Description

Title of Invention: METHOD FOR SCREENING INDUCED PLURIPOTENT STEM CELLS

Technical Field

[0001] The present invention relates to a method for screening induced pluripotent stem cells. More specifically, the present invention relates to a method for screening induced pluripotent stem cells exhibiting no differentiation resistance through confirmation of the expression of large intergenic non-coding RNA (lincRNA) or mRNA in induced pluripotent stem cells.

Background Art

[0002] In recent years, mouse and human induced pluripotent stem cells (iPS cells) have been successively established. Yamanaka et al., have induced iPS cells by introducing Oct3/4, Sox2, Klf4, and c-Myc genes into mouse-derived fibroblasts so as to enable the forced expression of such genes (WO 2007/069666 A1 and Takahashi, K. and Yamanaka, S., Cell, 126: 663-676 (2006)). Subsequently, it has been revealed that iPS cells can also be prepared using 3 of the above factors (excluding the c-Myc gene) (Nakagawa, M. et al., Nat. Biotechnol., 26: 101-106 (2008)). Furthermore, Yamanaka et al., have succeeded in establishing iPS cells by introducing the 4 above genes into human skin-derived fibroblasts, similarly to the case involving mice (WO 2007/069666 A1 and Takahashi, K. et al., Cell, 131: 861-872 (2007)). Meanwhile, Thomson et al.,'s group has prepared human iPS cells using Nanog and Lin28 instead of Klf4 and c-Myc (WO 2008/118820 A2 and Yu, J. et al., Science, 318: 1917-1920 (2007)). iPS cells can solve bioethical issues such as embryo disruption, and can be grown while maintaining their pluripotency, so that iPS cells are expected as grafting materials for regenerative medicine.

[0003] Meanwhile, even when the thus established iPS cells are induced to differentiate into specific tissue cells, the resulting cells may include undifferentiated (or insufficiently differentiated) cells having proliferation potency (Miura K. et al., Nat Biotechnol., 27: 743-745 (2009)). In such a case, there are concerns about tumorigenesis after grafting. Hence, a method for screening for an iPS cell line containing no cells that exhibit resistance to differentiation induction from among the thus established iPS cell lines has been desired.

Summary of Invention

Technical Problem

[0004] An object of the invention is to efficiently select safe iPS cells (induced pluripotent stem cells) suitable for clinical applications. Specifically, an object of the invention is

to provide a means for screening for a cell line exhibiting no differentiation resistance.

Solution to Problem

[0005] To achieve the above objects, the present inventors have examined RNA that is specifically expressed in iPS cell lines exhibiting differentiation resistance or RNA that is specifically expressed in iPS cell lines exhibiting no differentiation resistance using iPS cell lines exhibiting differentiation resistance and iPS cell lines exhibiting no differentiation resistance. It was thus confirmed that large intergenic non-coding RNA (lincRNA) or mRNA encoded by a specific genomic region is specifically expressed in iPS cell lines exhibiting differentiation resistance or iPS cell lines exhibiting no differentiation resistance.

[0006] Based on the above results, the present inventors have discovered that iPS cells exhibiting differentiation resistance can be screened for through the use of large intergenic non-coding RNA (lincRNA) or mRNA encoded by a specific genomic region as an indicator (marker), and thus have completed the present invention.

Thus, in one aspect, the present invention refers to a method for screening for a human induced pluripotent stem cell line exhibiting no differentiation resistance, comprising the steps of:

(i) measuring expression of at least one large intergenic non-coding RNA (lincRNA) or mRNA selected from group A and/or group B,

(ii) selecting a human induced pluripotent stem cell line in which the lincRNA or mRNA selected from group A expresses or the lincRNA or mRNA selected from group B does not express,

wherein the lincRNA or mRNA selected from group A is selected from the group consisting of

(A20) DLX5,

(A50) MEIS2,

(A53) lincRNA:chr17:21434064-21435857 reverse strand, and

(A58) GREB1L,

wherein the lincRNA or mRNA selected from Group B is selected from the group consisting of

(B4) C4orf51,

(B9) C7orf57,

(B10) lincRNA:chr7:124873114-124899839 reverse strand,

(B12) OC90,

(B13) lincRNA:chr8:133071643-133092468 reverse strand,

(B14) lincRNA:chr8:133073732-133075753 reverse strand,

(B15) HHLA1,

(B16) lincRNA:chr8:133076031-133093351 reverse strand,

(B17) lincRNA:chr8:133090096-133097869 reverse strand,

(B22) ABHD12B,

(B23) lincRNA:chr18:54721302-54731677 reverse strand,

(B27) ZNF541,

(B28) TBX1,

(B29) CXorf61, and

(B30) DB090170 TESTI4 Homo sapiens cDNA clone TESTI4038997 5', mRNA sequence [DB090170].

In another aspect, the present invention refers to a method for screening for a human induced pluripotent stem cell line exhibiting no differentiation resistance, comprising the following steps;

(i) measuring DNA-methylated state of LTR region located in at least one gene selected from group of (B4) C4orf51, (B15) HHLA1, and (B22) ABHD12B, and

(ii) selecting a human induced pluripotent stem cell line in which the LTR7 region is in a DNA-methylated state.

Also disclosed herein is the following [1] to [9].

[1] A method for screening for a human induced pluripotent stem cell line exhibiting *no differentiation resistance, comprising the steps of:*

(i) measuring expression of at least one large intergenic non-coding RNA (lincRNA) or mRNA selected from group A and/or group B,

(ii) selecting a human induced pluripotent stem cell line in which the lincRNA or mRNA selected from group A expresses or the lincRNA or mRNA selected from group B does not express;

Group A consisting of

(A1) lincRNA:chr1:852245-854050 reverse strand,

(A2) GPR177,

(A3) VTCN1,

(A4) lincRNA:chr1:142803013-142804254 reverse strand,

(A5) APOA2,
(A6) WNT6,
(A7) EPAS1,
(A8) COL3A1,
(A9) SLC40A1,
(A10) S100P,
(A11) HOPX,
(A12) GUCY1A3,
(A13) CDH10,
(A14) HAPLN1,

(A15) PITX1,
(A16) HAND1,
(A17) CGA,
(A18) AQP1,
(A19) DLX6,
(A20) DLX5,
(A21) SOX17,
(A22) FLJ45983,
(A23) PLCE1,
(A24) H19,
(A25) lincRNA:chr11:2016408-2017024 reverse strand,
(A26) lincRNA:chr11:2017517-2017651 forward strand,
(A27) IGF2,
(A28) P2RY6,
(A29) SLN,
(A30) NNMT,
(A31) APOA1,
(A32) ERP27,
(A33) LUM,
(A34) CCDC92,
(A35) CDX2,
(A36) FLJ41170,
(A37) MEG3,
(A38) lincRNA:chr14:101292469-101299626 forward strand,
(A39) lincRNA:chr14:101295637-101302637 forward strand,
(A40) lincRNA:chr14:101296681-101298460 forward strand,
(A41) lincRNA:chr14:101298129-101300147 forward strand,
(A42) lincRNA:chr14:101324825-101327247 forward strand,
(A43) MEG8,
(A44) lincRNA:chr14:101365673-101366049 forward strand,
(A45) lincRNA:chr14:101396955-101397357 forward strand,
(A46) lincRNA:chr14:101430757-101433381 forward strand,
(A47) lincRNA:chr14:101434059-101436282 forward strand,
(A48) lincRNA:chr14:101472355-101473369 forward strand,
(A49) DIO3,
(A50) MEIS2,
(A51) PRTG,
(A52) C17orf51,

(A53) lincRNA:chr17:21434064-21435857 reverse strand,

(A54) lincRNA:chr17:21435180-21454915 reverse strand,

(A55) lincRNA:chr17:21435959-21436405 reverse strand,

(A56) CCR7,

(A57) KRT23,

(A58) GREB1L,

(A59) GATA6,

(A60) TTR,

(A61) UCA1,

(A62) FLRT3,

(A63) lincRNA:chrX:73040495-73047819 reverse strand,

(A64) VGLL1,

(A65) RPS4Y1,

(A66) DDX3Y, and

(A67) RPS4Y2,

Group B consisting of

(B1) DMRTB1,

(B2) lincRNA:chr1:73430887-73446112 reverse strand,

(B3) lincRNA:chr1:73444697-73444997 reverse strand,

(B4) C4orf51,

(B5) PCDHA1,

(B6) lincRNA:chr6:95250854-95263604 reverse strand,

(B7) lincRNA:chr6:14280358-14285376 reverse strand,

(B8) lincRNA:chr6:14283301-14285685 reverse strand,

(B9) C7orf57,

(B10) lincRNA:chr7:124873114-124899839 reverse strand,

(B11) lincRNA:chr8:129599518-129624118 reverse strand,

(B12) OC90,

(B13) lincRNA:chr8:133071643-133092468 reverse strand,

(B14) lincRNA:chr8:133073732-133075753 reverse strand,

(B15) HHLA1,

(B16) lincRNA:chr8:133076031-133093351 reverse strand,

(B17) lincRNA:chr8:133090096-133097869 reverse strand,

(B18) lincRNA:chr8:138387843-138421643 reverse strand,

(B19) lincRNA:chr8:138418343-138425831 reverse strand,

(B20) *NDUFA4L2*,

(B21) lincRNA:chr13:54698462-54707001 reverse strand,

(B22) ABHD12B,

(B23) lincRNA:chr18:54721302-54731677 reverse strand,
(B24) ZNF208,
(B25) ZNF257,
(B26) ZNF676,
(B27) ZNF541,
(B28) TBX1,
(B29) CXorf61, and
(B30) DB090170 TESTI4 Homo sapiens cDNA clone TESTI4038997 5', mRNA sequence [DB090170].

