, and SOX gene.

[0080] Cells produced by the methods of the present invention are useful for causing differentiation into a variety of tissues and cells, and can be used in desired examinations, research, diagnosis, tests, treatments, and such. For example, induced stem cells are expected to be utilized in stem cell therapy. For example, reprogramming is induced by using somatic cells collected from patients, and then somatic stem cells and other somatic cells that are obtained by induction of differentiation can be transplanted into patients. Methods for inducing cellular differentiation are not particularly limited, and for example, differentiation can be induced by retinoic acid treatment, treatment with a variety of growth factors/cytokines, and treatment with hormones. Furthermore, the obtained cells can be used for detecting effects of the desired pharmaceutical agents and compounds, and this enables screening of pharmaceutical agents and compounds to be carried out.

Examples

[0081] Hereinbelow, the present invention is specifically described with reference to the Examples; however, it is not to be construed as being limited thereto

[Example 1]

Construction of pSeV(PM)/TS Δ F

[0082] The method for producing the Sendai virus vector pSeV(PM)/TS Δ F used in the present Example is shown below. In the present invention, "(PM)" refers to inserting a reprogramming gene between the P gene and the M gene, and "(HNL)" refers to inserting a reprogramming gene between the HN gene and the L gene. Furthermore, in

the present invention, "TS" refers to having G69E, T116A, and A183S mutations in the M protein, A262T, G264R, and K461G mutations in the HN protein, L511F mutation in the P protein, and N1197S and K1795E mutations in the L protein; and "ΔF" shows that the F gene is deleted. Hereinbelow, "SeV(PM)/TSΔF" refers to an F gene-deleted Sendai virus vector (Z strain) having G69E, T116A, and A183S mutations in the M protein; A262T, G264R, and K461G mutations in the HN protein; L511F mutation in the P protein; and N1197S and K1795E mutations in the L protein. This vector has an insertion site (*NotI* site) for gene introduction immediately downstream of the P gene (between the P gene and M gene). However, these are exemplary, and the present invention is not limited to these examples.

[0083] pSeV(PM)/TSΔF was constructed as follows. PCR was carried out (94°C for three minutes, and 25 cycles of [98°C for ten seconds, 55°C for 15 seconds, and 72°C for 12 minutes], followed by 72°C for seven minutes) using Litmus SallNheIfrg PmutMtsHNts ΔF-GFP delGFP (International Publication No. WO 2010/008054) as template, together with PMNOTI-F (5'-GAAATTTTCACCTAAGCGGCCGCAATGGCAGATATCTATAG -3') (SEQ ID NO: 10) and PMNOTI-R (5'-CTATAGATATCTGCCATTGCGGCCGCTTAGGTGAAATTTTC -3') (SEQ ID NO: 11). The approximately 11-kb PCR product obtained was treated with *DpnI*, and then *Escherichia coli* DH5α (ToYoBo Code No. DNA-903) was transformed with 20 μL of this reaction solution. A plasmid having the *NotI* sequence was selected by *NotI* digestion. Then, a clone that has the correct sequence was selected by sequencing. Thus, Litmus SallNheIfrg PmutMtsHNts(PM)-dF was obtained. Then, Litmus SallNheIfrg PmutMtsHNts(PM)-dF was digested with *SalI* and *NheI*, and the collected fragment (approximately 8 kbp) was ligated to a fragment (approximately 8 kbp) collected upon *SalI/NheI* digestion of the pSeV/ΔSallNheIfrg Lmut plasmid (International Publication No. WO 2003/025570) having two mutations in the L gene. Thus, pSeV (PM)/TSΔF was obtained. This vector has an insertion site (*NotI* site) for gene introduction between the P and M genes. The Sendai virus generated from a transcription product of this vector is referred to as SeV(PM)/TSΔF.

[Example 2]

Construction of pSeV18+KLF4/TSΔF

[0084] From a Jurkat cDNA library, the KIF4 gene was amplified by carrying out PCR using the PrimeStar HS DNA polymerase (Takara Bio Inc., Catalogue No. R010A), and this was inserted at the *NotI* site of a BlueScript plasmid vector to obtain the plasmid pBS-KS-KLF4 (WO2010/008054). pBS-KS-KLF4 was digested with *NotI* (37°C, 3 hours) and separated by electrophoresis on a 1% agarose gel. A band of approximately 1.5k bp was excised and purified with the Qiaquick Gel Extraction Kit (Quiagen, Catalogue No. 28706). The *NotI* fragment containing this KLF4 gene was cloned into the pSeV18+/TSΔF vector at the *NotI* site, and clones with the correct sequence were selected by sequencing to obtain pSeV18+KLF4/TSΔF. The Sendai virus generated from a transcription product of the pSeV18+/TSΔF vector is referred to as SeV18+/TSΔF, and the Sendai virus generated from a transcription product of the pSeV18+KLF4/TSΔF vector is referred to as SeV18+KLF4/TSΔF.

[Example 3]

Construction of pSeV(PM)/TS12ΔF

[0085] PCR (94°C for three minutes, and 30 cycles of [98°C for 10 seconds, 55°C for 15 seconds, and 72°C for 1.5 minutes], followed by 72°C for five minutes, and ∞ at 4°C) was carried out using PrimeStar, with pSeV(PM)/TSΔF as template, and primers F3208 (5'-AGAGAACAAGACTAAGGCTACC-3' (SEQ ID NO: 12)) and R3787 (5'-ACCTTGACAATCCTGATGTGG-3' (SEQ ID NO: 13)). The amplified band of about 600 bp was purified using the QIAquick PCR Purification Kit. PCR (94°C for three minutes, and 30 cycles of [98°C for 10 seconds, 55°C for 15 seconds, and 72°C for 1.5 minutes], followed by 72°C for five minutes, and ∞ at 4°C) was carried out using PrimeStar, with pSeV18+Oct3/4/TS12ΔF (WO 2010/008054; WO 2012/029770) as template, and primers F2001 (5'-

CCATCAACACTCCCCAAGGACC-3' (SEQ ID NO: 14)) and R3390 (5'-AGACGTGATGCGTTTGAGGCC-3' (SEQ ID NO: 15)). The amplified band of about 1.4 kbp was purified using the QIAquick PCR Purification Kit. These PCR products were mixed, and PCR (94°C for three minutes, and 30 cycles of [98°C for 10 seconds, 55°C for 15 seconds, and 72°C for two minutes], followed by 72°C for five minutes, and ∞ at 4°C) was carried out using PrimeStar, and the F2001 and R3787 primers. The PCR products were purified using the QIAquick PCR Purification Kit, digested with *Sall* and *NotI*, and then separated by agarose gel electrophoresis. Then, a band of about 1.6 kbp was excised, and purified using the QIAquick Gel Extraction Kit. pSeV(PM)/TSΔF was digested with *Sall* and *NotI*, and then separated by agarose gel electrophoresis. Then, a band of about 14.8 kbp was excised, and purified using the QIAquick Gel Extraction Kit. These two *Sall* and *NotI*-digested and purified products were used for ligation, and a clone that has the correct sequence was selected by sequencing to obtain pSeV(PM)/TS12ΔF. This vector has an insertion site (*NotI* site) for gene introduction between the P gene and M gene. The Sendai virus generated from a transcription product of this vector is referred to as SeV(PM)/TS12ΔF.

[Example 4]

Construction of SeV(PM)KOS/TS12ΔF

[0086] Plasmids for production of SeV/TS12ΔF vectors carrying the KLF4-OCT3/4-SOX2 genes were produced as shown below. pSeV(PM)/TSΔF was digested with *NotI*, then purified using the QIA quick PCR Purification Kit (QIAGEN catalogue No. 28106), and then subjected to BAP treatment. Then, this was purified using the QIAquick PCR Purification Kit. pSeV(PM)KOS/TSΔF was digested with *NotI*, separated by 1% agarose gel electrophoresis, a band of about 3.6 kbp was excised, and purification was carried out using the QIAquick Gel Extraction Kit (QIAGEN, catalog No. 28706). 100 μl of an elution solution attached to the kit was used for elution. The *NotI* fragment containing the KLF4 gene, the OCT3/4 gene, and the SOX2 gene was cloned into the *NotI* site of the pSeV(PM)/TS12ΔF vector, and a clone that has the correct sequence was selected by sequencing. Thus, pSeV(PM)KOS/TS12ΔF was obtained. The Sendai virus generated from a transcription product of this vector is referred to as SeV(PM)KOS/TS12ΔF.

[Example 5]

Generation of pSeV(HNL)c-rMyc/TS15ΔF

[0087] Litmus38TSΔF-P2(HNL) ΔGFP and pSeV/TSΔF-Linker L1361CL1558I (WO2010/008054; WO2012/029770) were individually digested with *Sall*-*NheI*, separated by agarose gel electrophoresis, and bands of 8.0 kbp and 8.3 kbp were excised individually and purified. These purified fragments were ligated to obtain pSeV(HNL)/TS15ΔF (a plasmid encoding the antigenome of SeV(HNL)/TS15ΔF). To the *NotI* site of this pSeV(HNL)/TS15ΔF was introduced a *NotI* fragment containing the c-rMyc gene which was excised and purified from pBS-KS-c-rMyc by *NotI* digestion, to obtain pSeV(HNL)c-rMyc/TS15ΔF. The nucleotide sequence and amino acid sequence of c-rMyc are shown in SEQ ID NOs: 8 and 9. c-rMyc has a378g, t1122c, t1125c, a1191g, and a1194g mutations. The Sendai virus generated from a transcription product of this vector is referred to as SeV(HNL)c-rMyc/TS15ΔF.

[Example 6]

Production of induced pluripotent stem cells (iPS cells) by the Sendai virus vector that retains genes for cellular reprogramming -1

[0088] Human neonatal foreskin-derived fibroblasts (BJ) from ATCC (CRL-2522; www.atcc.org) were cultured at 5 ×

10⁵ overnight in DMEM (GIBCO-BRL, 11995) containing 10 % FBS (GIBCO-BRL), penicillin (100 u/ml) and streptomycin (100 µg/ml; Nacalai Tesque, Code 26253-84), 10% FBS (Cell Culture Bioscience, Cat. No. 171012) (herein below referred to as 10%FBS/PS/DMEM) in a 5% CO₂ incubator at 37°C. Then, the cells were infected with the vectors under the conditions below.

Condition 1: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV18+KLF4/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30
SeV18+KLF4/TSΔF	MOI=3

Condition 2: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV18+KLF4/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30
SeV18+KLF4/TSΔF	MOI=10

Condition 3: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV18+/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30
SeV18+/TSΔF	MOI=3

Condition 4: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV18+/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30
SeV18+/TSΔF	MOI=10

Condition 5: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30

Condition 6: vector non-administration group (control group)

[0089] After the above vectors were administered to cells, they were cultured in a 5% CO₂ incubator at 37°C. Assessment was performed at n=2. The medium was exchanged with 10%FBS/PS/DMEM almost everyday. On day 6 of the infection, 1.0×10⁴ (cells) of the above vector-introduced cells detached using a 0.25% trypsin-EDTA solution were added to 1.25×10⁵ (cells) of mitomycin-treated feeder cells (for example, MEF) prepared in gelatin-coated 6-well plates, and they were cultured in a CO₂ incubator. Next day, the medium was changed from 10%FBS/PS/DMEM to a primate ES cell medium (ReproCell, RCHEMD001) with an addition of bFGF at 4 ng/ml, and the cells were cultured in a 5% CO₂ incubator at 37°C. The medium exchange was carried out everyday or once every two days. The medium could be the conditioned medium of the feeder cells.

[0090] On day 28 of the infection, the efficiency of iPS cell induction was observed by staining the activity of alkaline phosphatase which is an ES cell undifferentiation marker with NBCT/BCIP (PIERCE; NBT/BCIP, 1-Step, #34042) (Fig. 1). Compared with the test group that has no addition of the SeV18+KLF4/TSΔF vector (Condition 5 above), the test groups with addition of the SeV18+KLF4/TSΔF vector (Conditions 1 and 2 above) showed improvement of the efficiency of iPS cell induction in a manner dependent on the amount of the SeV18+KLF4/TSΔF vector added. Fig. 2 shows a graph of the average induction efficiency at n=2. The efficiency of iPS cell induction was improved about 6.9 times when the SeV18+KLF4/TSΔF vector was added at MOI=3, and about 12 times when it was added at MOI=10. The efficiency of iPS cell induction was improved about 3.2 times when the SeV18+ /TSΔF vector was added at MOI=3, and about 6 times when it was added at MOI=10.

[Example 7]

Production of induced pluripotent stem cells (iPS cells) by the Sendai virus vector that retains genes for cellular reprogramming -2

[0091] Human neonatal foreskin-derived fibroblasts (BJ) from ATCC (CRL-2522; www.atcc.org) were cultured in 6-well plates at 5×10^5 (cells) overnight in DMEM (GIBCO-BRL, 11995) containing 10 % FBS (GIBCO-BRL), penicillin (100 u/ml) and streptomycin (100 µg/ml; Nacalai Tesque, Code 26253-84), 10% FBS (Cell Culture Bioscience, Cat. No. 171012) (herein below referred to as 10%FBS/PS/DMEM) in a 5% CO₂ incubator at 37°C. Then, the cells were infected with the vectors under the conditions below. Assessment was performed at n=2.

Condition 1: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV18+KLF4/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=5
SeV(HNL)c-rMYC/TS15ΔF	MOI=5
SeV18+KLF4/TSΔF	MOI=5

Condition 2: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV1 8+KLF4/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30
SeV18+KLF4/TSΔF	MOI=10

Condition 3: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF

SeV(PM)KOS/TS12ΔF	MOI=5
SeV(HNL)c-rMYC/TS15ΔF	MOI=5

Condition 4: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30

Condition 5: vector non-administration group 1 (control group 1)

Condition 6: vector non-administration group 2 (control group 2)

[0092] After the above vectors were administered to cells, Conditions 1, 2 and 5 were cultured in a 5% CO₂ incubator at 37°C, and Conditions 3, 4 and 6 were cultured in a 5% CO₂ incubator at 35°C. The medium was exchanged with 10%FBS/PS/DMEM almost everyday. On day 6 of the infection, 1.0×10^4 (cells) of the above vector-introduced cells detached using a 0.25% trypsin-EDTA solution were added to 1.25×10^5 (cells) of mitomycin-treated feeder cells (for example, MEF) prepared in gelatin-coated 6-well plates, and they were cultured in a 5% CO₂ incubator at 37°C. Next day, the medium was changed from 10%FBS/PS/DMEM to a primate ES cell medium (ReproCell, RCHEMD001) with an addition of bFGF at 4 ng/ml, and the cells were cultured in a 5% CO₂ incubator at 37°C. The medium exchange was carried out everyday or once every two days. The medium could be the conditioned medium of the feeder cells.

[0093] On day 28 of the infection, the efficiency of iPS cell induction was observed by staining the activity of alkaline phosphatase which is an ES cell undifferentiation marker with NBCT/BCIP (PIERCE; NBT/BCIP, 1-Step, #34042) (Fig. 3). Compared with the test groups that have no addition of the SeV18+KLF4/TSΔF vector (Conditions 3 and 4 above), the test groups with addition of the SeV18+KLF4/TSΔF vector (Conditions 1 and 2 above) showed a high efficiency in the induction of iPS cells. In the vector non-administration groups (Conditions 5 and 6 above), the induction of iPS cells was not observed. Fig. 4 shows a graph of the average values from results obtained at n=2.

[Example 8]

Production of induced pluripotent stem cells (iPS cells) by the Sendai virus vector that retains genes for cellular reprogramming -3

[0094] Human adult skin-derived fibroblasts HDF (Applications, Inc. 106-05A-1388) were cultured in 6-well plates at 5×10^5 (cells) overnight in DMEM (GIBCO-BRL, 11995) containing 10 % FBS (GIBCO-BRL), penicillin (100 u/ml) and streptomycin (100 µg/ml; Nacalai Tesque, Code 26253-84), 10% FBS (Cell Culture Bioscience, Cat. No. 171012) (herein below referred to as 10%FBS/PS/DMEM) in a 5% CO₂ incubator at 37°C. Then, the cells were infected with the vectors under the conditions below. Assessment was performed at n=2.

Condition 1: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV18+KLF4/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=5
SeV(HNL)c-rMYC/TS15ΔF	MOI=5
SeV18+KLF4/TSΔF	MOI=5

Condition 2: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV18+KLF4/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30
SeV18+KLF4/TSΔF	MOI=10

Condition 3: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30

Condition 4: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF

SeV(PM)KOS/TS12ΔF	MOI=5
SeV(HNL)c-rMYC/TS15ΔF	MOI=5

Condition 5: vector non-administration group 1 (control group 1)

Condition 6: vector non-administration group 2 (control group 2)

[0095] After the above vectors were administered to cells, Conditions 1, 2 and 5 were cultured in a 5% CO₂ incubator at 37°C, and Conditions 3, 4 and 6 were cultured in a 5% CO₂ incubator at 35°C. The medium was exchanged with 10%FBS/PS/DMEM almost everyday. On day 6 of the infection, 1.0×10^5 (cells) of the above vector-introduced cells detached using a 0.25% trypsin-EDTA solution were added to 1.25×10^5 (cells) of mitomycin-treated feeder cells (for example, MEF) prepared in gelatin-coated 6-well plates, and they were cultured in a 5% CO₂ incubator at 37°C. Next day, the medium was changed from 10%FBS/PS/DMEM to a primate ES cell medium (ReproCell, RCHMD001) with an addition of bFGF at 4 ng/ml, and the cells were cultured in a 5% CO₂ incubator at 37°C. The medium exchange was carried out everyday or once every two days. The medium could be the conditioned medium of the feeder cells.