[2] The method according to [1], wherein the lincRNA or mRNA selected from group A is selected from the group consisting of
(A20) DLX5,
(A50) MEIS2,
(A53) lincRNA:chr17:21434064-21435857 reverse strand, and
(A58) GREB1L.

[3] The method according to [1], wherein the lincRNA or mRNA selected from group B is selected from the group consisting of
(B4) C4orf51,
(B9) C7orf57,
(B10) lincRNA:chr7:124873114-124899839 reverse strand,
(B12) OC90,
(B13) lincRNA:chr8:133071643-133092468 reverse strand,
(B14) lincRNA:chr8:133073732-133075753 reverse strand,
(B15) HHLA1,
(B16) lincRNA:chr8:133076031-133093351 reverse strand,
(B17) lincRNA:chr8:133090096-133097869 reverse strand,
(B22) ABHD12B,
(B23) lincRNA:chr18:54721302-54731677 reverse strand,
(B27) ZNF541,
(B28) TBX1,
(B29) CXorf61, and
(B30) DB090170 TESTI4 Homo sapiens cDNA clone TESTI4038997 5', mRNA sequence [DB090170].

[4] The method according to [1], wherein the lincRNA or mRNA selected from group B is selected from the group consisting of;
(B4) C4orf51,
(B15) HHLA1, and
(B22) ABHD12B.

[5] A method for screening for a human induced pluripotent stem cell line exhibiting no differentiation resistance, comprising the following steps;

(i) measuring DNA-methylated state of LTR region or neighborhood thereof located in at least one gene selected from group of (B4) C4orf51, (B15) HHLA1, and (B22) ABHD12B, and

(ii) selecting a human induced pluripotent stem cell line in which the LTR7 region is in a DNA-methylated state.

[6] A reagent for screening for a human induced pluripotent stem cell line exhibiting no differentiation resistance, containing a polynucleotide having at least 15 continuous nucleotides in the nucleotide sequence of at least one mRNA or LincRNA shown in the above group A or B, or a polynucleotide complementary thereto.

[7] The reagent according to [6], which is a microarray prepared by immobilizing, as a probe, a polynucleotide complementary to a polynucleotide having at least 15 continuous nucleotides in the nucleotide sequence of at least one mRNA or LincRNA shown in the above group A or B.

[8] A reagent for screening for a human induced pluripotent stem cell line exhibiting no differentiation resistance, containing an antibody that recognizes a protein encoded by at least one mRNA shown in the above group A or B.

[9] A kit for screening for a human induced pluripotent stem cell line exhibiting no differentiation resistance, containing the reagent according to any one of [6] to [8].

The present application claims priority from the U.S. Provisional Application No. 61/511,156 filed on July 25, 2011, and the contents of these patent applications are herein incorporated by reference.

Advantageous Effects of Invention

[0007] According to the present invention, human iPS cells exhibiting no differentiation resistance can be efficiently screened for. Therefore, the present invention is extremely useful for application of iPS cells to regenerative medicine.

Brief Description of Drawings

[0008] [fig.1]Fig.1 shows the result of measuring expression level of HHLA1, ABHD12B and C4orf51 of cell lines exhibiting differentiation resistance (shown as differentiation resistance): FB-RV3F-4, CB-RV4F-2, DP-EP6F-1, FB-RV3F-4 sub2, CB-RV4F-2 sub2 and DP-EP6F-1, and cell lines exhibiting no differentiation resistance (shown as normal): H1, FB-RV4F-2, FB-RV3F-1, FB-RV3F-4 sub6, CB-RV4F-2 sub1 and DP-EP6F-1 sub5, with quantitative PCR.

[fig.2]Fig. 2 is a schematic diagram showing locations of LTR region in C4orf51, HHLA1 and ABHD12B.

[fig.3]Fig.3 shows the results of the average methylation state of CG dinucleotide in

LTR7 region located in C4orf51, HHLA1or ABHD12B of 6 cell lines exhibiting differentiation resistance (shown as differentiation resistance): FB-RV3F-4, CB-RV4F-2, DP-EP6F-1, FB-RV3F-4 sub2, CB-RV4F-2 sub2 and DP-EP6F-1, and 6 cell lines exhibiting no differentiation resistance (shown as normal): H1, FB-RV4F-2, FB-RV3F-1, FB-RV3F-4 sub6, CB-RV4F-2 sub1 and DP-EP6F-1 sub5, by the Bisulfite method.

Description of Embodiments

[0009] 1. Method for screening human induced pluripotent stem cells (iPS cells)

The method for screening human induced pluripotent stem cells (iPS cells) of the present invention comprises using at least one lincRNA or mRNA shown in the following Table 1 (Group A) or 2 (Group B) as a marker for screening for an iPS cell line exhibiting no differentiation resistance.

[Table 1]

Group A

Marker name	Genbank Accession
lincRNA:chr1:852245-854050 reverse strand	
GPR177	NM_001002292
VTCN1	NM_024626
lincRNA:chr1:142803013-142804254 reverse strand	
APOA2	NM_001643
WNT6	NM_006522
EPAS1	NM_001430
COL3A1	NM_000090
SLC40A1	NM_014585
S100P	NM_005980
HOPX	NM_139211
GUCY1A3	NM_000856

CDH10	NM_006727
HAPLN1	NM_001884
PITX1	NM_002653
HAND1	NM_004821
CGA	NM_000735
AQP1	NM_198098
DLX6	NM_005222
DLX5	NM_005221
SOX17	NM_022454
FLJ45983	NR_024256
PLCE1	NM_016341
H19	NR_002196
lincRNA:chr11:2016408-2017024 reverse strand	
lincRNA:chr11:2017517-2017651 forward strand	
IGF2	NM_000612
P2RY6	NM_176798
SLN	NM_003063
NNMT	NM_006169
APOA1	NM_000039
ERP27	NM_152321
LUM	NM_002345
CCDC92	NM_025140
CDX2	NM_001265
FLJ41170	AK021542
MEG3	NR_003530
lincRNA:chr14:101292469-101299626 forward strand	
lincRNA:chr14:101295637-101302637 forward strand	
lincRNA:chr14:101296681-101298460 forward strand	
lincRNA:chr14:101298129-101300147 forward strand	
lincRNA:chr14:101324825-101327247 forward strand	

MEG8	NR_024149
lincRNA:chr14:101365673-101366049 forward strand	
lincRNA:chr14:101396955-101397357 forward strand	
lincRNA:chr14:101430757-101433381 forward strand	
lincRNA:chr14:101434059-101436282 forward strand	
lincRNA:chr14:101472355-101473369 forward strand	
DIO3	NM_001362
MEIS2	NM_170677
PRTG	NM_173814
C17orf51	NM_001113434
lincRNA:chr17:21434064-21435857 reverse strand	
lincRNA:chr17:21435180-21454915 reverse strand	
lincRNA:chr17:21435959-21436405 reverse strand	
CCR7	NM_001838
KRT23	NM_015515
GREB1L	NM_001142966
GATA6	NM_005257
TTR	NM_000371
UCA1	NR_015379
FLRT3	NM_198391
lincRNA:chrX:73040495-73047819 reverse strand	
VGLL1	NM_016267
RPS4Y1	NM_001008
DDX3Y	NM_001122665
RPS4Y2	NM_001039567

[Table 2]

Group B

Marker name	Genbank Accession
DMRTB1	NM_033067
lincRNA:chr1:73430887-73446112 reverse strand	
lincRNA:chr1:73444697-73444997 reverse strand	
C4orf51	NM_001080531
PCDHA1	NM_031410
lincRNA:chr6:95250854-95263604 reverse strand	
lincRNA:chr6:14280358-14285376 reverse strand	
lincRNA:chr6:14283301-14285685 reverse strand	
C7orf57	NM_001100159
lincRNA:chr7:124873114-124899839 reverse strand	
lincRNA:chr8:129599518-129624118 reverse strand	
OC90	NM_001080399
lincRNA:chr8:133071643-133092468 reverse strand	
lincRNA:chr8:133073732-133075753 reverse strand	
HHLA1	NM_001145095
lincRNA:chr8:133076031-133093351 reverse strand	
lincRNA:chr8:133090096-133097869 reverse strand	
lincRNA:chr8:138387843-138421643 reverse strand	
lincRNA:chr8:138418343-138425831 reverse strand	
NDUFA4L2	NM_020142
lincRNA:chr13:54698462-54707001 reverse strand	

ABHD12B	NM_181533
lincRNA:chr18:54721302-54731677 reverse strand	
ZNF208	NM_007153
ZNF257	NM_033468
ZNF676	NM_001001411
ZNF541	NM_001101419
TBX1	NM_080647
CXorf61	NM_001017978
DB090170 TESTI4 Homo sapiens cDNA clone TESTI4038997 5', mRNA sequence	DB090170

In the present invention, the term "lincRNA" refers to long-chain single-stranded RNA transcribed from a genome, which encodes no gene. LincRNA is denoted with chromosome No., the genome region represented by nucleotide No. described in the GenBank database, and transcriptional direction. For example, "chr1:852245-854050 reverse strand" means single-stranded RNA matching a sequence complementary to nucleotides 852245 to 854050 of chromosome 1 in the GenBank database.

[0010] In the present invention, the term "mRNA" may also refer to a precursor before splicing or mature mRNA after splicing. Examples of the sequence of mature mRNA include not only mRNAs having sequences corresponding to Accession Nos. of GenBank listed in Table 1 or 2, but also isoforms prepared by selective splicing. Also in the present invention, a polynucleotide (e.g., cDNA) from the mRNA or a protein encoded by the RNA can also be used as a marker.

[0011] Furthermore, in the present invention, the term "iPS cells" refers to stem cells artificially derived from somatic cells, which can be prepared by introducing a specific reprogramming factor in the form of DNA or protein into somatic cells and have properties almost equivalent to those of ES cells, such as pluripotency and proliferation potency based on self-replication (K. Takahashi and S. Yamanaka (2006) Cell, 126: 663-676; K. Takahashi et al. (2007), Cell, 131: 861-872; J. Yu et al. (2007), Science, 318: 1917-1920; Nakagawa, M. et al., Nat. Biotechnol. 26: 101-106 (2008); International Publication WO 2007/069666).

[0012] In the present invention, lincRNA or mRNA that can be recognized as a marker may be a polynucleotide comprising the full-length nucleotide sequence of the lincRNA or mRNA, a polynucleotide comprising a sequence complementary thereto, or, a fragment thereof. In the case of such a polynucleotide fragment, it preferably has a polynucleotide having at least 15 continuous nucleotides in the sequence of the

lincRNA or mRNA. Specific examples of such a polynucleotide having at least 15 nucleotides include a polynucleotide having a length of at least 18 continuous nucleotides, a polynucleotide having a length of at least 19 continuous nucleotides, a polynucleotide having a length of at least 20 continuous nucleotides, a polynucleotide having a length of at least 30 continuous nucleotides, a polynucleotide having a length of at least 40 continuous nucleotides, a polynucleotide having a length of at least 50 continuous nucleotides, a polynucleotide having a length of at least 60 continuous nucleotides, a polynucleotide having a length of at least 70 continuous nucleotides, and a polynucleotide having a length of at least 100 continuous nucleotides.

[0013] In the present invention, an iPS cell line exhibiting no differentiation resistance can be detected by measuring the degree of the expression of the above marker, and thus the iPS cell line can be screened for. More specifically, an iPS cell line expressing a marker of group A listed in Table 1 can be screened for as a cell line exhibiting no differentiation resistance, or an iPS cell line not expressing a marker of group B listed in Table 2 can be screened for as a cell line exhibiting no differentiation resistance.

[0014] Here, the expression "expressing a marker" refers to a situation in which a marker is detected by an arbitrary measuring method, and more preferably to a situation in which the thus obtained detection value is equivalent to or higher than a control detection value. Similarly, the expression "not expressing a marker" refers to a situation in which no marker is detected by an arbitrary measuring method, and more preferably to a situation in which the thus obtained detection value is equivalent to or lower than a control detection value. More specifically, when a marker of group A is used, a case in which a detection value is similar to that of a control ES cell line or an iPS cell line known to exhibit no differentiation resistance or a case in which a detection value is higher than that of an iPS cell line known to exhibit differentiation resistance indicates that a marker of group A is expressed. Meanwhile, when a marker of group B is used, a case in which the thus obtained detection value is similar to that of a control ES cell line or an iPS cell line known to exhibit no differentiation resistance or a case in which the thus obtained detection value is lower than that of an iPS cell line known to exhibit differentiation resistance indicates that a marker of group B is not expressed. Here, the expression "detection value high(er)" refers to a situation in which a detection value is 1.5 times, 2 times, 3 times, 4 times, or 5 times higher than a control value, for example, and more preferably, 5 or more times higher than the control value. The expression "detection value lower" refers to a situation in which a detection value is 2/3, 1/2, 1/3, 1/4, or 1/5 (or less) of the control value, for example, and more preferably, 1/5 (or less) of the control value.

[0015] In the present invention, examples of a method for measuring a marker include, but are not particularly limited to, a Northern blot method, in situ hybridization, RNase

protection assay, a microarray method, a PCR method, a real-time PCR method, a Western blot method, and flow cytometry.

[0016] In the case of a measuring method using hybridization, such as the Northern blot method, the full-length nucleotide sequence of the above marker or a polynucleotide complementary to a partial sequence thereof can be used as a probe. Here, the term "complementary polynucleotide (complementary strand, opposite strand)" refers to a polynucleotide that is in a complementary relationship with a subject sequence in terms of nucleotides on the basis of a base pair relationship such as A:T(U) or G:C. Examples of such a complementary strand include not only a complementary sequence completely complementary to the nucleotide sequence of a subject forward strand, but also a sequence having a complementary relationship such that it can hybridize to a subject forward strand under stringent conditions. In addition, stringent conditions can be determined based on the melting temperature (Tm) of a nucleic acid to be bound with a probe, as taught by Berger and Kimmel (1987, Guide to Molecular Cloning Techniques Methods in Enzymology, Vol. 152, Academic Press, San Diego CA). Washing conditions after hybridization are generally conditions of about "1 x SSC, 0.1% SDS, 37 degrees C," for example. Preferably a complementary strand can maintain a state of hybridizing to a subject forward strand even when washed under such conditions. Examples of even more stringent hybridization conditions include, but are not particularly limited to, washing conditions of about "0.5 x SSC, 0.1% SDS, 42 degrees C." Examples of more stringent hybridization conditions include conditions under which a forward strand and the complementary strand can maintain the hybridization state even when washed under washing conditions of about "0.1 x SSC, 0.1% SDS, 65 degrees C." Specific examples of such a complementary strand include a strand consisiting of a nucleotide sequence that is in a complete complementary relationship with a subject forward-strand nucleotide sequence, and a strand consisiting of a nucleotide sequence having at least 90%, preferably at least 95%, 96%, 97%, 98%, or 99% sequence identity with the strand. The probe size is a length of at least 15 continuous nucleotides, at least 18 continuous nucleotides, at least 19 continuous nucleotides, at least 20 continuous nucleotides, at least 30 continuous nucleotides, at least 40 continuous nucleotides, at least 50 continuous nucleotides, at least 60 continuous nucleotides, at least 70 continuous nucleotides, at least 100 continuous nucleotides, or full-length continuous nucleotides. Such a probe may be labeled with a radioisotope (e.g., ^{32}P and ^{33}P), a fluorescent substance (e.g., fluorescamine, rhodamine, Texas Red, dansyl, or derivatives thereof), a chemiluminescent substance, or an enzyme, for example.

[0017] Furthermore, a poly(oligo)nucleotide serving as the above probe is preferably provided in the form of a microarray with the poly(oligo)nucleotide immobilized on a

solid-phase support (substrate). Examples of a solid-phase support for a microarray include a glass substrate, a silicon substrate, a membrane, and beads, but the material, size, and shape thereof are not particularly limited. A method for forming a microarray is not particularly limited, and any method that can be used by persons skilled in the art may be employed herein. Examples thereof include a method (on-chip method) that involves directly synthesizing a probe on the surface of a solid-phase support and a method that involves binding a probe prepared in advance to the surface of a solid-phase support. A method that is generally employed when a probe is directly synthesized on the surface of a solid-phase support comprises performing selective synthesis of an oligonucleotide in a predetermined micro-matrix region using a protecting group to be selectively removed by light irradiation in combination with a photolithographic technique and a solid phase synthesis technique that are used for semiconductor manufacturing. Meanwhile, examples of a method that can be used herein, which comprises preparing a probe in advance and then binding it to the surface of a solid-phase support, include a method that comprises spotting a probe onto the surface of a solid-phase support that has been surface-treated with a polycationic compound or a silane coupling agent having an amino group, an aldehyde group, an epoxy group or the like using a spotter device depending on nucleic acid probe types or solid-phase support types, and a method that comprises synthesizing a probe having a reactive group introduced therein, spotting the probe onto the surface of a solid-phase support that has been surface-treated in advance so as to cause the formation of a reactive group, and thus binding and immobilizing the probe onto the surface of the solid-phase support via covalent bonding.

[0018] In another embodiment, when the above marker is specifically recognized and amplified, an oligonucleotide containing a nucleotide sequence of the marker or a sequence complementary to the nucleotide sequence can be used as a primer. A primer can be prepared by designing it based on each nucleotide sequence of the above marker using primer 3 (http://primer3.sourceforge.net/) or vector NTI (Infomax), for example, and then performing synthesis and purification. A primer is designed while avoiding a complementary sequence of the two primers so as to prevent a set of or a pair of primers (2 primers) consisting of a sense strand (5' terminal side) and an antisense strand (3' terminal side) from annealing to each other; and also avoiding palindrome so as to prevent the formation of a hairpin structure within a primer. The primer size is not particularly limited, as long as amplification and detection of the above marker are possible, and is a length of at least 15 nucleotides, preferably a length of 15 to 50 nucleotides, and more preferably a length of 20 to 35 nucleotides. A primer can be synthesized with a method known in the art as a method for synthesizing an oligonucleotide (e.g., a phosphotriethyl method and a phosphodiester method) using a

generally employed automatic DNA synthesizer. Such a primer may be labeled with a labeling substance similar to the above so as to facilitate the detection of amplification products.

[0019] In another embodiment, an antibody can be used when the above marker is recognized as a protein.

[0020] The form of the antibody of the present invention is not particularly limited and may be a polyclonal antibody or a monoclonal antibody the immunogen of which is a protein encoded by mRNA listed in Table 1 or 2, or, a chimeric antibody (e.g., a human/mouse chimeric antibody), a humanized antibody, or a human antibody, or, a fragment of these antibodies (e.g., Fab, Fab', $F(ab')_2$, Fc, Fv, and scFv), or, an antibody having antigen-binding property to a polypeptide comprising at least 8 continuous amino acids (e.g., 10 to 20 continuous amino acids) in the amino acid sequence of the protein.