[0096] On day 28 of the infection, the efficiency of iPS cell induction was observed by staining the activity of alkaline phosphatase which is an ES cell undifferentiation marker with NBCT/BCIP (PIERCE; NBT/BCIP, 1-Step, #34042) (Fig. 5). Compared with the test groups that have no addition of the SeV18+KLF4/TSΔF vector (Conditions 3 and 4 above), the test groups with addition of the SeV18+KLF4/TSΔF vector (Conditions 1 and 2 above) showed a high efficiency in the induction of iPS cells. In the vector non-administration groups (Conditions 5 and 6 above), the induction of iPS cells was not observed. Fig. 6 shows a graph of the average values from results obtained at n=2.

[Example 9]

Production of induced pluripotent stem cells (iPS cells) by the Sendai virus vector that retains genes for cellular reprogramming -4

[0097] Human fetal lung cell-derived fibroblasts (MRC-5; ATCC, CCL-171) were cultured in 6-well plates at 2×10^5 (cells) overnight in DMEM (GIBCO-BRL, 11995) containing 10 % FBS (GIBCO-BRL), penicillin (100 u/ml) and streptomycin (100 µg/ml; Nacalai Tesque, Code 26253-84), 10% FBS (Cell Culture Bioscience, Cat. No. 171012) (herein below referred to as 10%FBS/PS/DMEM) in a 5% CO₂ incubator at 37°C. Then, the cells were infected with the vectors under the conditions below. Assessment was performed at n=2.

Condition 1: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV18+KLF4/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=5
SeV(HNL)c-rMYC/TS15ΔF	MOI=5
SeV18+KLF4/TSΔF	MOI=5

Condition 2: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF+SeV18+KLF4/TSΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30
SeV18+KLF4/TSΔF	MOI=10

Condition 3: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF

SeV(PM)KOS/TS12ΔF	MOI=5
SeV(HNL)c-rMYC/TS15ΔF	MOI=5

Condition 4: SeV(PM)KOS/TS12ΔF+SeV(HNL)c-rMYC/TS15ΔF

SeV(PM)KOS/TS12ΔF	MOI=30
SeV(HNL)c-rMYC/TS15ΔF	MOI=30

Condition 5: vector non-administration group 1 (control group 1)

Condition 6: vector non-administration group 2 (control group 2)

[0098] After the above vectors were administered to cells, Conditions 1, 2 and 5 were cultured in a 5% CO₂ incubator at 37°C, and Conditions 3, 4 and 6 were cultured in a 5% CO₂ incubator at 35°C. The medium was exchanged with 10%FBS/PS/DMEM almost everyday. On day 6 of the infection, 1.0×10^5 (cells) of the above vector-introduced cells detached using a 0.25% trypsin-EDTA solution were added to 1.25×10^5 (cells) of mitomycin-treated feeder cells (for example, MEF) prepared in gelatin-coated 6-well plates, and they were cultured in a 5% CO₂ incubator at 37°C. Next day, the medium was changed from 10%FBS/PS/DMEM to a primate ES cell medium (ReproCell, RCHEMD001) with an addition of bFGF at 4 ng/ml, and the cells were cultured in a 5% CO₂ incubator at 37°C. The medium exchange was carried out everyday or once every two days. The medium could be the conditioned medium of the feeder cells.

[0099] On day 28 of the infection, the efficiency of iPS cell induction was observed by staining the activity of alkaline phosphatase which is an ES cell undifferentiation marker with NBCT/BCIP (PIERCE; NBT/BCIP, 1-Step, #34042) (Fig. 7). Compared with the test groups that have no addition of the SeV18+KLF4/TSΔF vector (Conditions 3 and 4 above), the test groups with addition of the SeV18+KLF4/TSΔF vector (Conditions 1 and 2 above) showed a high efficiency in the induction of iPS cells. In the vector non-administration groups (Conditions 5 and 6 above), the induction of iPS cells was not observed. Fig. 8 shows a graph of the average values from results obtained at n=2.

[Example 10]

Assessment of the *in vivo* pluripotency of the induced artificial pluripotent stem cells

[0100] The *in vivo* pluripotency was confirmed by teratoma formation in immunocompromised mice. The BJ cell-derived iPS cell clone BJ-1215K#1 or the HDF cell-derived iPS cell clone HDF-1215K#1 was inoculated subcutaneously to SCID mice, and tumor mass formation was confirmed about one month later. Approximately 2 months later, specimens were harvested, fixed in 10% formalin and embedded in paraffin. The tissue sections were stained with hematoxylin and eosin, and triploblastic differentiation was confirmed (Fig. 9).

[Example 11]

Karyotype analysis

[0101] Karyotype analysis of the BJ cell-derived iPS cell clone BJ-1215K#1, the HDF cell-derived iPS cell clone HDF-1215K#1, and the MRC-5 cell-derived iPS cell clone MRC5-1215K#1 at the 9th passage was performed in a request to Nihon Gene Research Laboratories Inc. The results showed that they have 46 chromosomes, and that the karyotype is normal (Fig. 10).

[Example 12]

Induction of iPS cells from mouse fibroblasts

[0102] Fibroblasts from 129+TER/SvJcl mice were inoculated to a 6-well plate at 5×10^5 cells/well, and cultured overnight in a CO₂ incubator (37°C, 5% CO₂). Cells in the wells for cell count measurement were detached using a trypsin-EDTA solution, and the number of cells per well was measured. The amount of vector was calculated based on this cell count. Each of the SeV(PM)KOS/TS12ΔF vector, SeV(HNL)c-rMYC/TS15ΔF vector and SeV18+KLF4/TSΔF vector was added to the 10%FBS/PS/DMEM medium at MOI=5, and vector solutions were prepared to be 1 ml final solutions. The culture solution was removed from the 6-well plate, followed by addition of the prepared vector solutions, and culturing was carried out for about 24 hours in a CO₂ incubator (37°C, 5% CO₂). The cell culture solution was then removed, 10%FBS/PS/DMEM medium was added (2 mL/well), and culturing was carried out for about 24 hours in a CO₂ incubator (37°C, 5% CO₂). The cell culture solution was removed, the ES cell medium was added (the 2i medium was prepared by mixing 250 ml of an ES cell medium (Neurobasal medium; Life technologies, Cat. No.21103-049), 250 ml of DMEM/F-12, GlutaMAX (Life technologies, Cat. No. 10565-018), 10 ml of B27 (Life technologies, Cat. No. 17504044), 5 ml of N2 (Life technologies, Cat. No. 17502-048), 3.5 μl of 2-mercaptoethanol (Sigma, Cat. No. M3148-100ML), 2.5 ml of L-Glutamine (Life technologies, Cat. No.25030-0841), 5 ml of penicillin-streptomycin (Nacalai Tesque, Cat. No. 26253-84), 100 μl of PD0325901 (prepared at 5 mM; Funakoshi, Cat. No. Axon1408), and 100μl of CT 99021 (prepared at 15 mM; Funakoshi, Cat. No. Axon1386). LIF (Millipore, Cat. No. ESG1106) was added to the 2i medium at a final concentration of 1000 U/ml, and this was used as a mouse ES cell medium (herein below referred to as 2i/LIF medium)), and culturing was carried out in a CO₂ incubator (37°C, 5% CO₂). Hereinafter until day 5 of the vector infection, the culturing was carried out while exchanging the medium with the 2i/LIF medium. On day 5 of the infection, feeder cells were inoculated into 6-well plates coated with a 0.1 % gelatin solution at 1.4×10^5 cells/well/2 ml 10%FBS/PS/DMEM and 10 cm dishes at 7×10^5 cells/dish/10 ml 10%FBS/PS/DMEM, and they were cultured in a CO₂ incubator (37°C, 5% CO₂). On day 6 of the infection, vector-infected cells were detached using trypsin-EDTA, the 2i/LIF medium was added to prepared feeder cells, cells were plated into 6-well plates at a proportion of 1×10^4 cells/well and 1×10^5 cells/well or into 10 cm dishes at a proportion of 1×10^4 cells/dish and 1×10^5 cells/dish, and cultured in a CO₂ incubator (37°C, 5% CO₂).

After this, induction of iPS cells was carried out with medium exchange with the 2i/LIF medium almost everyday. On day 21 of the infection, alkaline phosphatase staining was carried out (Fig. 11). iPS cell-like alkaline phosphatase-positive cells were measured, and the resulting induction efficiency was about 0.1%.

[Example 13]

Obtaining clones of mouse iPS cells

[0103] From the 10 cm dishes with cells seeded at 1×10^4 cells/dish and 1×10^5 cells/dish in Example 12, 6 clones and 18 clones were picked up, respectively, on day 16 of the infection, and they were passaged and cultured. Removal of the Sendai virus vector was confirmed by staining with an anti-SeV antibody (Fig. 12). The results shows that nine Sendai virus vector-negative clones were obtained (clone numbers: 5-2, 5-6, 5-7, 5-11, 5-13, 5-14, 5-15, 5-16 and 5-17).

[Example 14]

Gene expression analysis by immunostaining

[0104] The mouse iPS cells that were induced in Example 12 and confirmed to be negative of the Sendai virus vectors in Example 13 were assessed by immunostaining with an anti-NANOG antibody and an anti-OCT4 antibody (Fig. 13). The results show that NANOG and OCT4 of three iPS cell clones (5-2, 5-7 and 5-17) were stained similarly as mouse ES cells, which are the positive control. NANOG and OCT4 of mouse fibroblasts, which are the negative control, were not stained. Accordingly, the assessed iPS cells were shown to be expressing the NANOG and OCT4 genes at the protein level.

[Example 15]

Assessment of pluripotency

[0105] Mouse iPS cells that were induced in Example 12 and confirmed to be negative of the Sendai virus vectors in Example 13 were assessed for their pluripotency.

[0106] Embryoid bodies were formed by culturing in a serum medium that does not contain LIF (Fig. 14). The same was with mouse ES cells.

[0107] After embryoid body formation, the cells were passaged in gelatin-coated plates and cultured in a serum medium containing no LIF, and the cellular nucleus was stained by GATA4 (a transcription factor which is a marker of the endoderm) staining on day 4 (Fig. 15).

[0108] Culturing was continued in a serum medium containing no LIF, and passaging was done in gelatin-coated plates. On day 10, tissues that are thought to be pulsating cardiac muscles were observed. On day 14, the cells were fixed and immunostained using an antibody against α -Actinin (Fig. 16). The cells were stained overall. Among them, cells strongly stained were observed in some regions.

[0109] On day 4 of culturing in a 2i medium containing no LIF and passaging in gelatin-coated plates, differentiation into nerve cells that are β III tubulin-positive (Fig. 17) and tyrosine hydroxylase-positive (Fig. 18) was shown. These

results showed that it is possible to induce from mouse cells, iPS cells that have triploblastic differentiation ability by using the Sendai virus vectors of the present invention.

[Example 16]

Induction of iPS cells from human monocytes -1

[0110] Induction of iPS cells from human CD14⁺ monocytes was performed. The monocytes used were purchased from LONZA (Cat. No. 2W-400C). The monocytes were cultured in IMDM containing 10% Fetal Bovine Serum and penicillin-streptomycin (hereinafter referred to as 10%FBS/PS/IMDM). The frozen monocyte cells were thawed in a 37°C water bath, transferred to 50 ml Falcon tubes containing 10 ml of 10%FBS/PS/IMDM, and mixed gently. After centrifugation at 100g, 5 minutes, room temperature, the supernatant was removed, and 10 ml of 10%FBS/PS/IMDM was added and mixed. The cell number was counted using a hemocytometer. The cells were inoculated into 6-well plates at 5×10^5 cells/well/2 ml 10%FBS/PS/IMDM, and cultured in a CO₂ incubator (37°C, 5% CO₂).

[0111] After collection of the cells in Eppendorf tubes and centrifugation, the supernatant was removed, 500 µl of 10%FBS/PS/IMDM was added and mixed, and the cell number was counted. The amount of vector required was calculated from the cell number, and vector solutions were prepared as vector solutions with a final volume of 500µl, by mixing 10%FBS/PS/IMDM with three types of vectors: SeV(PM)KOS/TS12ΔF vector, SeV(HNL)c-rMYC/TS15ΔF vector and SeV18+KLF4/TSΔF vector so that the MOI becomes 5, 10, 30 and 50. The cell solution and the vector solutions were mixed and added to 6-well plates, and cultured overnight in a CO₂ incubator (37°C, 5% CO₂). Next day, 10%FBS/PS/IMDM was added at 1 ml/well, and the cells were cultured for two days in a CO₂ incubator (37°C, 5% CO₂). The medium was exchanged with 10%FBS/PS/IMDM until day 3 and day 4 of the infection. The cells were detached using trypsin-EDTA on day 6 of the infection, suspended in 10%FBS/PS/IMDM, added onto the feeder cells, and cultured in a CO₂ incubator (37°C, 5% CO₂). Next day, the medium was exchanged with a primate ES cell medium (ReproCell, RCHEMD001) with an addition of bFGF at 4 ng/ml. Then, culturing was continued until day 28 of the vector infection with medium exchange. The state of the cells during the induction process is shown in Fig. 19. On day 28, alkaline phosphatase staining was carried out, and induction of alkaline phosphatase-positive colonies was observed (Fig. 20). The efficiency of iPS cell induction became higher in a manner dependent on the vector quantity, and was about 0.01% to 0.03% (Fig. 21).

[Example 17]

Induction of iPS cells from human peripheral blood mononuclear cells

[0112] Peripheral blood mononuclear cells were prepared by the Ficoll method from blood provided by two volunteers (as Donors 1 and 2). Culturing was carried out using a medium (PBMC+ medium) prepared by adding cytokines (100 ng/mL SCF, 100 ng/mL FLT-3 Ligand, 20 ng/mL Thrombopoetin, 10 ng/mL IL-6) to the PBMC medium (medium prepared by adding StemPro-34 Nutrient, L-Glutamine and penicillin-streptomycin to StemPro-34 SFM). Vector infection was performed on day 4 of the culturing. The cell number of peripheral blood mononuclear cells was measured, and the vector quantity was calculated so that the MOI becomes 5, 10, 30 and 50. Vector solutions were prepared by adding the calculated vectors to the PBMC+ medium. After removal of the PBMC culture solution and addition of the vector solutions, the cells were cultured in a CO₂ incubator (37°C, 5% CO₂). The cells were detached using trypsin-EDTA on day 3 of the vector infection, suspended in the PBMC+ medium, added onto the feeder cells, and cultured in a CO₂ incubator (37°C, 5% CO₂). From the next day until day 6 of the infection, the medium was exchanged with the PBMC medium, and the cells were cultured in a CO₂ incubator (37°C, 5% CO₂). On day 7 of the infection, half of the medium was removed, and an equivalent amount of a primate ES cell medium (ReproCell, RCHEMD001) with an addition of bFGF at 4 ng/ml was added, and the cells were cultured in a CO₂ incubator (37°C,

5% CO₂). After this, culturing was done using the primate ES cell medium (ReproCell, RCHEMD001) with an addition of bFGF at 4 ng/ml, and iPS-like cells were observed on day 21 of the infection (Fig. 22 and Fig. 23). Then, alkaline phosphatase staining was carried out (Fig. 24). The results of calculating the efficiency of inducing alkaline phosphatase-positive iPS-like cells showed that an induction efficiency of 0.1%-0.8% was achieved, although the induction efficiency differed depending on the donor and the amount of vector (Fig. 25).

[Example 18]

Induction of iPS cells from human monocytes -2

[0113] Peripheral blood mononuclear cells were prepared by the Ficoll method from blood provided by two volunteers (as Donors 3 and 4). From these peripheral blood mononuclear cells, CD14+ cells were separated as the starting material by using the EasySep positive selection Human CD14 positive Selection kit (Veritas, Cat. No. 18058). By FACS analysis using an anti-CD14 antibody, about 15% of the peripheral blood mononuclear cells were CD14-positive cells, and it was confirmed that after separation, the purification was improved to 85% or above. These cells were infected with three types of vectors: SeV(PM)KOS/TS12ΔF vector, SeV(HNL)c-rMYC/TS15ΔF vector and SeV18+KLF4/TSΔF vector so that the MOI becomes 5, 10, 30 and 50, and induction of iPS cells was carried out by the same method as shown in Example 16. These results confirmed induction of alkaline phosphatase stain-positive iPS-like cells (Fig. 26, Fig. 27, Fig. 28), and the induction efficiency was 0.12% at maximum (Fig. 29).

Industrial Applicability

[0114] By the present invention, it is possible to significantly improve the induction efficiency of pluripotent stem cells by using a vector that contains the KLF gene, OCT gene, and SOX gene in this order. It is useful that pluripotent stem cells can be induced with high efficiency without low-temperature culturing, and use of a temperature-sensitive vector, in particular, enables prompt vector removal in induction of pluripotent stem cells. With the methods of the present invention, one can expect to obtain more physiologically favorable pluripotent stem cells because low-temperature stimulus is not applied to the cells.