Methods for producing the above antibody are known and the antibody of the present invention can be produced according to these conventional methods (Current protocols in Molecular Biology edit. Ausubel et al., (1987) Publish. John Wiley and Sons. Section 11.12-11.13).

[0021] Specifically, when the antibody of the present invention is a polyclonal antibody, it can be obtained by synthesizing a protein encoded by mRNA listed in Table 1 or 2, which has been expressed and purified according to a conventional method using Escherichia coli or the like, or an oligopeptide having a partial amino acid sequence thereof, immunizing a non-human animal such as a domestic rabbit with the resultant, and then obtaining the antibody from the serum of the immunized animal according to a conventional method. Meanwhile, in the case of a monoclonal antibody, it can be obtained by subjecting hybridoma cells (prepared by cell fusion of myeloma cells with spleen cells obtained from the above-immunized non-human animal) to HAT selection and affinity assay with a target polypeptide (Current protocols in Molecular Biology edit. Ausubel et al., (1987) Publish. John Wiley and Sons. Section 11.4-11.11), for example. The thus obtained antibody may be labeled with a fluorescent substance (e.g, fluorescamine, rhodamine, Texas Red, dansyl, or a derivative thereof), a chemiluminescent substance, or an enzyme, for example.

[0022] Moreover, a protein to be used for antibody preparation can be obtained by, based on the gene sequence information from the Genbank database, DNA cloning, construction of each plasmid, transfection to a host, culturing the transformant, and collecting the protein from the culture product. These procedures can be performed according to methods known by persons skilled in the art or methods described in documents (Molecular Cloning, T. Maniatis et al., CSH Laboratory (1983), DNA Cloning, DM. Glover, IRL PRESS (1985)), for example. Specifically, such a protein can be obtained

by preparing recombinant DNA (expression vector) that enables gene expression in desired host cells, introducing the DNA into host cells for transformation, culturing the transformant, and then collecting the target protein from the thus obtained culture product.

[0023] Furthermore, in the present invention, method for screening iPS cells exhibiting no differentiation resistance may also be performed by measuring DNA-methylated state of LTR region or neighborhood thereof located in the candidate gene bodies including intron and exon. At this time, LTR means the repeat sequence derived from retrovirus. For example, as the LTR subfamilies such as LTR1, LTR1B, LTR5, LTR7, LTR8, LTR16A1, LTR16C, LTR26, LTR26E, MER48, and MLT2CB are known. Preferable LTR subfamily is LTR7 of human endogenous retroviruses (HERV)-H family in this invention. The LTR7 is located in 658 loci in gene bodies of the whole human genome. The sequence of LTR7 is shown in SEQ NO: 1. In this invention, the sequence of LTR7 include sequence having at least 90%, preferably at least 95%, 96%, 97%, 98%, or 99% sequence identity with the strand.

[0024] Examples of candidate genes are a marker of group B listed in Table 2. A preferable example of such gene is selected from the group of C4orf51, HHLA1 and, ABHD12B. Examples of LTR region in C4orf51, HHLA1, and ABHD12B is shown in SEQ NO: 2, 3, 4, 5, 6 and 7.

[0025] Example of the method of measuring DNA-methylated state involves hydrolyzing unmethylated cytosine using bisulfite. Concretely, the methods include a method that involves performing bisulfite treatment, PCR, and then sequencing, a method that involves using methylation-specific oligonucleotide (MSO) microarrays, or methylation-specific PCR that involves causing PCR primers to recognize a difference between a sequence before bisulfite treatment and the sequence after bisulfite treatment and then determining the presence or the absence of methylated DNA based on the presence or the absence of PCR products. In addition to these methods, by chromosome immunoprecipitation using a DNA methylation-specific antibody, DNA-methylated regions can be detected from specific regions by extracting DNA sequences within DNA-methylated regions, performing PCR, and then performing sequencing.

Upon screening iPS cells exhibiting no differentiation resistance, subject iPS cells in which the DNA-methylated state in the above LTR region located in the candidate gene bodies is in a DNA-methylated state can be selected as iPS cells exhibiting no differentiation resistance. Here, the expression, "the DNA-methylated state" refers to, for example, a state in which the detected methylated CpGs in the subject region account for 50%, 60%, 70%, 80%, 90% or more, preferably 100% of all detected CpGs.

As an example of a method for detecting the percentage of methylated CpGs in one arbitrarily selected cell are sequenced. Hence, the percentage can be calculated by repeatedly sequencing a template to which a PCR product has been cloned a plurality of times such as 2 or more times, preferably 5 or more times, and more preferably 10 or more times and then comparing the number of sequenced clones with the number of clones for which DNA methylation has been detected. When a pyrosequencing method is employed, the percentage can also be directly determined by measuring amout of cytosine or thymine (the amount of cytosine means amount of methylated DNAs and the amount of thymine means amount of unmethylated DNAs).

Upon screening for iPS cells exhibiting no differentiation resistance with the use of the above markers, iPS cells to be subjected to screening may be prepared by introducing a specific reprogramming factor in the form of DNA or protein into somatic cells, according to a previously established method.

[0026] A reprogramming factor may be composed of a gene that is specifically expressed in ES cells, a gene product thereof, or non-coding RNA, a gene playing an important role in maintaining undifferentiation of ES cells, a gene product thereof, or non- coding RNA, or a low-molecular-weight compound. Examples of a gene(s) encompassed by a reprogramming factor include Oct3/4, Sox2, Sox1, Sox3, Sox15, Sox17, Klf4, Klf2, c-Myc, N-Myc, L-Myc, Nanog, Lin28, Fbx15, ERas, ECAT15-2, Tcl1, beta-catenin, Lin28b, Sall1, Sall4, Esrrb, Nr5a2, Tbx3, and Glis1. These reprogramming factors may be used independently or in combination.

[0027] Examples of combinations of reprogramming factors include the combinations as described in WO2007/069666, WO2008/118820, WO2009/007852, WO2009/032194, WO2009/058413, WO2009/057831, WO2009/075119, WO2009/079007, WO2009/091659, WO2009/101084, WO2009/101407, WO2009/102983, WO2009/114949, WO2009/117439, WO2009/126250, WO2009/126251, WO2009/126655, WO2009/157593, WO2010/009015, WO2010/033906, WO2010/033920, WO2010/042800, WO2010/050626, WO2010/056831, WO2010/068955, WO2010/098419, WO2010/102267, WO2010/111409, WO2010/111422, WO2010/115050, WO2010/124290, WO2010/147395, and WO2010/147612, as described in Huangfu D, et al. (2008), Nat. Biotechnol., 26: 795-797, Shi Y, et al. (2008), Cell Stem Cell, 2: 525-528, Eminli S, et al. (2008), Stem Cells. 26:2467-2474, Huangfu D, et al. (2008), Nat Biotechnol. 26:1269-1275, Shi Y, et al. (2008), Cell Stem Cell, 3, 568-574, Zhao Y, et al. (2008), Cell Stem Cell, 3:475-479, Marson A, (2008), Cell Stem Cell, 3, 132-135, Feng B, et al. (2009), Nat Cell Biol. 11:197-203, R.L. Judson et al., (2009), Nat. Biotech., 27:459-461, Lyssiotis CA, et al. (2009), Proc Natl Acad Sci U.S.A. 106: 8912-8917, Kim JB, et al. (2009), Nature. 461: 649-643, Ichida JK, et al. (2009), Cell Stem Cell. 5: 491-503, Heng JC, et

al. (2010), Cell Stem Cell. 6: 167-74, Han J, et al. (2010), Nature. 463: 1096-100, Mali P, et al. (2010), Stem Cells. 28: 713-720, Maekawa M, et al. (2011), Nature. 474: 225-9.

Examples of the above reprogramming factor also include factors to be used for improving the efficiency for establishment such as histone deacetylase (HDAC) inhibitors {e.g., low-molecular weight inhibitors such as valproic acid (VPA), trichostatin A, sodium butyrate, MC 1293, and M344, and nucleic acid expression inhibitors such as siRNA and shRNA against HDAC (e.g., HDAC1 siRNA Smartpool™ (Millipore) and HuSH 29mer shRNA Constructs against HDAC1 (OriGene))}, MEK inhibitors (e.g., PD184352, PD98059, U0126, SL327, and PD0325901), Glycogen synthase kinase-3 inhibitors (e.g., Bio and CHIR99021), DNA methyl transferase inhibitors (e.g., 5-azacytidine), histone methyltransferase inhibitors (e.g., low-molecular-weight inhibitors such as BIX-01294, and nucleic acid expression inhibitors such as siRNA and shRNA against Suv39hl, Suv39h2, SetDBl, and G9a), L-channel calcium agonists (e.g., Bayk8644), butyric acid, TGF beta inhibitors or ALK5 inhibitors (e.g., LY364947, SB431542, 616453, and A-83-01), p53 inhibitors (e.g., siRNA and shRNA against p53), ARID3A inhibitors (e.g., siRNA and shRNA against ARID3A), miRNA (e.g., miR-291-3p, miR-294, miR-295, and mir-302), Wnt Signaling (e.g., soluble Wnt3a), neuropeptide Y, prostaglandins (e.g., prostaglandin E2 and prostaglandin J2), hTERT, SV40LT, UTF1, IRX6, GLISl, PITX2, and DMRTBl. In the Description, these factors to be used for improving the efficiency for establishment are not particularly distinguished from reprogramming factors.