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- [WO2010008054A](#) [0004] [0037] [0058] [0083] [0084] [0085] [0087]
- [WO2012029770A](#) [0004] [0085] [0087]
- [EP2612911A](#) [0005]
- [WO200769666A](#) [0018] [0057] [0061] [0062] [0064] [0070] [0071] [0071] [0071] [0071] [0074] [0075] [0075]
- [WO0070055A](#) [0024] [0030] [0030] [0048] [0052]
- [WO0070070A](#) [0030] [0030] [0030] [0048] [0053]

- [WO2003025570A](#) [0037] [0053] [0083]
- [WO9716539A](#) [0046] [0053]
- [WO9716538A](#) [0046]
- [WO0118223A](#) [0046]
- [WO03025570A](#) [0046]
- [WO2005071092A](#) [0046]
- [WO2006137517A](#) [0046] [0053]
- [WO2007083644A](#) [0046]
- [WO2008007581A](#) [0046]
- [WO200580598A](#) [0061]
- [US4873192A](#) [0065]
- [WO2007069666A](#) [0078]

Non-patent literature cited in the description

- **TAKAHASHI, K.YAMANAKA, S.**Cell, 2006, vol. 126, 663-676 [0005]
- **MAHERALI, N. et al.**Cell Stem Cell, 2007, vol. 1, 55-70 [0005]
- **OKITA, K. et al.**Nature, 2007, vol. 448, 313-317 [0005]
- **WERNIG, M. et al.**Nature, 2007, vol. 448, 318-324 [0005]
- **TAKAHASHI, K.**Cell, 2007, vol. 131, 861-872 [0005]
- **YU, J. et al.**Science, 2007, vol. 318, 1917-1920 [0005]
- **LOWRY, W.E. et al.**Proc. Natl. Acad. Sci. USA, 2008, vol. 105, 2883-2888 [0005]
- **PARK, I.H. et al.**Nature, 2008, vol. 451, 141-146 [0005]
- **MASAKI, H. et al.**Stem Cell Res., 2008, vol. 1, 105-115 [0005] [0071]
- **STADTFELD, M. et al.**Science, 2008, vol. 322, 945-949 [0005]
- **OKITA, K. et al.**Science, 2008, vol. 322, 949-953 [0005]
- **YU, J. et al.**Science, 2009, vol. 324, 797-801 [0005]
- **ZHOU, W. et al.**stem cells, 2009, vol. 27, 2667-2674 [0005]
- **ICHISAKA T. et al.**Nature, 2007, vol. 448, 7151313-7 [0018]
- **Virus**, 2007, vol. 57, 129-36 [0026]
- **Annu. Rev. Genet.**, 1998, vol. 32, 123-162 [0026]
- **Fields virology**Lippincott-Raven200100001305-1340 [0026]
- **Microbiol. Immunol.**, 1999, vol. 43, 613-624 [0026]
- **Field Virology**199600001205-1241 [0026]
- **Medical Journal of Osaka University**, 1955, vol. 6, 11-15 [0030]
- **J. Virol.**, 1994, vol. 68, 8413-8417 [0030]
- **LI, H.-O. et al.**J. Virol., 2000, vol. 74, 146564-6569 [0030] [0030]
- **INOUE et al.**J. Virol., 2003, vol. 77, 3238-3246 [0032] [0041]
- **INOUE, M. et al.**J. Virol., 2003, vol. 77, 3238-3246 [0033] [0034]
- **WRIGHT, K. E. et al.**Virus Res., 2000, vol. 67, 49-57 [0034]
- **J. Virology**, 1981, vol. 39, 519-528 [0044]
- **HASAN, M. K. et al.**J. Gen. Virol., 1997, vol. 78, 2813-2820 [0046]
- **KATO, A. et al.**EMBO J., vol. 16, 578-587 [0046]
- **YU, D. et al.**Genes Cells, 1997, vol. 2, 457-466 [0046]
- **DURBIN, A. P. et al.**Virology, 1997, vol. 235, 323-332 [0046]
- **WHELAN, S. P. et al.**Proc. Natl. Acad. Sci. USA, 1995, vol. 92, 8388-8392 [0046]
- **SCHNELL. M. J. et al.**EMBO J., 1994, vol. 13, 4195-4203 [0046]
- **RADECKE, F. et al.**EMBO J., 1995, vol. 14, 5773-5784 [0046]
- **LAWSON, N. D. et al.**Proc. Natl. Acad. Sci. USA, vol. 92, 4477-4481 [0046]
- **GARCIN, D. et al.**EMBO J., 1995, vol. 14, 6087-6094 [0046]
- **KATO, A. et al.**Genes Cells, 1996, vol. 1, 569-579 [0046]

- **BARON, M. D. BARRETT, T. J.** Virol., 1997, vol. 71, 1265-1271 [0046]
- **BRIDGEN, A. ELLIOTT, R. M.** Proc. Natl. Acad. Sci. USA, 1996, vol. 93, 15400-15404 [0046]
- **TOKUSUMI, T. et al.** Virus Res., 2002, vol. 86, 33-38 [0046]
- **LI, H.-O. et al.** J. Virol., 2000, vol. 74, 6564-6569 [0046]
- Uirusu-gaku Jikken-gaku Kakuron (Detailed Virology Experiments) Maruzen 19820000 [0046]
- **MAEKAWA et al.** Nature, 2011, vol. 474, 225-229 [0055] [0062] [0070] [0071]
- **BLELLOCH R. et al.** Cell Stem Cell, 2007, vol. 1, 245-247 [0057] [0071]
- **FUSAKI et al.** Proc Jpn Acad Ser B Phys Biol Sci., 2009, vol. 85, 348-362 [0058]
- Techniques in Molecular Biology MacMillan Publishing Company 19830000 [0065]
- **KUNKEL** Proc. Natl. Acad. Sci. USA, 1985, vol. 82, 488-492 [0065]
- **KUNKEL et al.** Methods Enzymol., 1987, vol. 154, 367-382 [0065]
- **SAMBROOK et al.** Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press 19890000 [0065]
- **DAYHOFF et al.** Atlas of Protein Sequence and Structure Natl. Biomed. Res. Found. 19780000 [0065]
- **S. HENIKOFF J. G. HENIKOFF** Proc. Acad. Natl. Sci. USA, 1992, vol. 89, 10915-10919 [0066]
- **ALTSCHUL, S. F. et al.** J. Mol. Biol., 1990, vol. 215, 403-410 [0067]
- **ALTSCHUL S. F. et al.** Nature Genet., 1993, vol. 3, 266-272 [0067]
- **MADDEN, T. L. et al.** Meth. Enzymol., 1996, vol. 266, 131-141 [0067]
- **ALTSCHUL S. F. et al.** Nucleic Acids Res., 1997, vol. 25, 3389-3402 [0067]
- **ZHANG J. MADDEN T. L.** Genome Res., 1997, vol. 7, 649-656 [0067]
- **TATIANA A et al.** FEMS Microbiol Lett., 1999, vol. 174, 247-250 [0067]
- **TAKAHASHI, K. YAMANAKA S.** Cell, 2006, vol. 126, 663-676 [0071]
- **LOWRY WE et al.** Proc Natl Acad Sci USA, 2008, vol. 105, 82883-8 [0071]
- **NAKAGAWA M. et al.** Nat Biotechnol., 2008, vol. 26, 1101-6 [0073]
- **WERING M. et al.** Cell Stem Cell, 2008, vol. 2, 110-2 [0073]
- **Y. ZHAO et al.** Cell Stem Cell, 2008, vol. 3, 5475-479 [0073]
- **N. MAHERALIK. HOCHEDFINGER** Cell Stem Cell, 2008, vol. 3, 6595-605 [0073]
- **PARK IH et al.** Nature, 2008, vol. 451, 7175141-6 [0073] [0078]
- **KUBICEK, S. et al.** Mol. Cell, 2007, vol. 25, 473-481 [0073]
- **SHI Y et al.** Cell Stem Cell, 2008, vol. 2, 6525-8 [0073]
- **FIERS, W.** Nature, 1978, vol. 273, 5658113-120 [0074]
- **PARK IH. et al.** Nature, 2008, vol. 451, 7175141-6 [0074]
- Mol Cell Biol., 2003, vol. 23, 82699-708 [0074]
- **NANOG** Cell, 2003, vol. 113, 631-642 [0074]
- Nature, 2003, vol. 423, 541-545 [0074]
- Development, 2003, vol. 130, 1673-1680 [0074] [0074]
- Nat Med, 2004, vol. 10, 155-63 [0074]
- **YU J. et al.** Science, 2007, vol. 318, 58581917-20 [0074]
- **LIAO J et al.** Cell Res., 2008, vol. 18, 5600-3 [0074]
- **HANNA J** Cell., 2008, vol. 133, 2250-64 [0074]
- **HUANGFU D et al.** Nat Biotechnol., 2008, [0075]
- Nat. Biotechnol., 2008, vol. 26, 795-797 [0075]
- **HUANGFU, D. et al.** Nat Biotechnol., 2008, vol. 26, 111269-75 [0075]
- **AOI T. et al.** Science, 2008, [0078]
- Science, 2008, vol. 321, 5889699-702 [0078]
- **TAKAHASHI K et al.** Cell, 2007, vol. 131, 861-872 [0078]
- **STADTFELD M et al.** Curr Biol., 2008, [0078]
- Curr Biol., 2008, vol. 18, 12890-4 [0078]
- **HANNA J. et al.** Cell, 2008, vol. 133, 2250-64 [0078]

PATENTKRAV

1. In vitro fremgangsmåde til fremstilling af pluripotente stamceller, hvor fremgangsmåden omfatter

5

indføring af en temperatur-følsom Sendai virusvektor, som omfatter, i én vektor, KLF genet, OCT genet og SOX genet i denne rækkefølge, og en Sendai virusvektor, som omfatter KLF genet men ikke OCT genet og SOX genet, og dyrkning i området fra 36,5 til 37,5°C,

10

hvor den temperaturfølsomme Sendai virusvektor, som omfatter, i én vektor, KLF genet, OCT genet og SOX genet i denne rækkefølge, er en F gen-deleteret Sendai virusvektor, som omfatter G69E, T116A og A183S mutationer i M proteinet, A262T, G264R og K461G mutationer i HN proteinet, D433A, R434A, K437A og L511F mutationer i P proteinet og N1197S og K1795E mutationer i L

15

proteinet, og hvor Sendai virusvektoren, som omfatter KLF genet, men ikke OCT genet og SOX genet, er en F gen-deleteret Sendai virusvektor, som omfatter G69E, T116A og A183S mutationer i M proteinet, A262T, G264R og K461G mutationer i HN proteinet, L511F mutation i P proteinet og N1197S og K1795E mutationer i L

20

2. Sammensætning til inducering af pluripotente stamceller, hvor sammensætningen omfatter:

25

(a) en temperaturfølsom Sendai virusvektor, som omfatter, i en vektor, KLF genet, OCT genet og SOX genet i denne rækkefølge, som er en F gen-deleteret Sendai virusvektor, som omfatter G69E, T116A og A183S mutationer i M proteinet, A262T, G264R og K461G mutationer i HN proteinet, D433A, R434A, K437A og L511F mutationer i P proteinet og N1197S og K1795E mutationer i L proteinet; og

30

(b) en Sendai virusvektor, som omfatter KLF genet men ikke OCT genet og SOX genet, som er en F gen-deleteret Sendai virusvektor, som omfatter G69E, T116A og A183S mutationer i M proteinet, A262T, G264R og K461G mutationer i HN proteinet, L511F mutation i P proteinet og N1197S og K1795E mutationer i L proteinet.

35

3. Sammensætning ifølge krav 2, som yderligere omfatter en temperatur-følsom

Sendai virusvektor indsat med et MYC gen, fortrinsvis hvor den MYC gen indsatte temperaturfølsomme Sendai virusvektor er en F gen-deleteret Sendai virusvektor, som omfatter G69E, T116A og A183S mutationer i M proteinet, A262T, G264R og K461G mutationer i HN proteinet, D433A, R434A, K437A og L511F mutationer i P proteinet og L1361C, L1558I, N1197S og K1795E mutationer i L proteinet.

4. In vitro fremgangsmåde til inducering af pluripotente stamceller, omfattende indføring af sammensætningen ifølge krav 2 i celler og dyrkning af cellerne i området fra 36,5 til 37,5°C.

10

5. Sæt til inducering af pluripotente stamceller, hvor sættet omfatter:

(a) en temperaturfølsom Sendai virusvektor, som omfatter, i en vektor, KLF gen, OCT gen og SOX gen i denne rækkefølge, som er en F gen-deleteret Sendai virusvektor, som omfatter G69E, T116A og A183S mutationer i M proteinet, A262T, G264R og K461G mutationer i HN proteinet, D433A, R434A, K437A og L511F mutationer i P proteinet og N1197S og K1795E mutationer i L proteinet og

(b) en Sendai virusvektor, som omfatter KLF gen men ikke OCT gen og SOX gen, som er en F gen-deleteret Sendai virusvektor, som omfatter G69E, T116A og A183S mutationer i M proteinet, A262T, G264R og K461G mutationer i HN proteinet, L511F mutation i P proteinet og N1197S og K1795E mutationer i L proteinet.

25 6. Sæt ifølge krav 5, som yderligere omfatter en temperaturfølsom Sendai virusvektor indsat med et MYC gen.

DRAWINGS

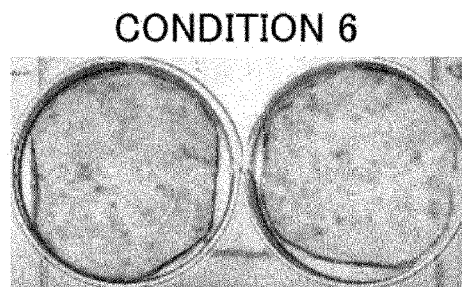
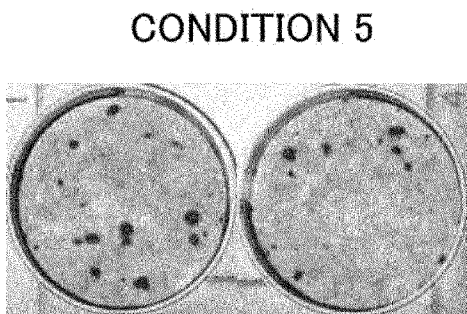
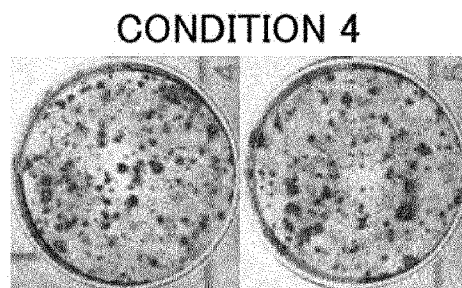
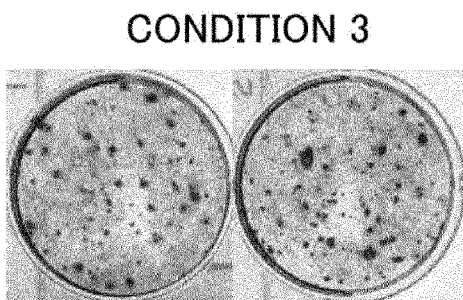
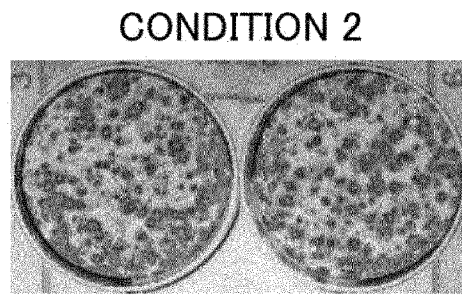
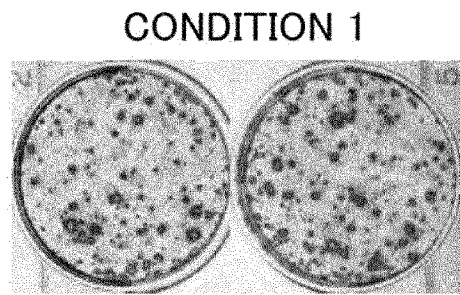