[0028] When a reprogramming factor is in the form of protein, it may be introduced into somatic cells by techniques such as lipofection, fusion with a cell membrane-permeable peptide (e.g., HIV-derived TAT and polyarginine), or microinjection.

[0029] Meanwhile, when a reprogramming factor is in the form of DNA, it can be introduced into somatic cells with a technique using a vector such as a virus, a plasmid, or an artificial chromosome, lipofection (or liposome transfetion), or microinjection, for example. Examples of viral vectors include a retrovirus vector, a lentivirus vector (Cell, 126, pp. 663-676, 2006; Cell, 131, pp. 861-872, 2007; Science, 318, pp. 1917-1920, 2007), an adenovirus vector (Science, 322, 945-949, 2008), an adeno-associated virus vector, and a Sendai virus vector (WO 2010/008054). Furthermore, examples of artificial chromosome vectors include a human artificial chromosome (HAC), a yeast artificial chromosome (YAC), and a bacterial artificial chromosome (BAC or PAC), As a plasmid, a plasmid for mammalian cells can be used (Science, 322: 949-953, 2008). A vector to be used herein can contain regulatory sequences such as a promoter, an enhancer, a ribosome binding sequence, a terminator, and a polyadenylation site so that a nuclear reprogramming substance can be expressed. Fur-

thermore, if necessary, such a vector can further contain a selection marker sequence such as a drug resistance gene (e.g., a kanamycin resistance gene, an ampicillin resistance gene, and a puromycin resistance gene), a thymidine kinase gene, and a diphtheria toxin gene, and a reporter gene sequence such as a green fluorescent protein (GFP), beta glucuronidase (GUS), and FLAG. Moreover, the above vector can further cotain LoxP sequences at the 5'-end and 3'-end of a gene encoding a reprogramming factor or a gene encoding a reprogramming factor linked to a promoter, which allows it to cleave the gene after introduction into somatic cells.

[0030] Furthermore, when a reprogramming factor is in the form of RNA, it may be introduced into somatic cells with techniques such as lipofection or microinjection. To suppress degradation, RNA with 5-methylcytidine and pseudouridine (TriLink Biotechnologies) incorporated therein may also be used (Warren L, (2010) Cell Stem Cell, 7: 618-630).

Examples of culture solutions for inducing iPS cells include 10% to 15% FBS-containing DMEM, DMEM/F12, or DME culture solutions (these culture solutions may further appropriately contain LIF, penicillin/streptomycin, puromycin, L-glutamine, nonessential amino acids, beta-mercaptoethanol, or the like) or commercially available culture solutions {e.g., a culture solution for culturing mouse ES cells (TX-WES culture solution, Thromb-X), a culture solution for culturing primate ES cells (a culture solution for primate ES/iPS cells, ReproCELL), and serum free medium (mTeSR, Stemcell Technology)}.

[0031] An example of culture methods is as follows. Somatic cells are brought into contact with a reprogramming factor on a DMEM or DMEM/F12 culture solution containing 10% FBS at 37 degrees C in the presence of 5% CO_2 and are cultured for about 4 to 7 days. Subsequently, the cells are reseeded on feeder cells (e.g., mitomycin C-treated STO cells or SNL cells). About 10 days after contact between somatic cells and the reprogramming factor, cells are cultured in a culture solution for primate ES cell culture containing bFGF. About 30 to 45 days or more after the contact, iPS cell-like colonies can be formed.

[0032] Alternatively, cells may be cultured at 37 degrees C in the presence of 5% CO_2 using a DMEM culture solution containing 10% FBS (which may further appropriately contain LIF, penicillin/streptomycin, puromycin, L-glutamine, nonessential amino acids, beta-mercaptoethanol, and the like) on feeder cells (e.g., mitomycin C-treated STO cells or SNL cells). After about 25 to 30 days or more, ES-like colonies can be formed. Examples of desirable methods include a method that involves using directly, instead of feeder cells, somatic cells to be reprogrammed (Takahashi K, et al., (2009), PLoS One, 4: e8067 or WO2010/137746) or an extracellular matrix (e.g., Laminin-5 (WO2009/123349) and matrigel (BD)).

Another example in addition to these examples is a culture method that involves culturing with serum-free medium (Sun N, et al., (2009), Proc Natl Acad Sci U.S.A., 106: 15720-15725). Furthermore, iPS cells may also be established under hypoxia conditions (0.1% or more, 15% or less oxygen concentration) in order to increase the efficiency for establishment (Yoshida Y, et al., (2009), Cell Stem Cell, 5: 237-241 or WO2010/013845).

[0033] During the above culture, a culture solution is exchanged with a fresh culture solution once a day from day 2 after the start of culture. In addition, the number of somatic cells to be used for nuclear reprogramming is not limited, but ranges from approximately 5 x 10^3 cells to approximately 5 x 10^6 cells per culture dish (100 cm^2).

[0034] iPS cells can be selected depending on the shapes of the thus formed colonies. Meanwhile, when a drug resistance gene to be expressed in conjunction with a gene that is expressed when somatic cells are reprogrammed (e.g., Oct3/4 or Nanog) is introduced as a marker gene, cells are cultured in a culture solution (selection culture solution) containing a suitable medical agent, so that the thus established iPS cells can be selected. Furthermore, iPS cells can be selected through observation with a fluorescence microscope when a marker gene is a fluorescent protein gene, through addition of a luminescent substrate when a marker gene is a luminescent enzyme gene, or through addition of a chromogenic substrate when a marker gene is a chromogenic enzyme gene.

[0035] The term "somatic cells" as used herein may refer to all animal cells (preferably, mammalian cells including human cells) excluding germ-line cells (e.g., ova, oocytes, and ES cells) or totipotent cells. Examples of somatic cells include, but are not limited to, any fetal somatic cells, neonate somatic cells, and mature healthy or pathogenic somatic cells, or, any primary cultured cells, passaged cells, and established cell lines. Specific examples of somatic cells include (1) tissue stem cells (somatic stem cells) such as neural stem cells, hematopoietic stem cells, mesenchymal stem cells, and dental pulp stem cells, (2) tissue precursor cells, (3) differentiated cells such as lymphocytes, epithelial cells, endothelial cells, muscle cells, fibroblasts (e.g., skin cells), hair cells, hepatocytes, gastric mucosal cells, enterocytes, spleen cells, pancreatic cells (e.g., pancreatic exocrine cells), brain cells, pneumocytes, renal cells, and fat cells.

2. Reagent and kit for screening human induced pluripotent stem cell line

The present invention further provides a reagent for screening for a human induced pluripotent stem cell line. The reagent for screening of the present invention contains at least one type of probe, primer, or antibody for recognition of the above-described marker. Such a reagent can be used for producing a kit in combination with other reagents or apparatuses. The kit of the present invention may contain a reagent for RNA extraction, a reagent for gene extraction, a reagent for chromosome extraction, or

the like. Also, the kit of the present invention may contain a means for discrimination analysis for discrimination between a cell line exhibiting differentiation resistance and a cell line exhibiting no differentiation resistance, such as documents or instructions containing procedures for discrimination analysis, a program for implementing the procedures for discrimination analysis by a computer, the program list, a recording medium containing the program recorded therein, which is readable by the computer (e.g., flexible disk, optical disk, CD-ROM, CD-R, and CD-RW), and an apparatus or a system (e.g., computer) for implementation of discrimination analysis.

Examples

[0036] The present invention will next be described in detail by way of Examples, which should not be construed as limiting the scope of the present invention.

Example 1

[0037] 1. Cell

As human ES cells, KhES1, KhES3 (Suemori H, et al., Biochem Biophys Res Commun. 345: 926-32, 2006) and H9 (Thomson, J.A., et al., Science 282: 1145-1147, 1998) were used.

[0038] As human iPS cells, 9 families and 39 clones prepared by the following method were used.

(i) Six (6) factors (OCT3/4, SOX2, KLF4, L-Myc, LIN28, and p53shRNA) were introduced into CD34 positive cells (WO2010/131747) extracted from umbilical cord blood using an episomal vector (Okita K, et al., Nat Methods, 8: 409-12, 2011), so that four CB-EP6F clones were prepared.

(ii) Four (4) factors (OCT3/4, SOX2, KLF4, and c-MYC) were introduced into CD34 positive cells extracted from umbilical cord blood using a retrovirus, so that three CB-RV4F clones were prepared (WO2010/131747).

(iii) Four (4) factors (OCT3/4, SOX2, KLF4, and c-MYC) were introduced into CD34 positive cells (WO2010/131747) extracted from umbilical cord blood using the Sendai virus (Seki T, et al., Cell Stem Cell, 7: 11-4, 2010), so that five CB-SV4F clones were prepared.

(iv) Six (6) factors (OCT3/4, SOX2, KLF4, L-Myc, LIN28, and p53shRNA) were introduced into dental pulp stem cells using an episomal vector, so that two DP-EP6F clones were prepared (Okita K, et al., Nat Methods, 8: 409-12, 2011).

(v) Six (6) factors (OCT3/4, SOX2, KLF4, L-Myc, LIN28, and p53shRNA) were introduced into fibroblasts using an episomal vector, so that three FB-EP6F clones were prepared (Okita K, et al., Nat Methods, 8: 409-12, 2011).

(vi) Three (3) factors (OCT3/4, SOX2, and KLF4) were introduced into fibroblasts using retrovirus, so that four FB-RV3F clones were prepared (Okita K, et al., Nat

Methods, 8: 409-12, 2011).

(vii) Four (4) factors (OCT3/4, SOX2, KLF4, and c-MYC) were introduced into fibroblasts using retrovirus, so that eleven FB-RV4F clones were prepared (Okita K, et al., Nat Methods, 8: 409-12, 2011).

(viii) Six (6) factors (OCT3/4, SOX2, KLF4, L-Myc, LIN28, and p53shRNA) were introduced into T cells (Seki T, et al., Cell Stem Cell, 7: 11-4, 2010) included in peripheral blood mononuclear cells (PBMC) using an episomal vector, so that four PM-EP6F clones were prepared (Okita K, et al., Nat Methods, 8: 409-12, 2011).

(ix) Four (4) factors (OCT3/4, SOX2, KLF4, and c-MYC) were introduced into T cells included in peripheral blood mononuclear cells (PBMC) using Sendai virus, so that four PM-SV4F clones were prepared (Seki T, et al., Cell Stem Cell, 7: 11-4, 2010).

2. Confirmation of differentiation resistance

To confirm differentiation resistance of ES cells and iPS cells, the aforementioned cells were subjected to differentiation induction to result in neural cells using a modified SFEBq method comprising the following steps.

(1) 10 micromolar Y27632 (WAKO) was added to a culture solution of ES cells or iPS cells and then the solution was left to stand for 3 hours.

(2) Feeder cells were removed using a CTK solution (collagenase-trypsine-KSR), the resultant was treated with Accumax (Innovate cell technologies) and then disintegrated into single cells, and the resulting cells were plated on a 96-well plate (Lipidure-coat U96w, NOF Corporation) at 9,000 cells/150 microliter/well.

(3) Cells were cultured in DMEM/F12 (Invitrogen) containing 10 micromolar Y-27632, 2 micromolar Dorsomorphin (Sigma), 10 micromolar SB431542 (Sigma), 5% KSR (Invitrogen), MEM-Non essential amino acid solution (Invitrogen), L-glutamine (Invitrogen), and 2-Mercaptoethanol (Invitrogen). One-half the medium was exchanged every 3 or 4 days with medium lacking Y-27632, Dorsomorphin, and SB431542, followed by 14 days of culture. Subsequently, the thus obtained neural cells were isolated, fixed in 37% formalin, stained with Alexa Fluor 488 Mouse anti-Oct3/4 (BD Pharmingen), and then analyzed using a flow cytometer. In the cases of CB-RV4F-2, DP-EP6F-1, FB-RV3F-3, FB-RV3F-4, FB-RV4F-5, and FB-RV4F-11, Oct3/4 positive cells were contained at 5% or higher even after differentiation induction. These iPS cells were used as the cells of cell lines exhibiting differentiation resistance. The results are shown in Table 3.

[0039]

[Table 3]

Content of Oct3/4 positive cells in each iPS cell line

patent cell name	Souce	Factor	method	Oct3/4 positive cells (%)										deter mi nation
				1st	2nd	3rd	4th	5th	6th	7th	8th	9th	Max rate	
KhES1				0	0.8	0.08	0.13	0.07	0.01	1.7	0.59	0	1.7	
KhES3				0.2									0.2	o
H1				0.13	0.09								0.13	o
H9				0	0.05	0.1	0.05	0	0.3				0.3	o
CB-EP6F-1	CB	OSKUL+ shp53	Episomal plasmid	0.3	0.01								0.3	o
CB-EP6F-2	CB	OSKUL+ shp53	Episomal plasmid	0.3	0.07								0.3	o
CB-EP6F-3	CB	OSKUL+ shp53	Episomal plasmid	0.1	0.03								0.1	o
CB-EP6F-4	CB	OSKUL+ shp53	Episomal plasmid	4.7	0.1								4.7	
CB-RV4F-1	CB	OSKM	Retro virus	0	0.35	0.49							0.49	o
CB-RV4F-2	CB	OSKM	Retro virus	10.74	8.69	19.01	9.6	14.44	12.78				19.01	x
CB-RV4F-3	CB	OSKM	Retro virus	0	0.39	0.04							0.39	o

CB-SV4F-1	CB	OSKM	Sendai virus	0.9	0.7	0.7							0.9	o
CB-SV4F-2	CB	OSKM	Sendai virus	0	0.1	0.1							0.1	o
CB-SV4F-3	CB	OSKM	Sendai virus	0.3	0.1								0.3	o
CB-SV4F-4	CB	OSKM	Sendai virus	0.2	0.3								0.3	o
CB-SV4F-5	CB	OSKM	Sendai virus	0.1	0								0.1	o
DP-EP6F-1	dental pulp	OSKUL+shp53	Episomal plasmid	13.61	13.91	2.42	6.1	2.18	1.3	1.96			13.91	x
DP-EP6F-2	dental pulp	OSKUL+shp53	Episomal plasmid	0.01	0.06	0.08							0.08	o
FB-EP6F-1	Fibro	OSKUL+shp53	Episomal plasmid	0	0.02								0.02	o
FB-EP6F-2	Fibro	OSKUL+shp53	Episomal plasmid	0.16	0.02								0.16	o
FB-EP6F-3	Fibro	OSKUL+shp53	Episomal plasmid	0.11	0.05	0.1							0.11	o
FB-RV3F-1	Fibro	OSK	Retro virus	0	0	0.1							0.1	o
FB-RV3F-2	Fibro	OSK	Retro virus	0.28	0.1								0.28	o
FB-RV3F-3	Fibro	OSK	Retro virus	0	7.64	14.25	1.19						14.25	x
FB-RV3F-4	Fibro	OSK(M)	Retro virus	1.24	12.41	8.4	8.9	14.9	14.16	4			14.9	x
FB-RV4F-1	Fibro	OSKM	Retro virus	0.05	0.4								0.4	o
FB-RV4F-2	Fibro	OSKM	Retro virus	0.15	0.26	0	0	0	0.01	0.04			0.26	o
FB-RV4F-3	Fibro	OSKM	Retro virus	0.92	3.62	11.2							11.2	x

FB-RV4F-4	Fibro	OSKM	Retro virus	0.04	0.04								0.04	o
FB-RV4F-5	Fibro	OSKM	Retro virus	17.47	5.19	12.6	8.3	6.94	17.1				17.47	x
FB-RV4F-6	Fibro	OSKM	Retro virus	0	3.64								3.64	
FB-RV4F-7	Fibro	OSKM	Retro virus	0.01	0.07								0.07	o
FB-RV4F-8	Fibro	OSKM	Retro virus	0	0.09								0.09	o
FB-RV4F-9	Fibro	OSKM	Retro virus	0	0.05								0.05	o
FB-RV4F-10	Fibro	OSKM	Retro virus	0.03	0.02								0.03	o
FB-RV4F-11	Fibro	OSKM	Retro virus	1.11	13.06	14.39							14.39	x
PB-EP6F-1	PBMN	OSKUL+ shp53	Episomal plasmid	0.1	0.04	0.13							0.13	o
PB-EP6F-2	PBMN	OSKUL+ shp53	Episomal plasmid	4.7	0.02								4.7	
PB-EP6F-3	PBMN	OSKUL+ shp53	Episomal plasmid	0.2	0.02								0.2	o
PB-EP6F-4	PBMN	OSKUL+ shp53	Episomal plasmid	0.1	0.94								0.94	o
PB-SEP4F-1	PBMN	OSKM	Sendai virus	0.1	0.1								0.1	o
PB-SV4F-2	PBMN	OSKM	Sendai virus	0.1	0.2								0.2	o
PB-SV4F-3	PBMN	OSKM	Sendai virus	0.1	1.3	1.3							1.3	
PB-SV4F-4	PBMN	OSKM	Sendai virus	0.1	0.2	0.4							0.4	o

"o" means the clone exhibiting no differentiation resistance.

"x" measns the clone exhibiting differentiation resistance.

3. Identification of differentiation resistance marker

RNA was collected from 5 iPS cell lines exhibiting differentiation resistance (CB-RV4F-2, DP-EP6F-1, FB-RV3F-3, FB-RV3F-4, and FB-RV4F-5) and 27 iPS cell lines (including ES cells) exhibiting no differentiation resistance. RNA expression was

measured using microarrays (Human GE G3 8x60k, Agilent). Table 4 shows marker groups that were expressed in cell lines exhibiting no differentiation resistance at a level 5 or more times higher than that in cell lines exhibiting differentiation resistance. Similarly, Table 5 shows marker groups that were expressed in cell lines exhibiting no differentiation resistance at a level 5 or more times lower than that in cell lines exhibiting differentiation resistance. Here, four markers, the P value of each of which obtained by t-test was 0.05 or less, were confirmed from Table 4 (DLX5, MEIS2, lincRNA:chr17:21434064-21435857 reverse strand, GREB1L) and 12 markers, the P value of each of which obtained by t-test was 0.05 or less, were confirmed from Table 5 (C4orf51, C7orf57, lincRNA:chr7:124873114-124899839 reverse strand, OC90, lincRNA:chr8:133071643-133092468 reverse strand, lincRNA:chr8:133076031-133093351 reverse strand, ABHD12B, lincRNA:chr18:54721302-54731677 reverse strand, ZNF541, TBX1, CXorf61, DB090170 TESTI4 Homo sapiens cDNA clone TESTI4038997 5', mRNA sequence [DB090170]).