FIG. 1

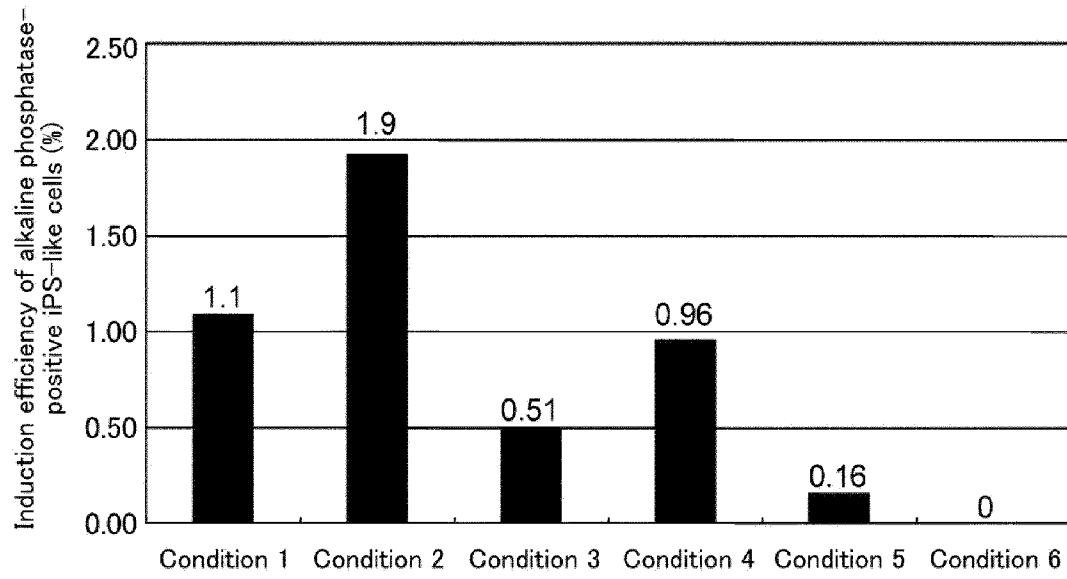


FIG. 2

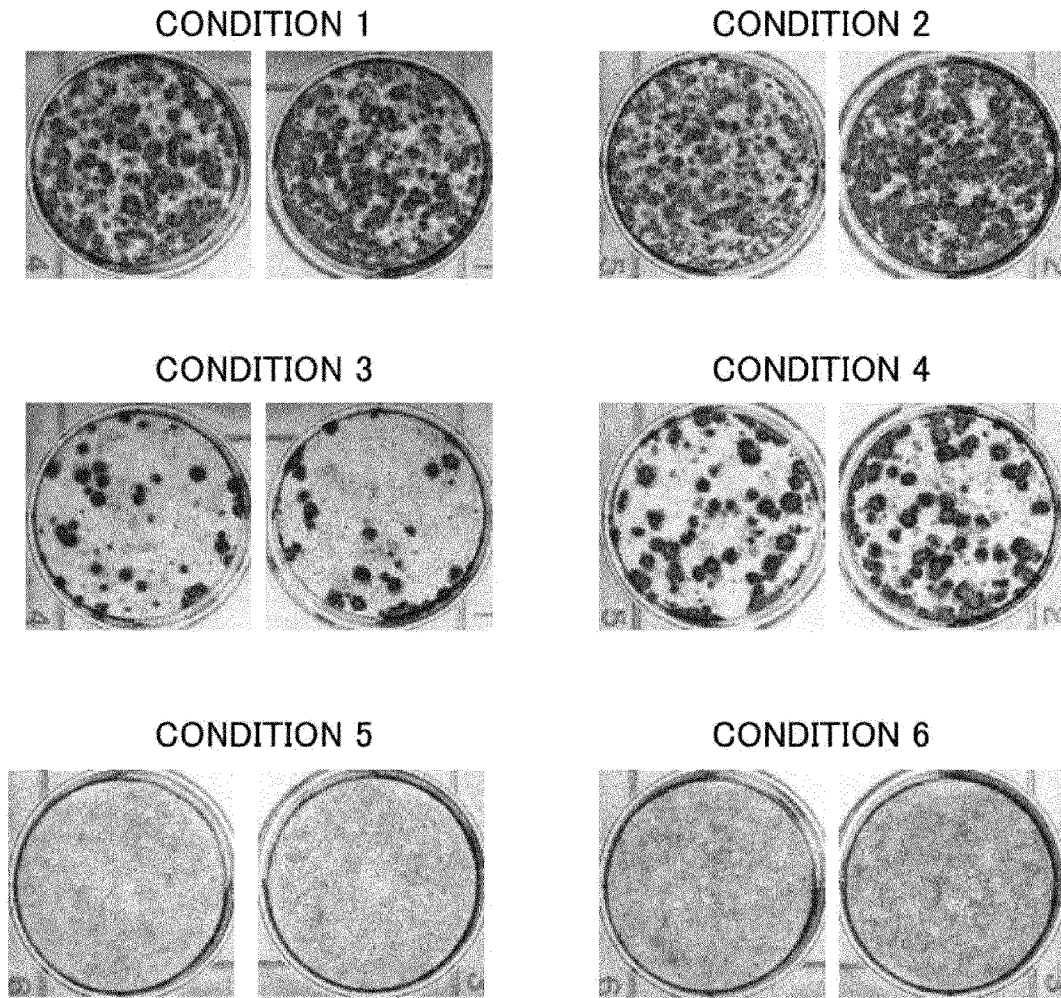


FIG. 3

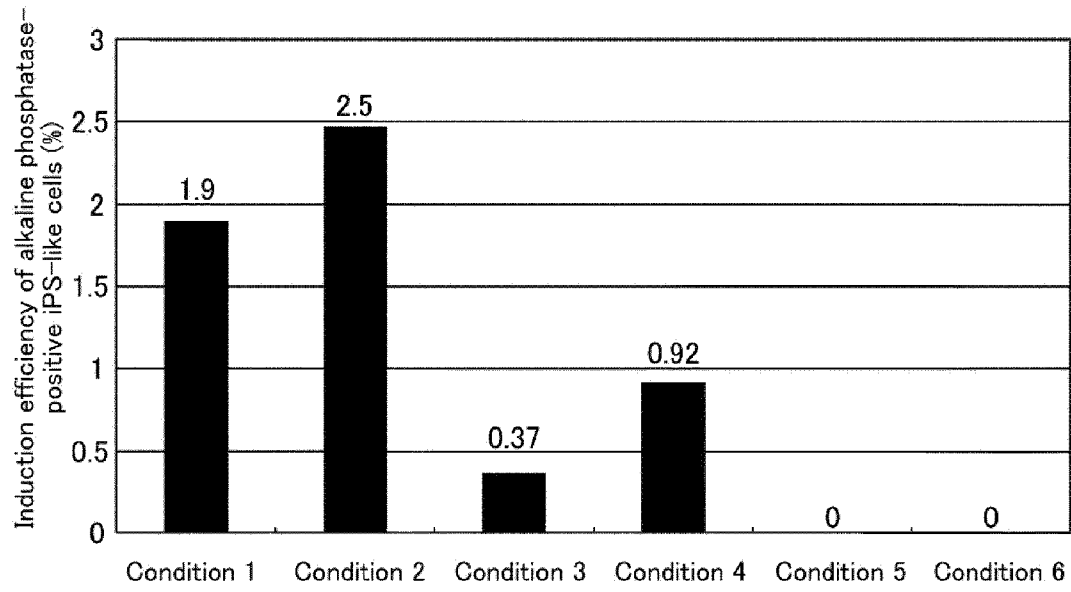


FIG. 4

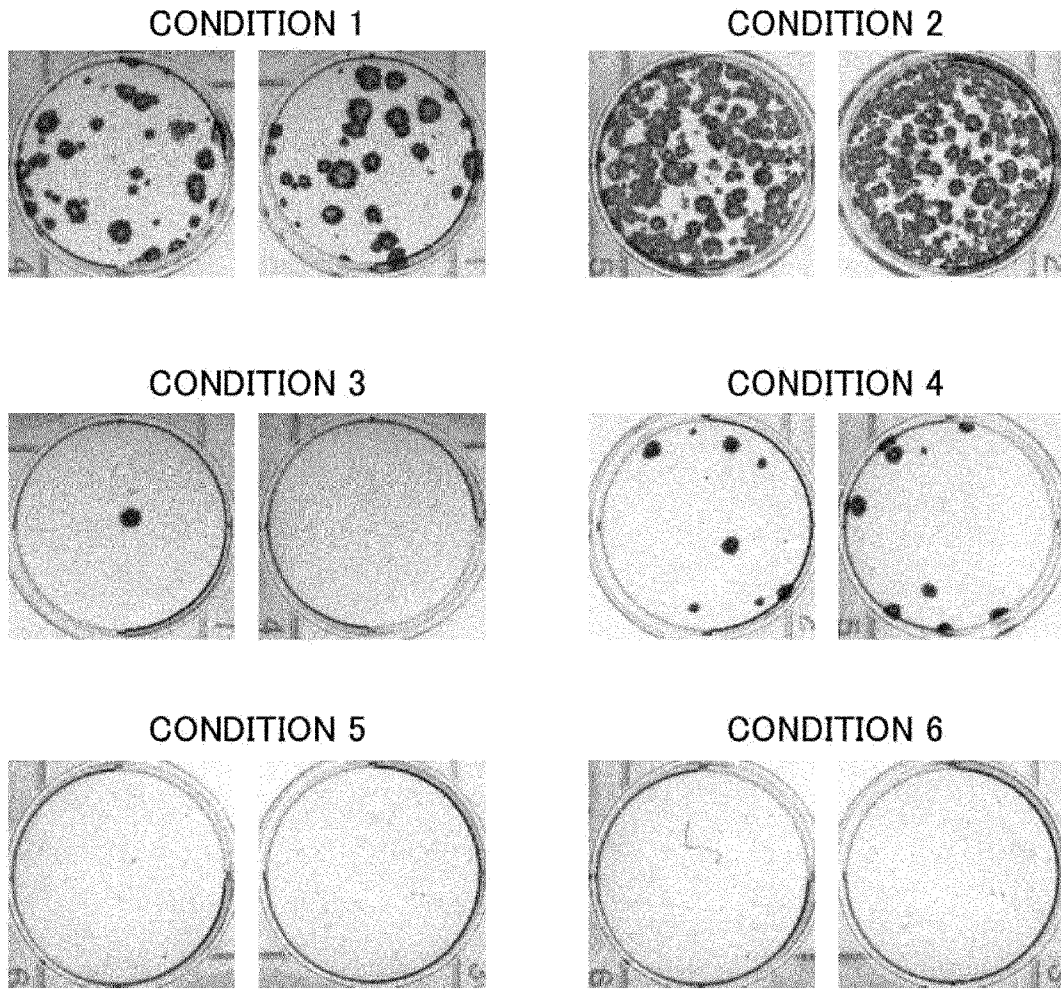


FIG. 5

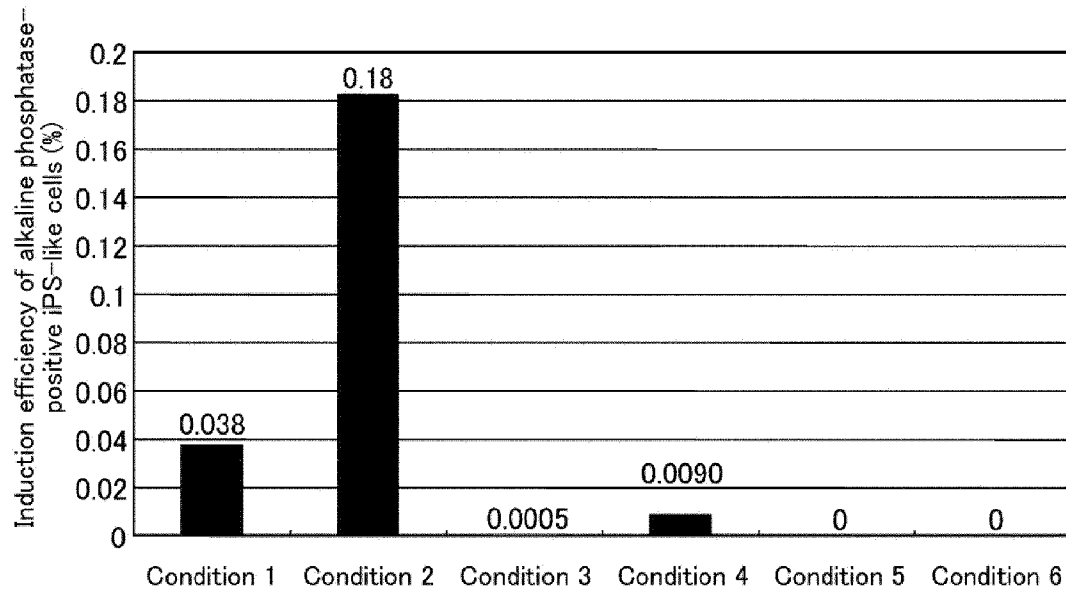


FIG. 6

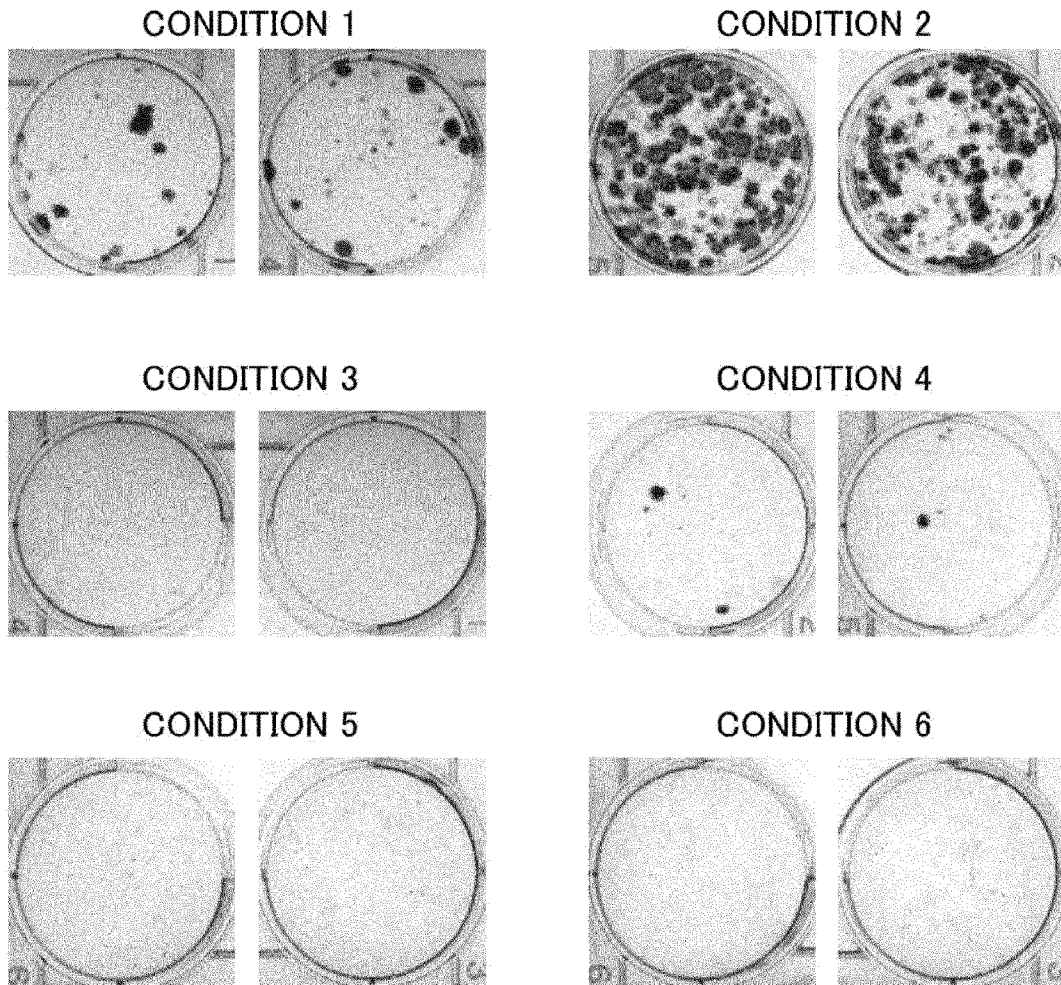


FIG. 7

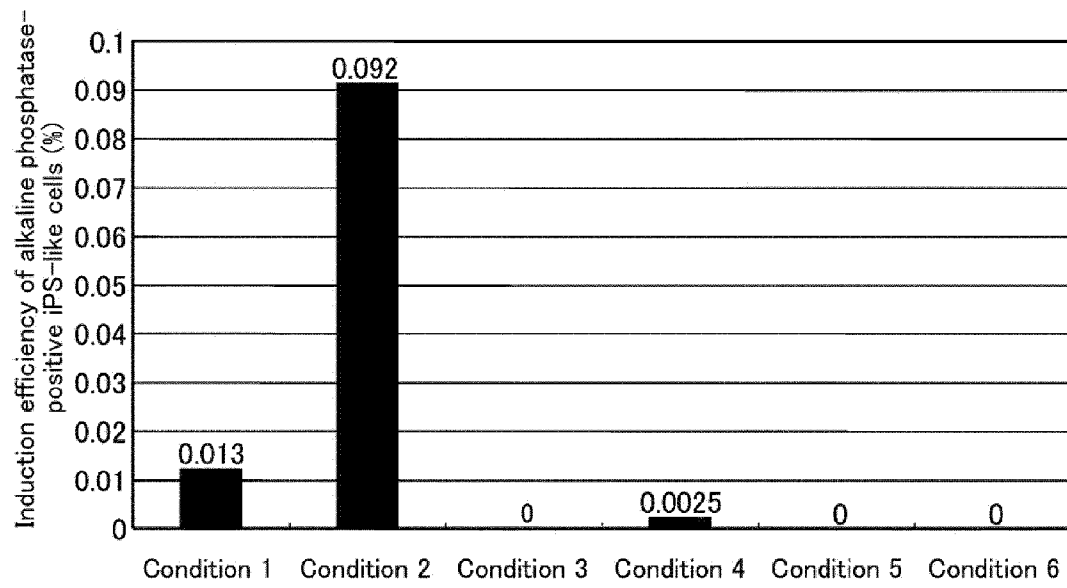


FIG. 8

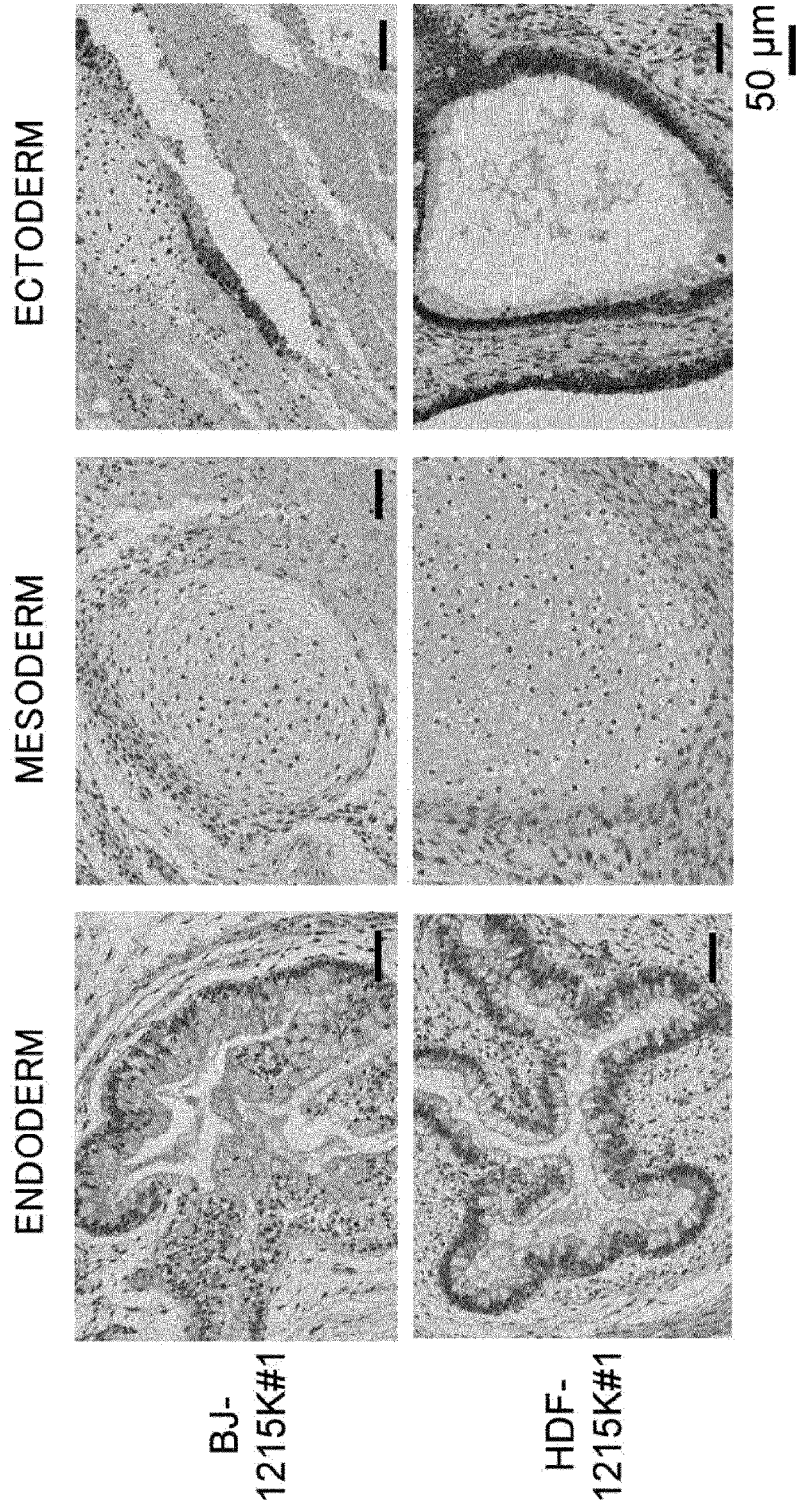
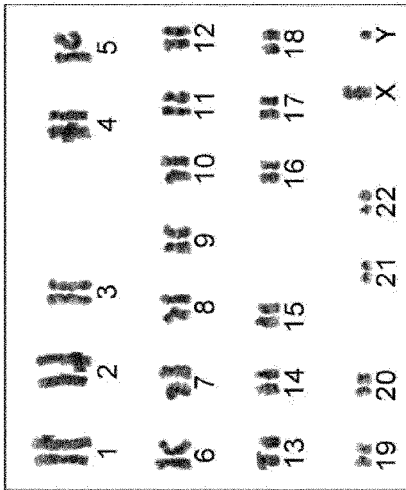
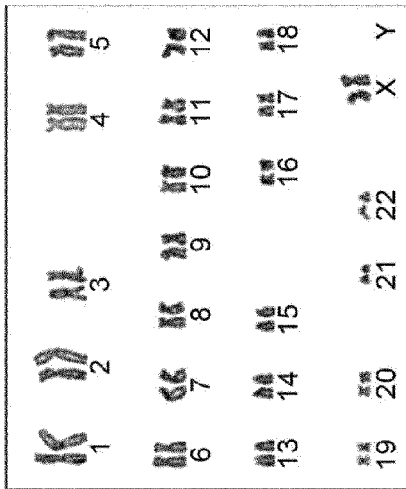


FIG. 9

BJ-1215K#1



HDF-1215K#1



MRC5-1215K#1

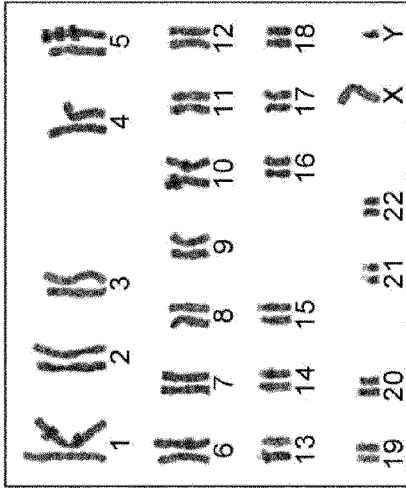


FIG. 10

Day 21 after vector infection

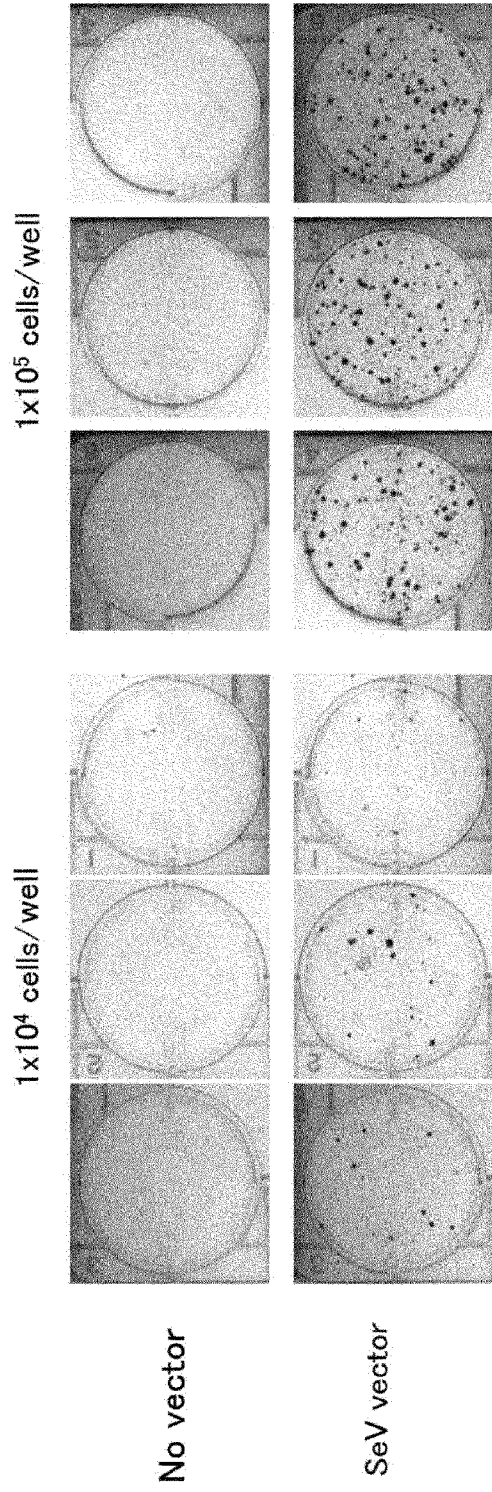


FIG. 11

Clone names : 5-2, 5-5, 5-6, 5-7, 5-11, 5-12, 5-13, 5-14, 5-15 → P9day4
Clone names : 5-16, 5-17 → P10day4