[Table 4-1]

Markers for cell lines exhibiting no differentiation resistance

Marker name	Genbank Accession	Chromosome Number	Strand Direction	Region	P<0.05
lincRNA:chr1:852245-854050 reverse strand		chr1	-	852,245-854,050	
GPR177	NM_001002292	chr1	-	29,518,977-29,543,121	
VTCN1	NM_024626	chr1	-	117,686,209-117,753,549	
lincRNA:chr1:142803013-142804254 reverse strand		chr1	-	142,803,013-142,804,254	
APOA2	NM_001643	chr1	-	161,192,063-161,193,418	
WNT6	NM_006522	chr2	+	23,961,932-23,965,019	
EPAS1	NM_001430	chr2	+	46,524,541-46,613,842	
COL3A1	NM_000090	chr2	+	189,839,099-189,877,472	
SLC40A1	NM_014585	chr2	-	190,425,316-190,445,537	
S100P	NM_005980	chr4	+	6,695,566-6,698,897	
HOPX	NM_139211	chr4	-	57,514,154-57,547,872	
GUCY1A3	NM_000856	chr4	+	156,587,862-156,658,214	
CDH10	NM_006727	chr5	-	24,487,209-24,645,085	
HAPLN1	NM_001884	chr5	-	82,934,017-83,016,896	
PITX1	NM_002653	chr5	-	134,363,424-134,369,964	
HAND1	NM_004821	chr5	-	153,854,532-153,857,824	
CGA	NM_000735	chr6	-	87,795,222-87,804,824	
AQP1	NM_198098	chr7	+	30,951,415-30,965,131	

[0040]

[Table 4-2]

Marker name	Genbank Accession	Chromosome Number	Strand Direction	Region	P<0.05
DLX6	NM_005222	chr7	+	96,635,290-96,640,352	
DLX5	NM_005221	chr7	-	96,649,702-96,654,143	○
SOX17	NM_022454	chr8	+	55,370,495-55,373,456	
FLJ45983	NR_024256	chr10	-	8,092,413-8,095,447	
PLCE1	NM_016341	chr10	+	95,753,746-96,088,149	
H19	NR_002196	chr11	-	2,016,406-2,019,065	
lincRNA:chr11:2016408-2017024 reverse strand		chr11	-	2,016,408-2,017,024	
lincRNA:chr11:2017517-2017651 forward strand		chr11	+	2,017,517-2,017,651	
IGF2	NM_000612	chr11	-	2,150,350-2,182,439	
P2RY6	NM_176798	chr11	+	72,975,570-73,009,664	
SLN	NM_003063	chr11	-	107,578,101-107,582,787	
NNMT	NM_006169	chr11	+	114,166,535-114,183,238	
APOA1	NM_000039	chr11	-	116,706,469-116,708,338	
ERP27	NM_152321	chr12	-	15,066,976-15,091,463	
LUM	NM_002345	chr12	-	91,497,232-91,505,542	
CCDC92	NM_025140	chr12	-	124,420,955-124,457,163	
CDX2	NM_001265	chr13	-	28,536,278-28,543,317	
FLJ41170	AK021542	chr14	+	81,527,645-81,529,369	
MEG3	NR_003530	chr14	+	101,292,445-101,327,363	

[0041]

[Table 4-3]

Marker name	Genbank Accession	Chromosome Number	Strand Direction	Region	P<0.05
lincRNA:chr14:101292469-101299626 forward strand		chr14	+	101,292,469-101,299,626	
lincRNA:chr14:101295637-101302637 forward strand		chr14	+	101,295,637-101,302,637	
lincRNA:chr14:101296681-101298460 forward strand		chr14	+	101,296,681-101,298,460	
lincRNA:chr14:101298129-101300147 forward strand		chr14	+	101,298,129-101,300,147	
lincRNA:chr14:101324825-101327247 forward strand		chr14	+	101,324,825-101,327,247	
MEG8	NR_024149	chr14	+	101,361,107-101,373,305	
lincRNA:chr14:101365673-101366049 forward strand		chr14	+	101,365,673-101,366,049	
lincRNA:chr14:101396955-101397357 forward strand		chr14	+	101,396,955-101,397,357	
lincRNA:chr14:101430757-101433381 forward strand		chr14	+	101,430,757-101,433,381	
lincRNA:chr14:101434059-101436282 forward strand		chr14	+	101,434,059-101,436,282	
lincRNA:chr14:101472355-101473369 forward strand		chr14	+	101,472,355-101,473,369	
DIO3	NM_001362	chr14	+	102,027,688-102,029,789	
MEIS2	NM_170677	chr15	-	37,183,232-37,393,500	○
PRTG	NM_173814	chr15	-	55,903,738-56,035,177	
C17orf51	NM_001113434	chr17	-	21,431,571-21,454,941	
lincRNA:chr17:21434064-21435857 reverse strand		chr17	-	21,434,064-21,435,857	○
lincRNA:chr17:21435180-21454915 reverse strand		chr17	-	21,435,180-21,454,915	
lincRNA:chr17:21435959-21436405 reverse strand		chr17	-	21,435,959-21,436,405	
CCR7	NM_001838	chr17	-	38,710,021-38,721,736	

[0042]

[Table 4-4]

Marker name	Genbank Accession	Chromosome Number	Strand Direction	Region	P<0.05
KRT23	NM_015515	chr17	-	39,078,952-39,093,836	
GREB1L	NM_001142966	chr18	+	18,822,203-19,102,791	○
GATA6	NM_005257	chr18	+	19,749,416-19,782,227	
TTR	NM_000371	chr18	+	29,171,730-29,178,987	
UCA1	NR_015379	chr19	+	15,939,757-15,946,230	
FLRT3	NM_198391	chr20	-	14,304,639-14,318,313	
lincRNA:chrX:73040495-73047819 reverse strand		chrX	-	73,040,495-73,047,819	
VGLL1	NM_016267	chrX	+	135,614,311-135,638,966	
RPS4Y1	NM_001008	chrY	+	2,709,623-2,734,997	
DDX3Y	NM_001122665	chrY	+	15,016,019-15,032,390	
RPS4Y2	NM_001039567	chrY	+	22,917,954-22,942,918	

[0043]

[Table 5-1]

Markers for cell lines exhibiting differentiation resistance

Marker name	Genbank Accession	Chromosome Number	Strand Direction	Region	P<0.05
DMRTB1	NM_033067	chr1	+	53,925,072-53,933,158	
lincRNA:chr1:73430887-73446112 reverse strand		chr1	-	73,430,887-73,446,112	
lincRNA:chr1:73444697-73444997 reverse strand		chr1	-	73,444,697-73,444,997	
C4orf51	NM_001080531	chr4	+	146,601,356-146,653,949	○
PCDHA1	NM_031410	chr5	+	140,165,876-140,391,929	
lincRNA:chr6:95250854-95263604 reverse strand		chr6	-	95,250,854-95,263,604	
lincRNA:chr6:14280358-14285376 reverse strand		chr6	-	14,280,358-14,285,376	
lincRNA:chr6:14283301-14285685 reverse strand		chr6	-	14,283,301-14,285,685	
C7orf57	NM_001100159	chr7	+	48,075,117-48,100,894	○
lincRNA:chr7:124873114-124899839 reverse strand		chr7	-	124,873,114-124,899,839	○
lincRNA:chr8:129599518-129624118 reverse strand		chr8	-	129,599,518-129,624,118	
OC90	NM_001080399	chr8	-	133,036,467-133,071,627	○
lincRNA:chr8:133071643-133092468 reverse strand		chr8	-	133,071,643-133,092,468	○
lincRNA:chr8:133073732-133075753 reverse strand		chr8	-	133,073,732-133,075,753	
HHLA1	NM_001145095	chr8	-	133,073,733-133,117,512	
lincRNA:chr8:133076031-133093351 reverse strand		chr8	-	133,076,031-133,093,351	○
lincRNA:chr8:138387843-138421643 reverse strand		chr8	-	138,387,843-138,421,643	
lincRNA:chr8:138418343-138425831 reverse strand		chr8	-	138,418,343-138,425,831	

[0044]

[Table 5-2]

Marker name	Genbank Accession	Chromosome Number	Strand Direction	Region	P<0.05
NDUFA4L2	NM_020142	chr12	-	57,628,686-57,634,545	
lincRNA:chr13:54698462-54707001 reverse strand		chr13	-	54,698,462-54,707,001	
ABHD12B	NM_181533	chr14	+	51,338,878-51,371,688	○
lincRNA:chr18:54721302-54731677 reverse strand		chr18	-	54,721,302-54,731,677	○
ZNF208	NM_007153	chr19	-	22,148,897-22,193,745	
ZNF257	NM_033468	chr19	+	22,235,266-22,273,905	
ZNF676	NM_001001411	chr19	-	22,361,903-22,379,753	
ZNF541	NM_001101419	chr19	-	48,023,947-48,059,113	○
TBX1	NM_080647	chr22	+	19,744,226-19,771,116	○
CXorf61	NM_001017978	chrX	-	115,592,852-115,594,137	○
DB090170 TESTI4 Homo sapiens cDNA clone TESTI4038997 5', mRNA sequence [DB090170]	DB090170	chrX	-		○

Example 2

[0045] (1) Cell

The above four iPS cell lines exhibiting differentiation resistance (CB-RV4F-2, DP-EP6F-1, FB-RV3F-4, and FB-RV4F-5) were seeded and then the thus obtained colonies were picked up, so that 15 subclones were obtained from CB-RV4F-2, 15 subclones were obtained from DP-EP6F-1, 10 subclones were obtained from FB-RV3F-4, and 11 subclones were obtained from FB-RV4F-5.