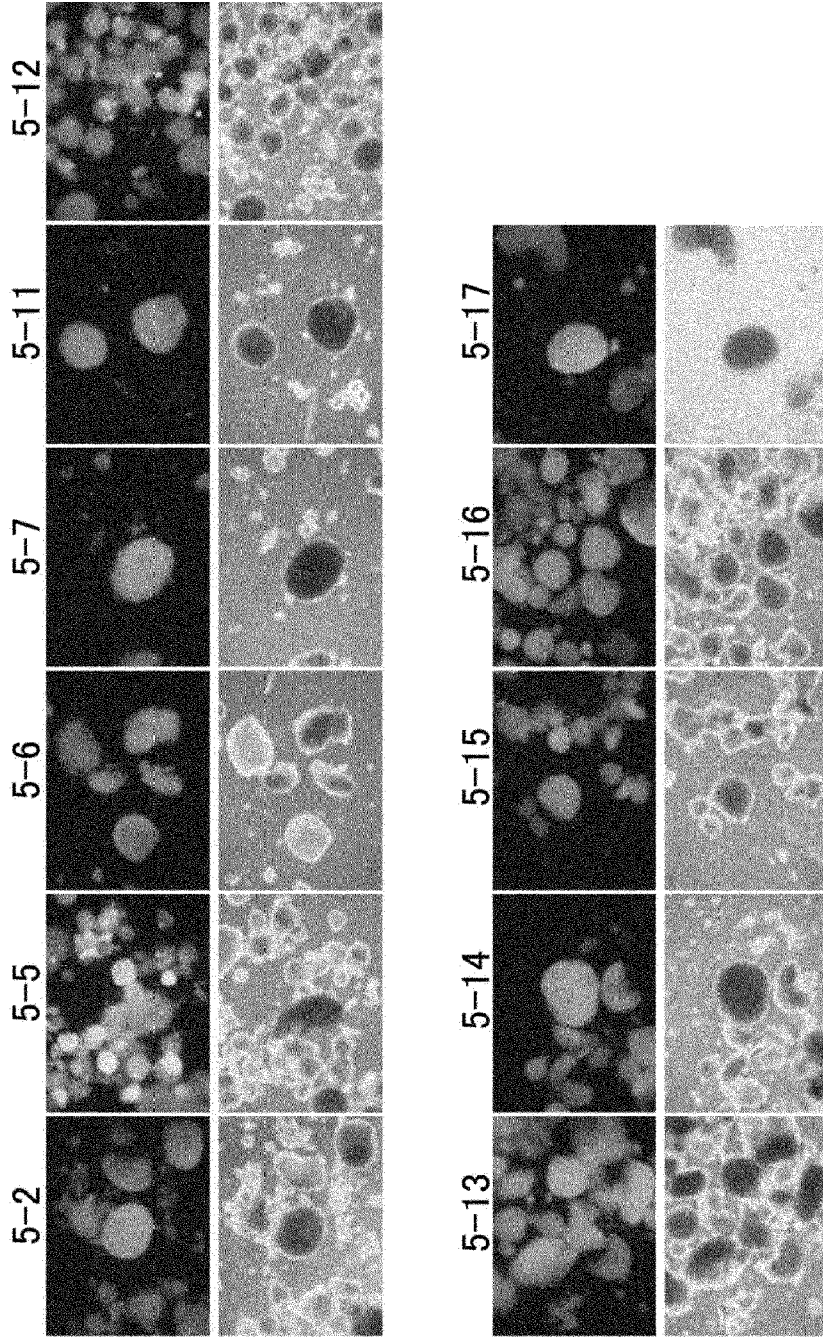


FIG. 12

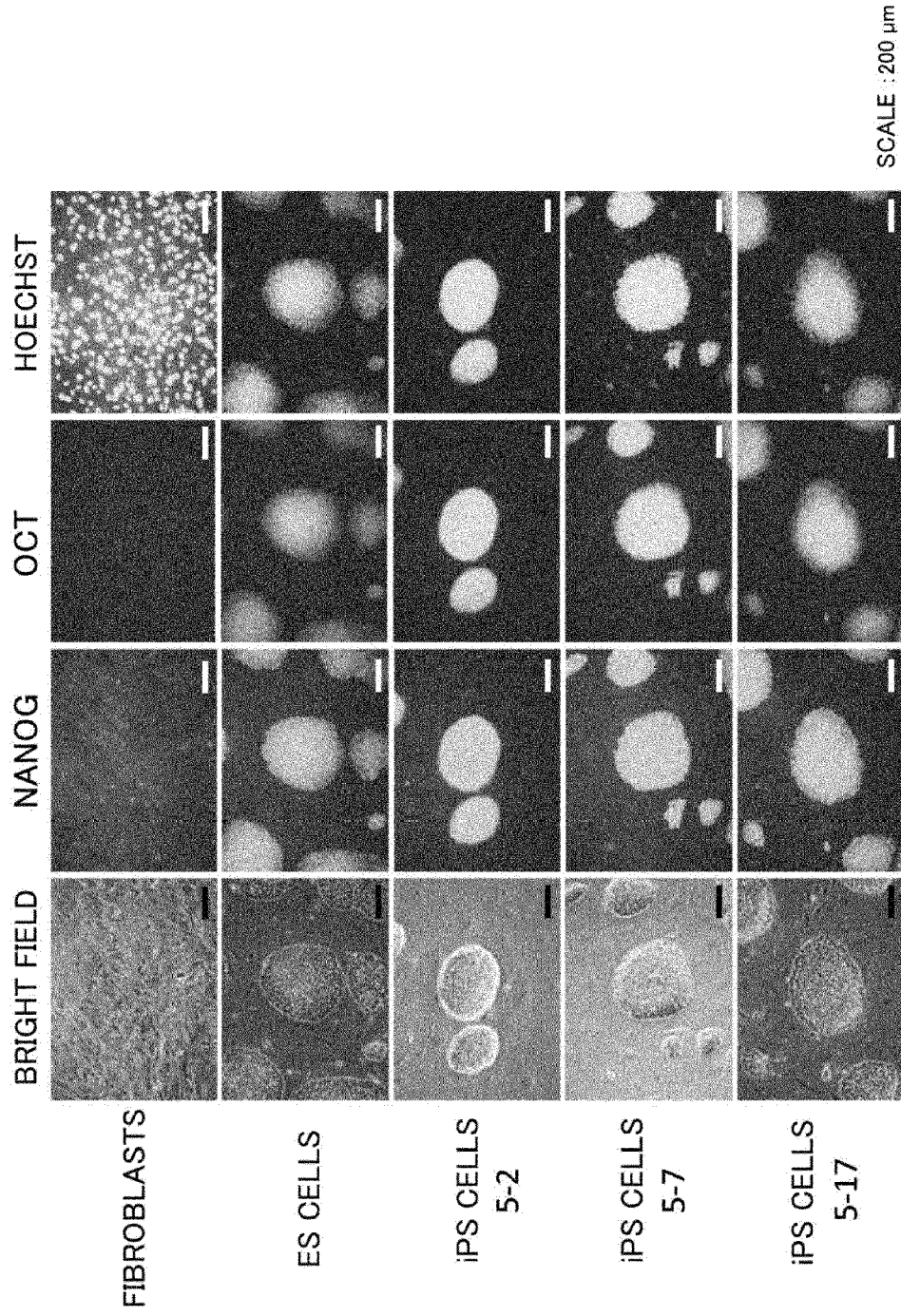


FIG. 13

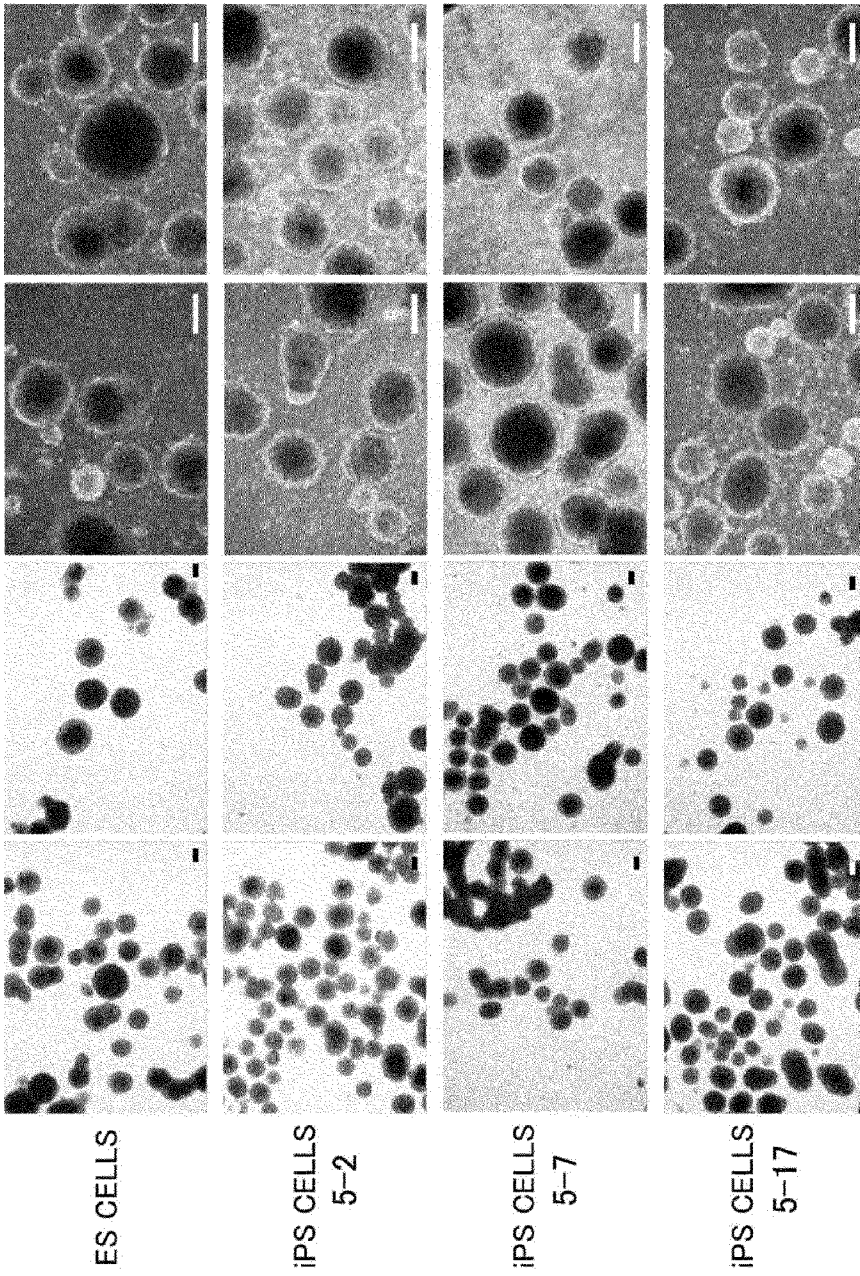


FIG. 14

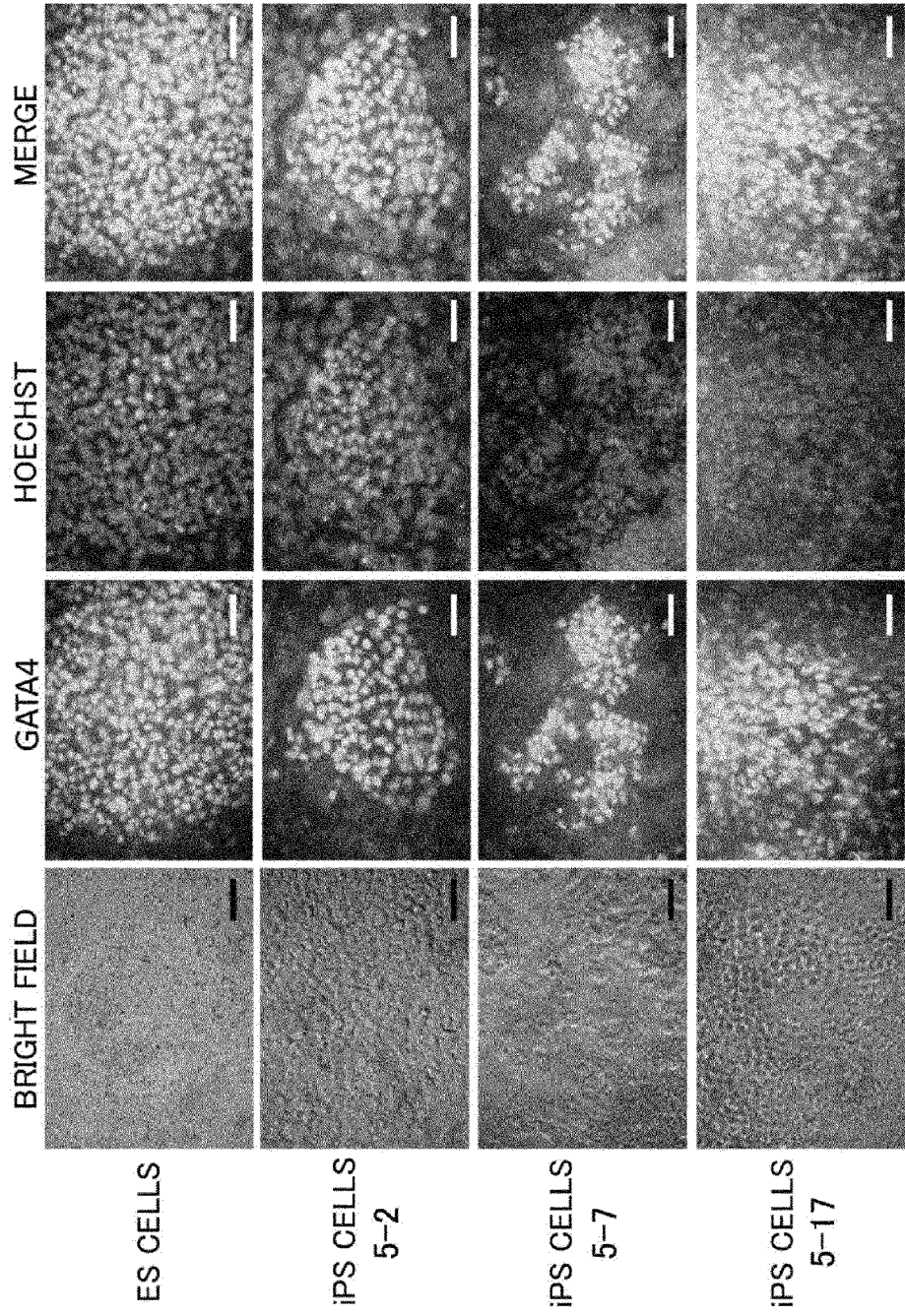
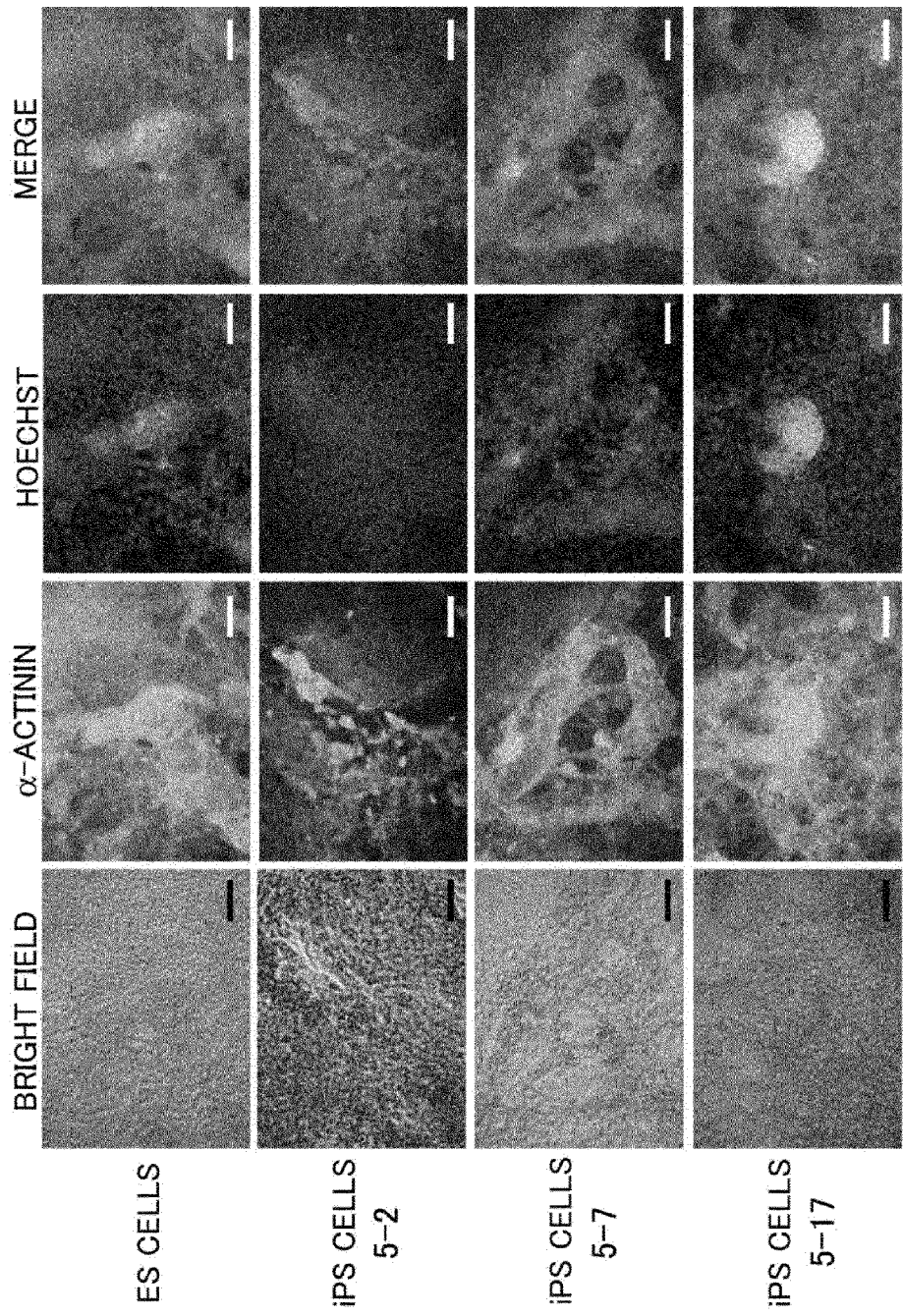


FIG. 15



SCALE = 100 μ m

FIG. 16

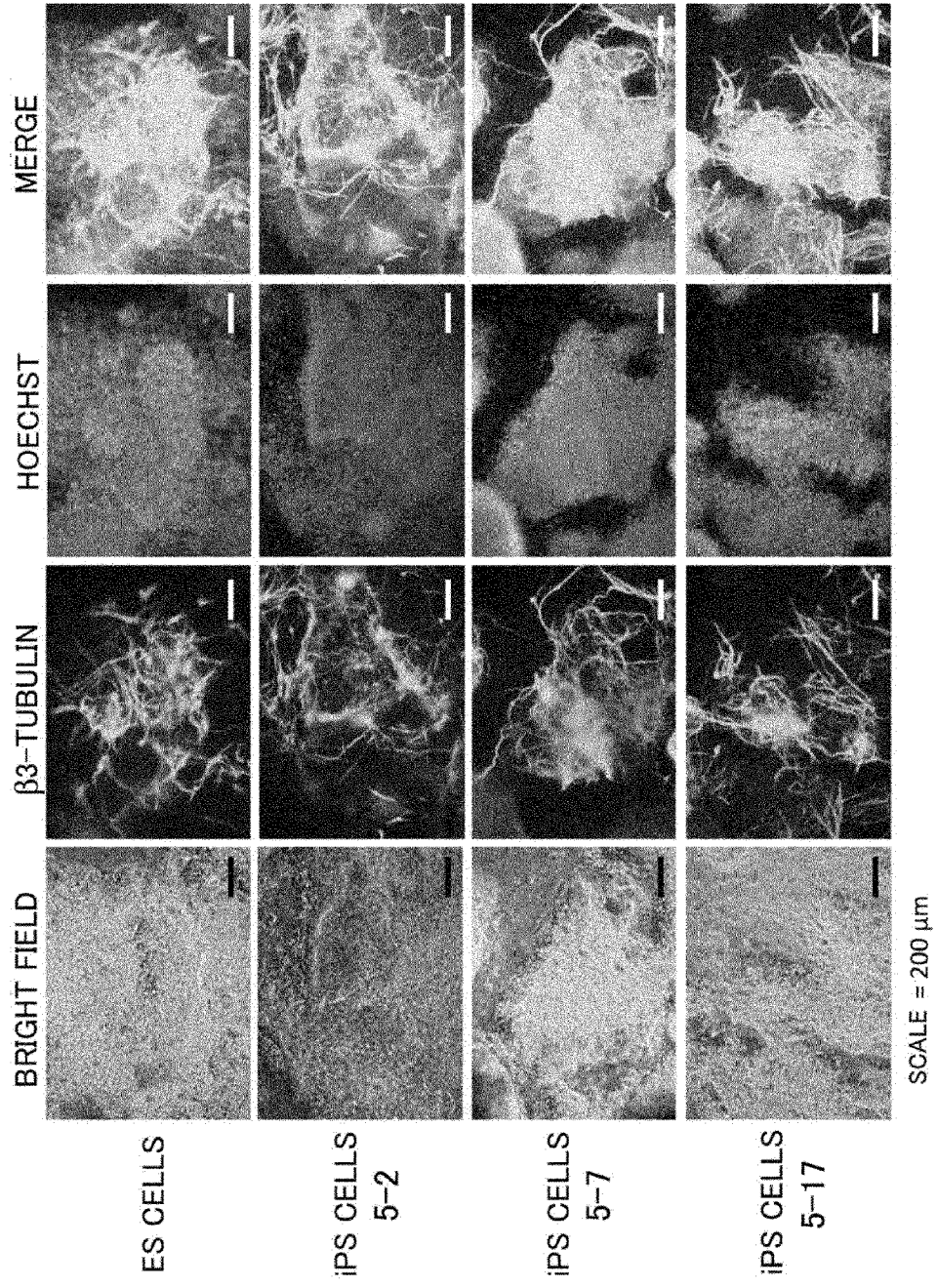


FIG. 17

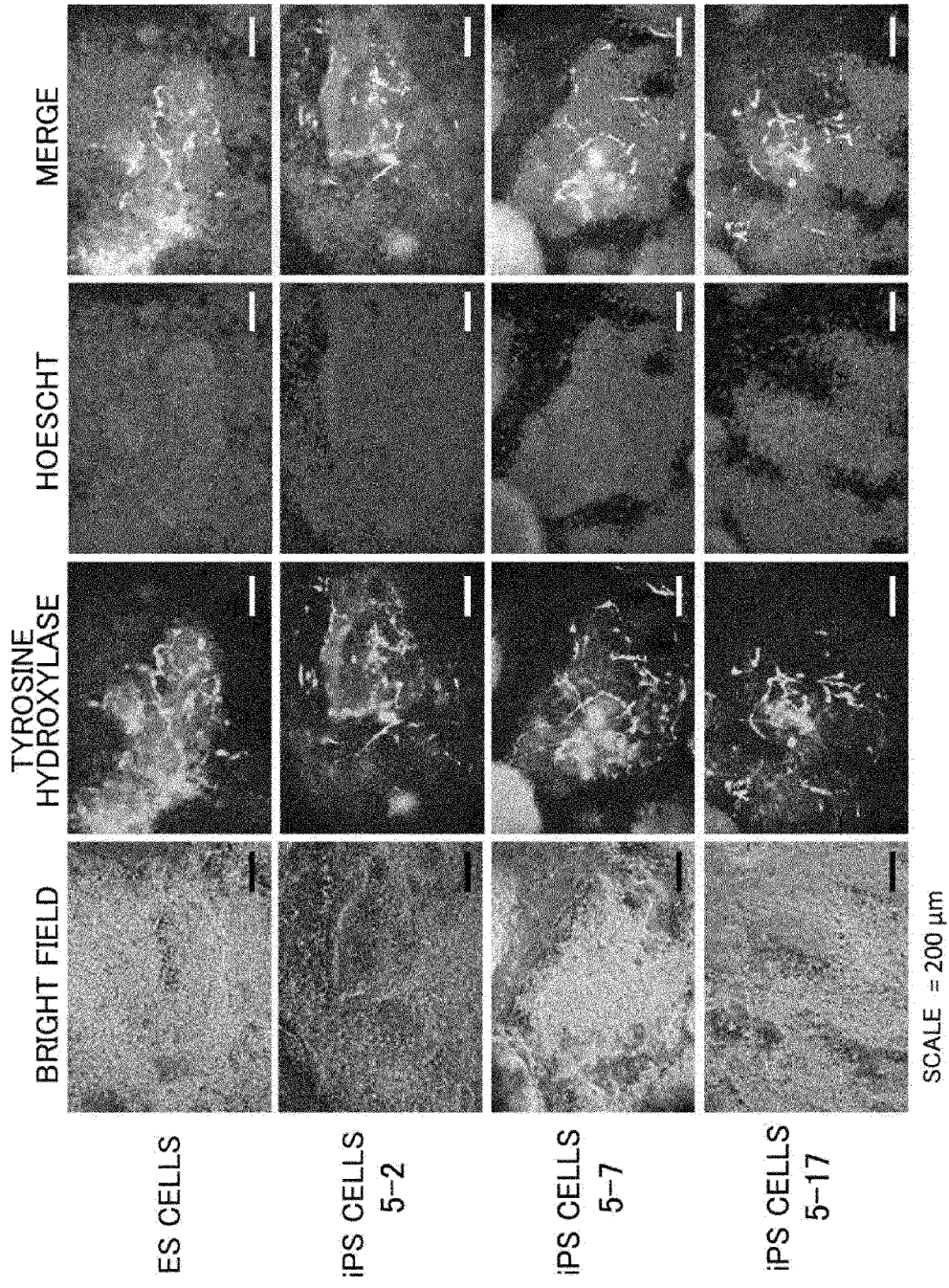


FIG. 18

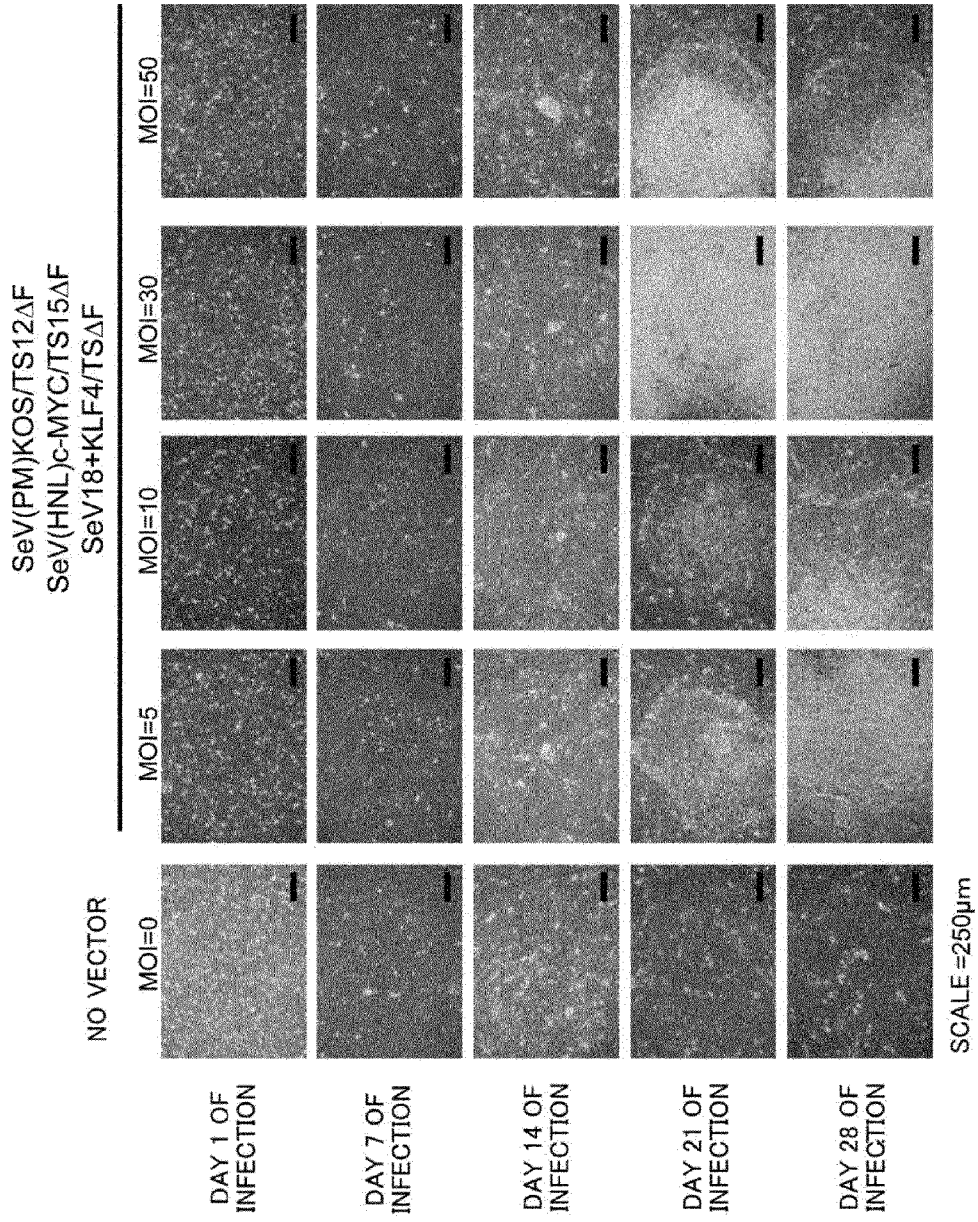


FIG. 19

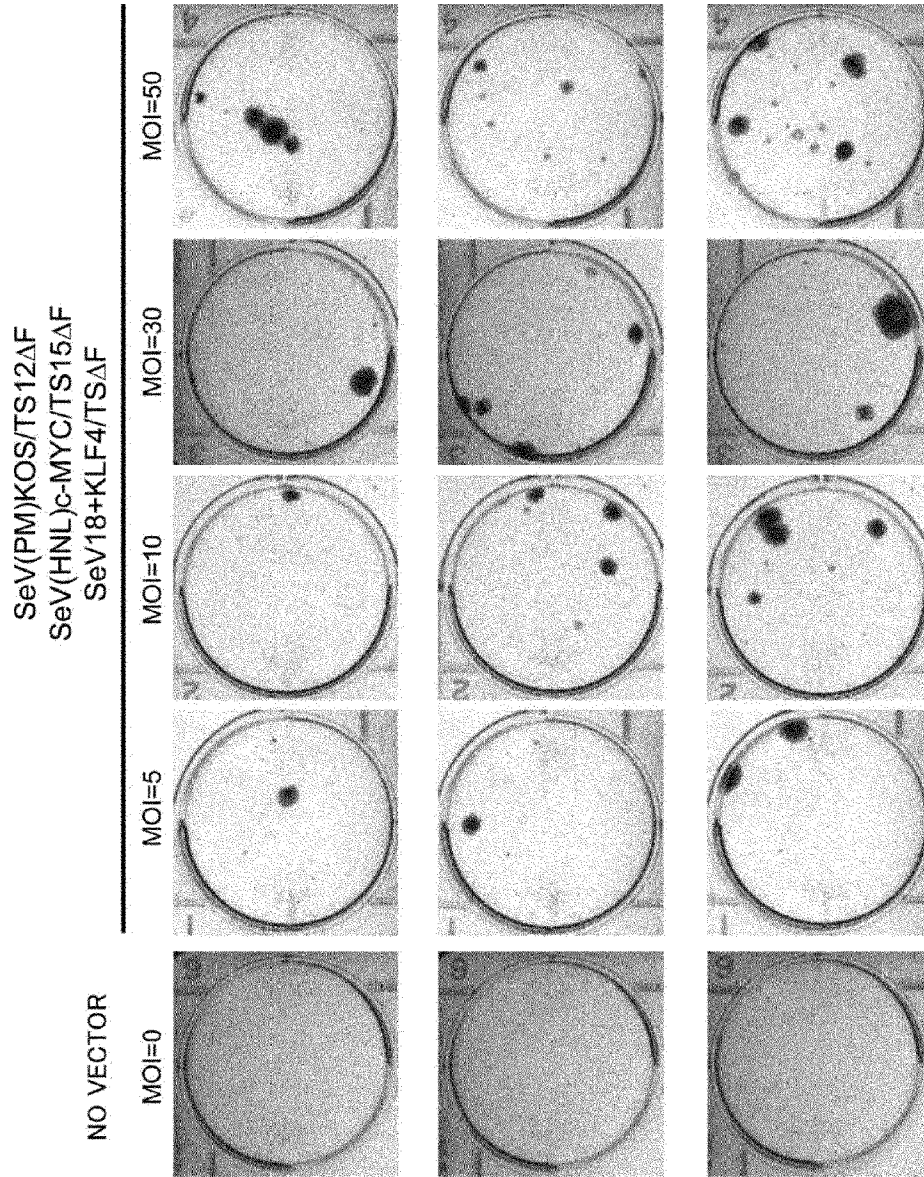


FIG. 20

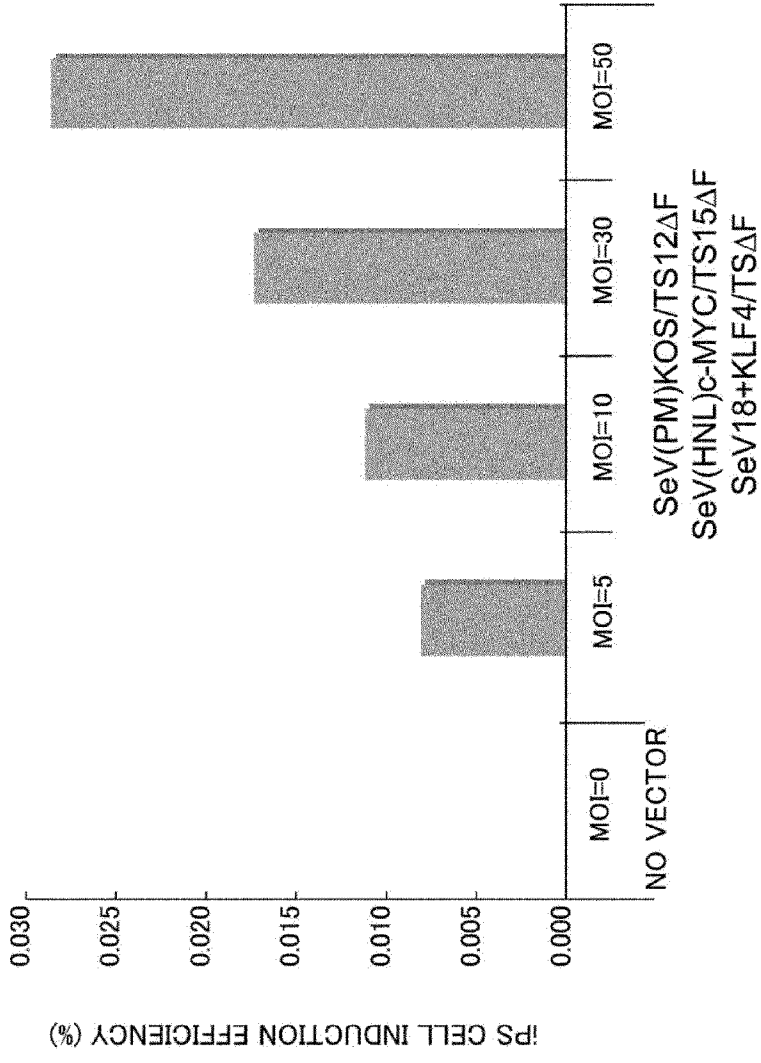


FIG. 21

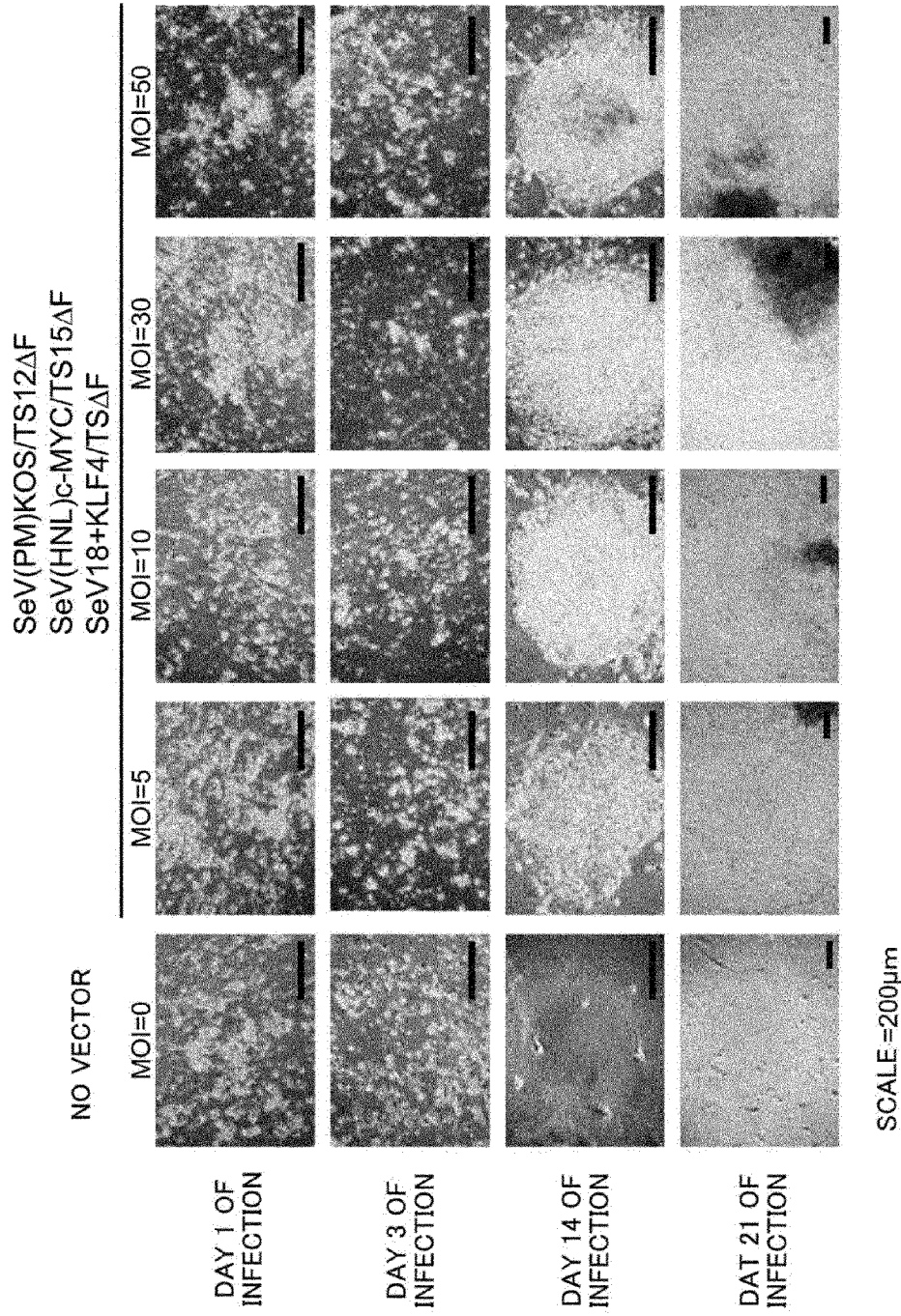
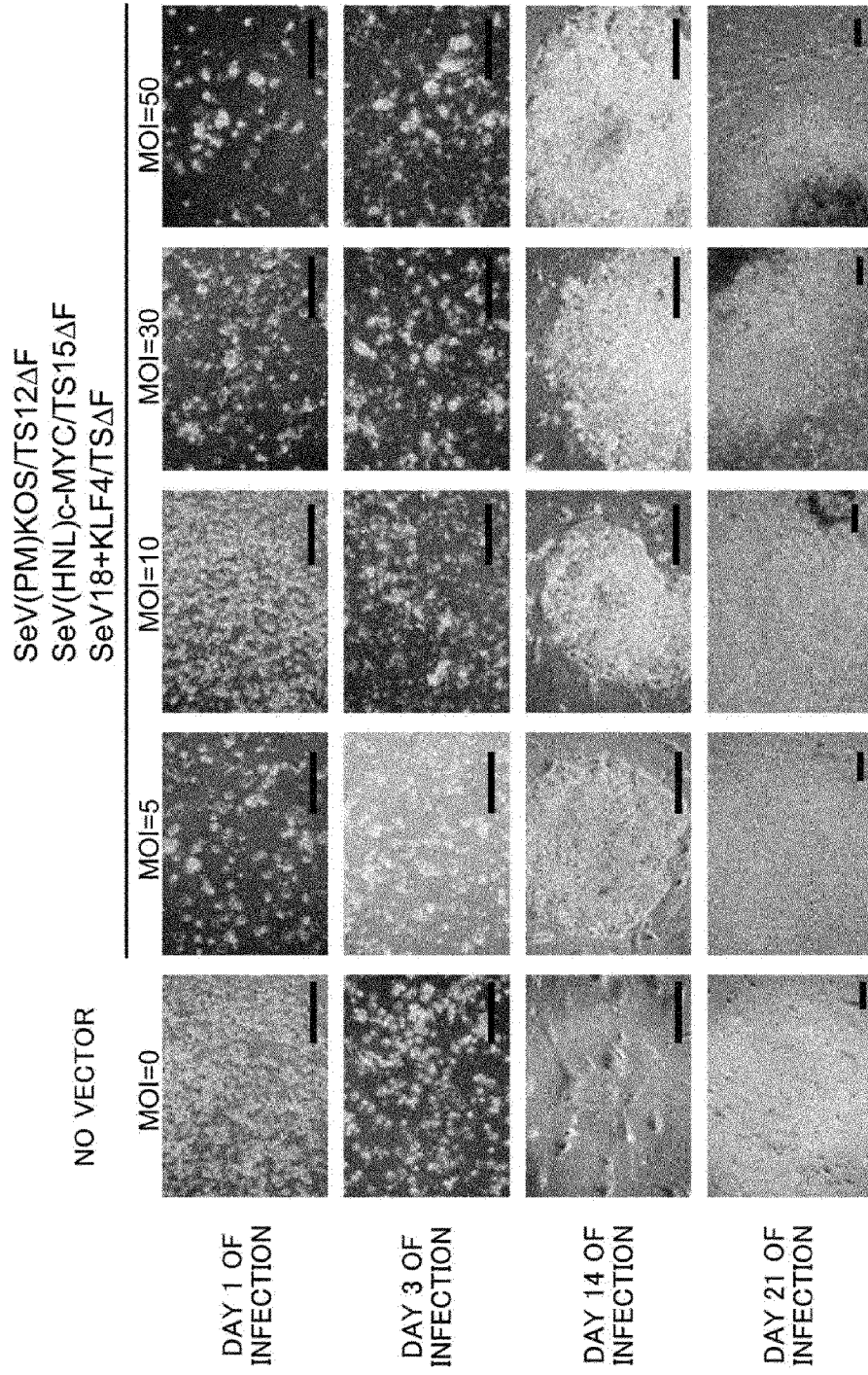


FIG. 22



SCALE=200μm

FIG. 23

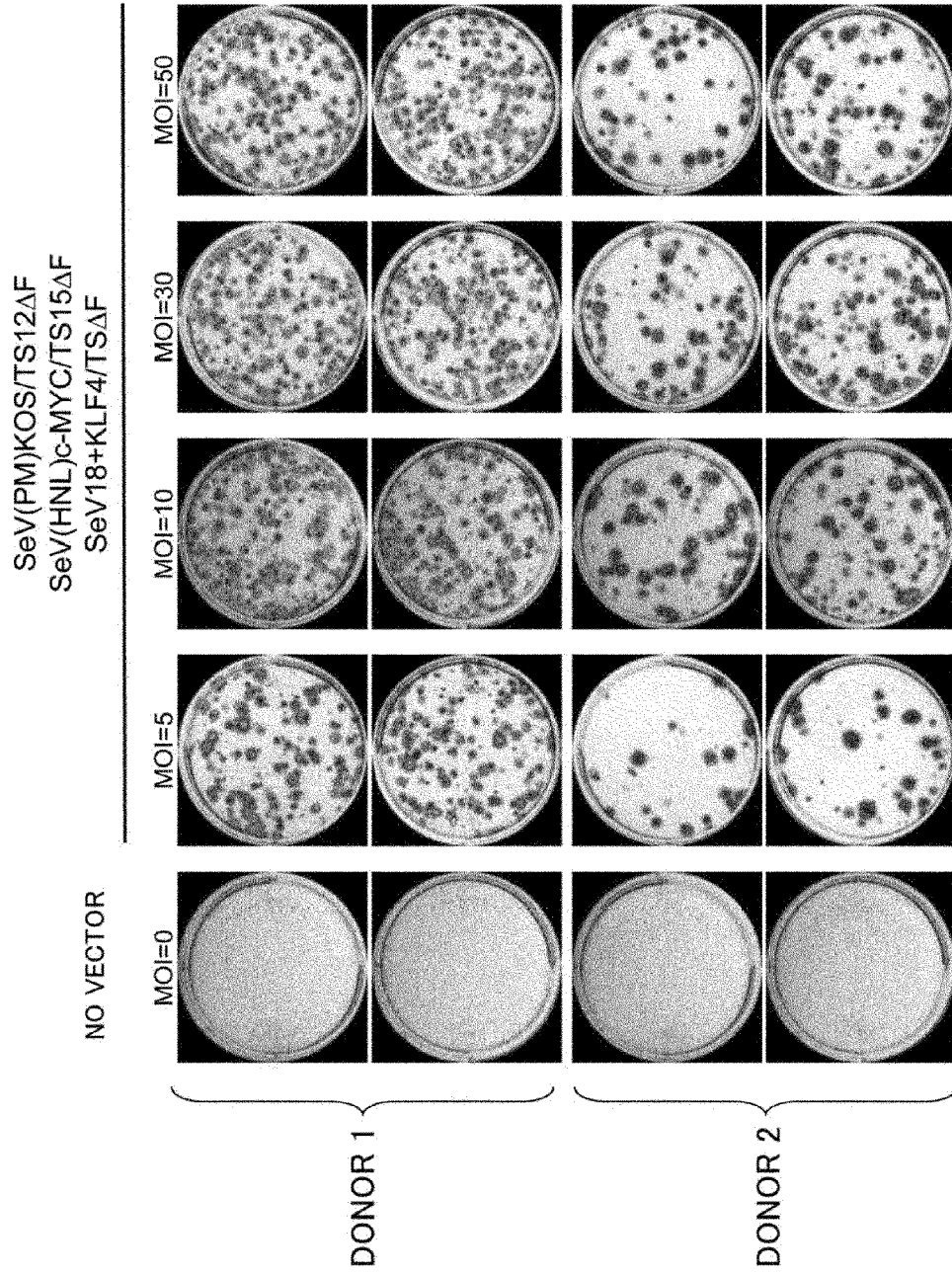


FIG. 24

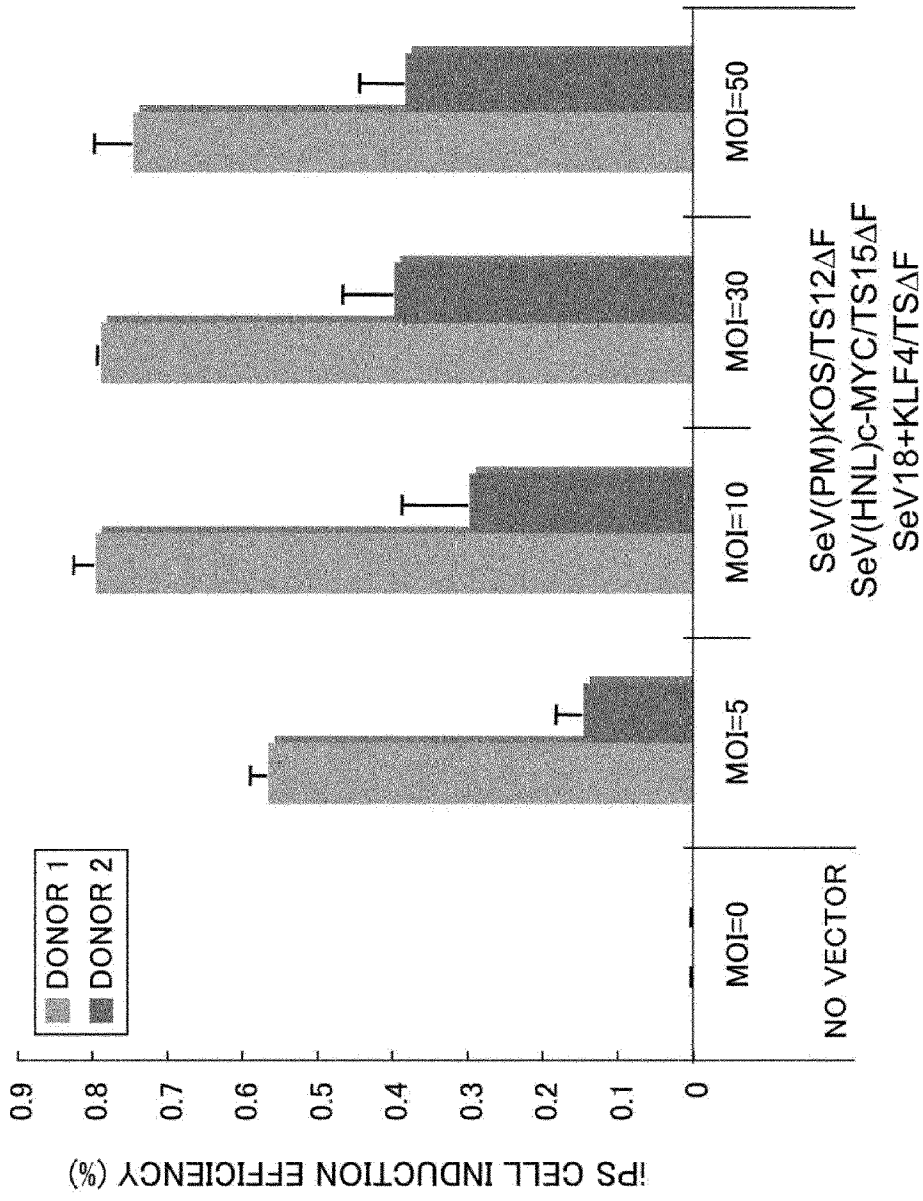


FIG. 25

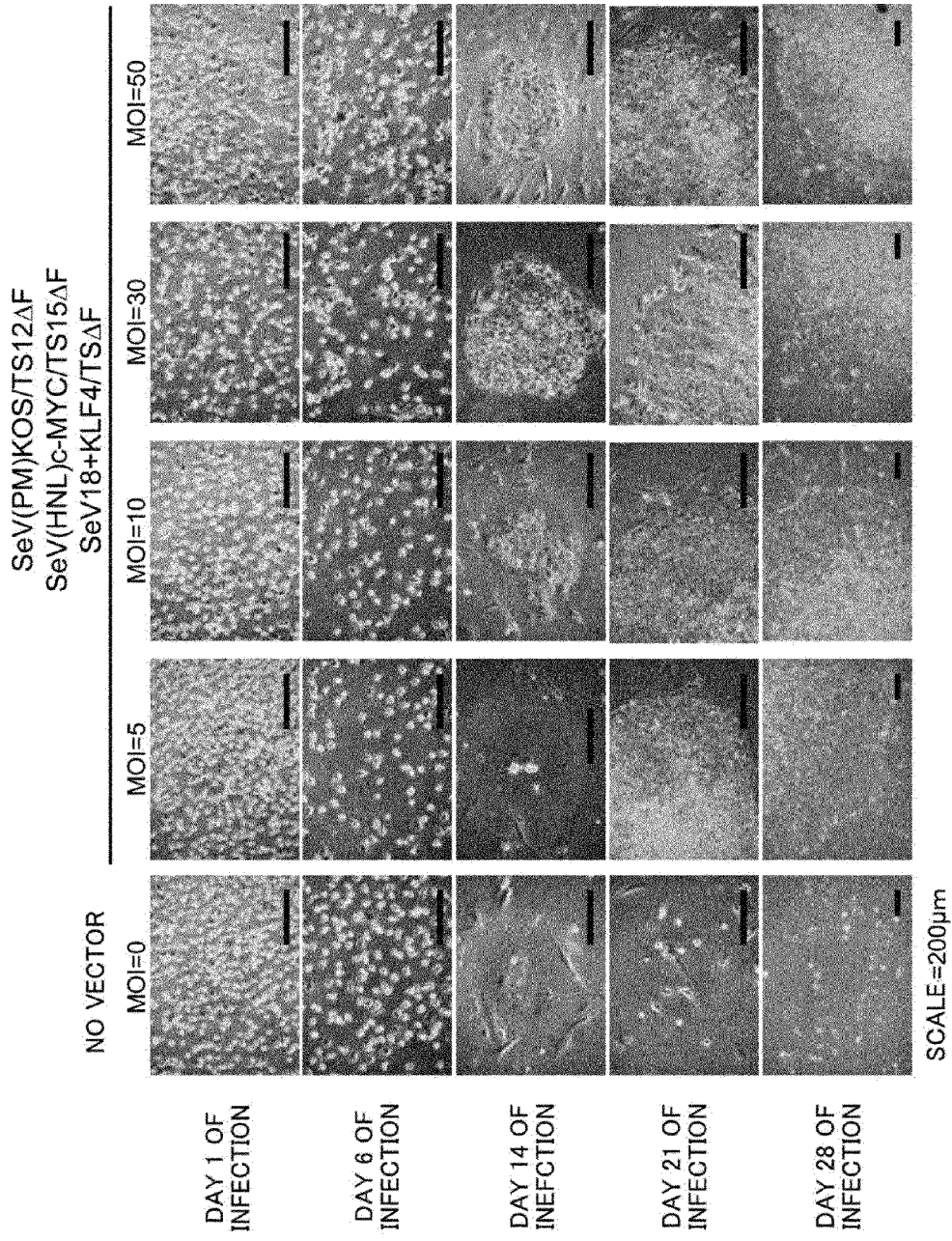


FIG. 26

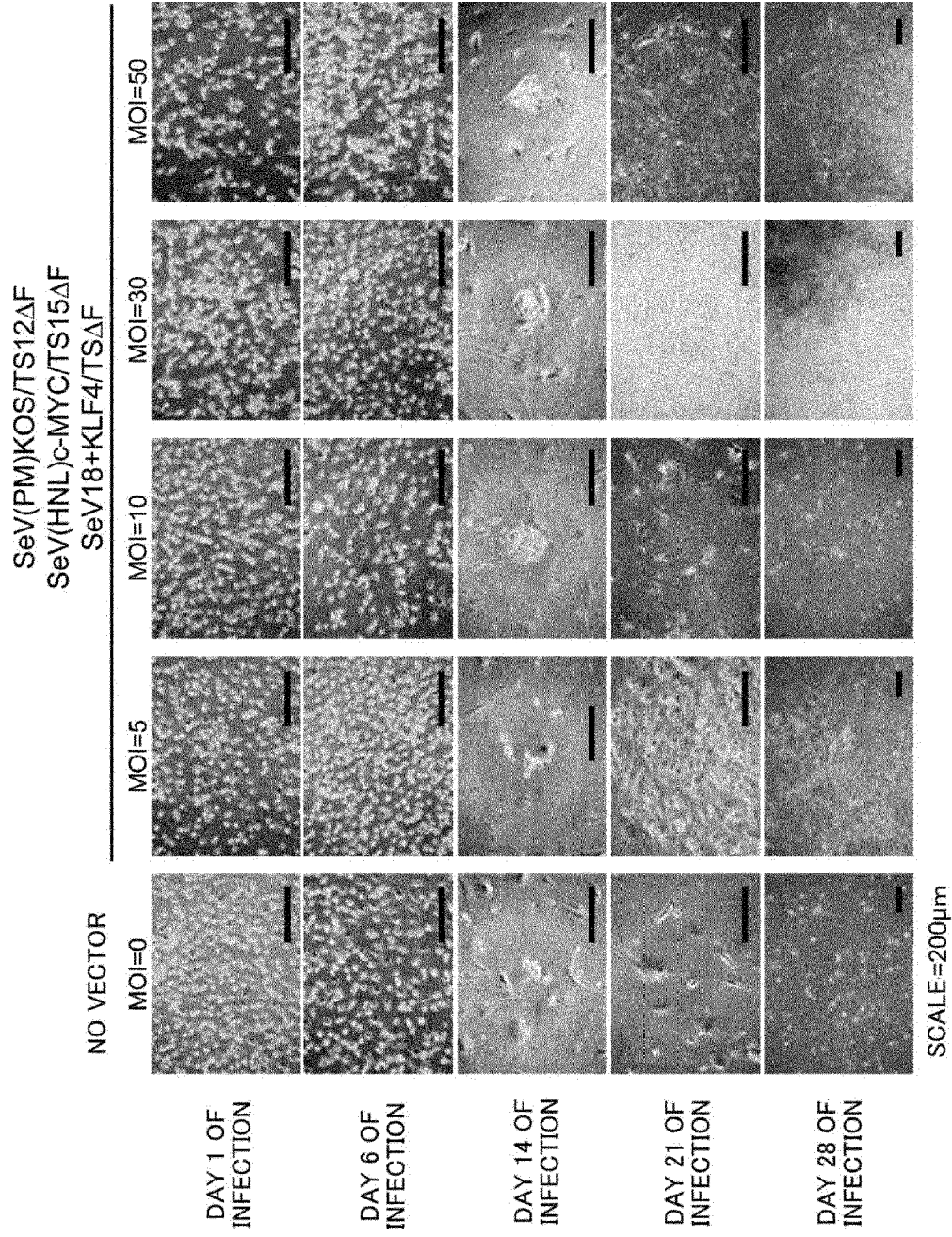


FIG. 27

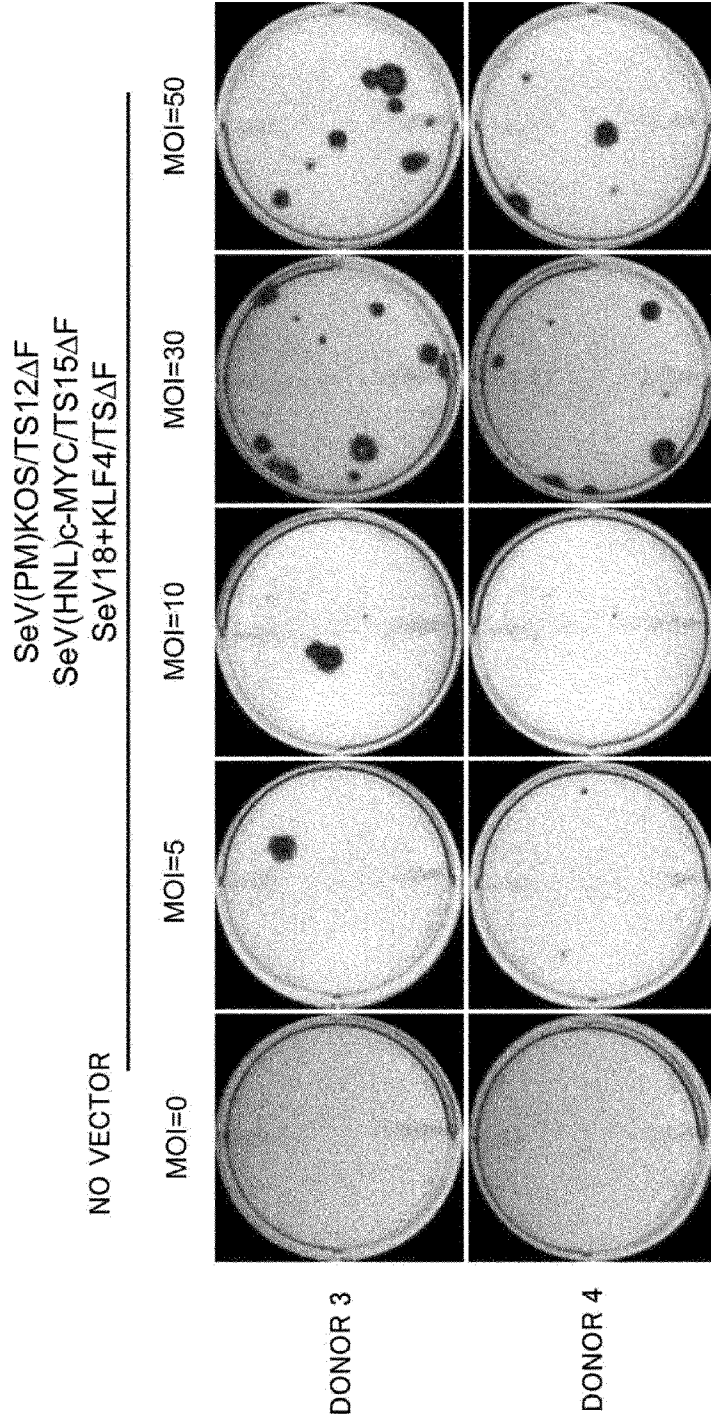


FIG. 28

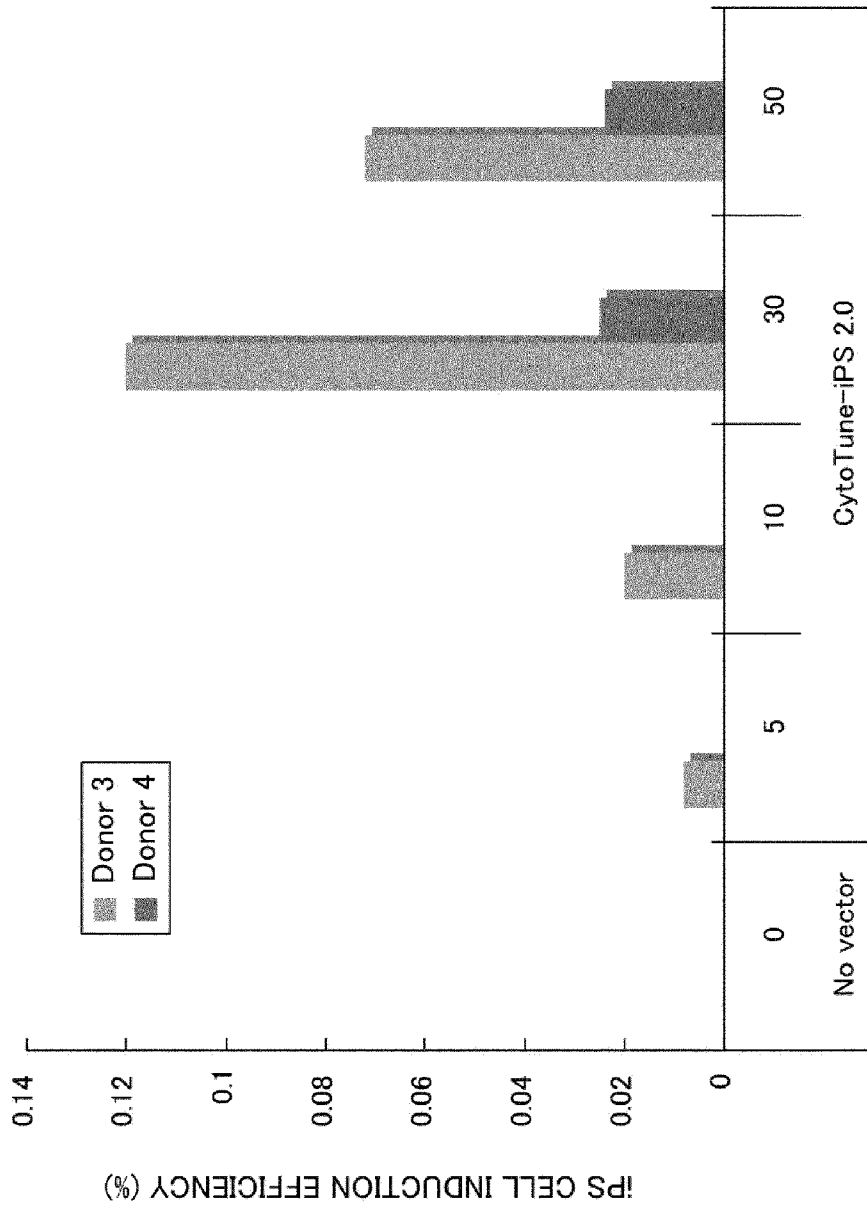


FIG. 29

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.