(2) Confirmation of differentiation resistance

To confirm the differentiation resistance of ES cells and iPS cells, differentiation induction to neural cells was performed using the above modified SFEBq method, and then the contents of TRA-1-60 positive cells were examined using a flow cytometer. As a result, 12 out of 15 CB-RV4F-2 subclones contained TRA-1-60 positive cells at 1% or more after induction of cell differentiation to neural cells (Table 6). Similarly,

12 out of 15 DP-EP6F-1 subclones (Table 7), 8 out of 10 FB-RV3F-4 subclones (Table 8), and 3 out of 11 FB-RV4F-5 subclones (Table 9) contained TRA-1-60 positive cells at 1% or more. These 35 subclones found to contain TRA-1-60 positive cells at 1% or more were screened for as iPS cell lines exhibiting differentiation resistance.

[0046] [Table 6]

TRA-1-60 positive cell content in CB-RV4F-2 subclone

subclone name	TRA-1-60 positive cells (%)			
	1st try	2nd try	3rd try	Average
CB-RV4F-2 sub1	0.1	0.1	0.1	0.1
CB-RV4F-2 sub2	24.6	10.1	11.5	15.4
CB-RV4F-2 sub3	17.2	14.7	4.2	12.03333
CB-RV4F-2 sub4	8.4	20	41.8	23.4
CB-RV4F-2 sub5	12	13.8	11	12.26667
CB-RV4F-2 sub6	20.7	15	14	16.56667
CB-RV4F-2 sub7	25.1	21.8	24.1	23.66667
CB-RV4F-2 sub8	10	4.6	2	5.533333
CB-RV4F-2 sub9	9.6	3.5	1.9	5
CB-RV4F-2 sub10	17.5	11.8	15.5	14.93333
CB-RV4F-2 sub11	0.1	0.3	0.1	0.166667
CB-RV4F-2 sub12	28.8	23.8	15.7	22.76667
CB-RV4F-2 sub13	23.1	21.5	12.1	18.9
CB-RV4F-2 sub14	14.2	7.3	11.8	11.1
CB-RV4F-2 sub15	0	0.5	0.1	0.2
CB-RV4F-2	27.3	26	8	20.43333
H9	0.2	0.1	0.2	0.166667
khES1	0	0	0.3	0.1
khES3	0.1	0	0.1	0.066667

[0047]

[Table 7]

TRA-1-60 positive cell content in DP-EP6F-1 subclone

subclone name	TRA-1-60 positive cells (%)
DP-EP6F-1 sub1	8.8
DP-EP6F-1 sub2	21
DP-EP6F-1 sub3	48.3
DP-EP6F-1 sub4	11.4
DP-EP6F-1 sub5	0.6
DP-EP6F-1 sub6	8.1
DP-EP6F-1 sub7	43.9
DP-EP6F-1 sub8	9.5
DP-EP6F-1 sub9	0.2
DP-EP6F-1 sub10	22
DP-EP6F-1 sub11	0.1
DP-EP6F-1 sub12	44.1
DP-EP6F-1 sub13	41.3
DP-EP6F-1 sub14	10.9
DP-EP6F-1 sub15	16.9
DP-EP6F-1	53.5
H9	0.1
khES3	0.1

[0048] [Table 8]

TRA-1-60 positive cell content in FB-RV3F-4 subclone

subclone name	TRA-1-60(%)			
	1st try	2nd try	3rd try	Average
FB-RV3F-4 sub1	10.9	29.1	9.9	16.63333
FB-RV3F-4 sub2	9.4	28.3	8.5	15.4
FB-RV3F-4 sub3	7.7	26	6.7	13.46667
FB-RV3F-4 sub4	0	0.1	0	0.033333
FB-RV3F-4 sub5	0.1	0	0	0.033333
FB-RV3F-4 sub6	6.7	12.9	7.5	9.033333
FB-RV3F-4 sub7	14	12.5	6.6	11.03333
FB-RV3F-4 sub8	12.9	25.9	24.8	21.2
FB-RV3F-4 sub9	7.5	9.5	5.2	7.4
FB-RV3F-4 sub10	21.4	32	21	24.8
FB-RV3F-4	30.8	41.4	19.9	30.7
H9	0.1	0	0.1	0.066667
khES1	0.1	0.1	0	0.066667
khES3	0	0.1	0	0.033333

[0049]

[Table 9]

TRA-1-60 positive cell content in FB-RV4F-5 subclone

subclone name	TRA-1-60 positive cells (%)		
	1st try	2nd try	Average
FB-RV4F-5 sub1	0.1	0.8	0.45
FB-RV4F-5 sub2	3.8	13.1	8.45
FB-RV4F-5 sub3	0.3	0.5	0.4
FB-RV4F-5 sub4	0.1	0.1	0.1
FB-RV4F-5 sub5	14.8	20.9	17.85
FB-RV4F-5 sub6	38.9	19.6	29.25
FB-RV4F-5 sub7	0.1	0	0.05
FB-RV4F-5 sub8	0.1	0.2	0.15
FB-RV4F-5 sub9	0.4	0.1	0.25
FB-RV4F-5 sub10	0.2	0.7	0.45
FB-RV4F-5 sub11	0.1	0.8	0.45
FB-RV4F-5	2.8	7.7	5.25
H9	0.2	0.1	0.15
khES1	0.1	0.3	0.2
khES3	0.1	0.3	0.2

Identification of differentiation resistance marker

RNAs were extracted from the 35 subclones exhibiting differentiation resistance and 16 subclones exhibiting no differentiation resistance, which had been screened for by the above method, and then the expression level of each RNA was examined using microarrays. As a result, lincRNA and mRNA were expressed at significantly high levels in subclones exhibiting differentiation resistance, as shown in Table 10.

[0050]

[Table 10]

Marker name	Genbank Accession	Chromosome Number	Strand Direction	Region	P<0.05
OC90	NM_001080399	chr8	-	133,036,467-133,071,627	○
lincRNA:chr8:133071643-133092468 reverse strand		chr8	-	133,071,643-133,092,468	○
lincRNA:chr8:133073732-133075753 reverse strand		chr8	-	133,073,732-133,075,753	○
HHLA1	NM_001145095	chr8	-	133,073,733-133,117,512	○
lincRNA:chr8:133076031-133093351 reverse strand		chr8	-	133,076,031-133,093,351	○
lincRNA:chr8:133090096-133097869 reverse strand		chr8	-	133,090,096-133,097,869	○
ABHD12B	NM_181533	chr14	+	51,338,878-51,371,688	○

Example 3

[0051] RNA expression of C4orf51, HHLA1 and ABHD12B contained in the Table 5 (Example 1) and Table 10 (Example 2) as the markers for cell lines exhibiting differentiation resistance was measured in 6 clones exhibiting differentiation resistance and 6 clones exhibiting no differentiation resistance with quantitative PCR (Fig. 1). It was confirmed that these marker genes were expressed in the clone exhibiting differentiation resistance as the same manner of former result. Furthermore, it was known that these genes have the LTR7 region in their gene bodies (Fig. 2). Consequently, percentage of methylated cytosines in CpG dinucleotide in LTR7 region or neighborhood thereof was measured with Pyrosequencing (Fig. 3). Briefly, pyrosequencing was carried out with primers designed with the Pyromark Assay Design Software 2.0 (Qiagen). The primer sequence and the CpG dinucleotide in LTR7 region is shown in Tables 11 and 12. PCR was performed in a 25 microliter reaction mix containing 25ng bisulfite-converted DNA, 1X Pyromark PCR Master Mix (Qiagen), 1X Coral Load Concentrate (Qiagen), and 0.3 micromolar forward and 5' biotinylated reverse primers. PCR conditions were 45 cycles of 95 degrees C for 30 s, 50 degrees C for 30 s, and 72 degrees C for 30 s. PCR product was bound to streptavidin sepharose beads (Amersham Biosciences), and then was purified, washed, denatured, and washed again. Then, 0.3 micromol/L pyrosequencing primer was annealed to the purified PCR product. Pyrosequencing reactions were performed in the PSQ HS 96 Pyrosequencing System. The degree of methylation was expressed as percentage of methylated

cytosines divided by the sum of methylated and unmethylated cytosines (percentage of 5mC). To validate PCR pyrosequencing assay, each CpG dinucleotide position was assayed in triplicate and their averages were used in final analysis. As the result, the methylated status of CpG dinucleotide position in the LTR7 region or neighborhood thereof located in C4orf51, HHLA1 and ABHD12B gene bodies were significantly high level.

[0052] [Table 11]

Region	primer	sequence	SEQ ID NO
HHLA1 3'LTR-1 (pos.1,2)	forward primer	TGTGAAAGTTTTTTTTTGGTTTATTTTG	8
	reverse primer	CCTCTCCAAAACTCTAATACATATCTT	9
	pyrosequencing primer	ACAATAAAACTATTTATTTCACCT	10
HHLA1 3'LTR-2 (pos.3,4)	forward primer	AAAGTTTGTTTGGTGGTTTTTT	11
	reverse primer	AAAAAAATTAATCTCCTCCATATACCTT	12
	pyrosequencing primer	TTGTTTGGTGGTTTTTTTA	13
ABHD12B before 5'LTR (pos.1,2,3)	forward primer	TGTGTATTAATGTATGGTTAATTTTGGTAA	14
	reverse primer	CAAACCATCTAAACAAATACCTACAA	15
	pyrosequencing primer	GTTGTTTTTTATGTAGTGTTT	16
ABHD12B 5'LTR (pos.4)	forward primer	TGTGTATTAATGTATGGTTAATTTTGGTAA	14
	reverse primer	CAAACCATCTAAACAAATACCTACAA	15
	pyrosequencing primer	TTAGGTTTTTGAGTTTAAGTTAA	17
ABHD12B 3'LTR (pos.1,2)	forward primer	AAGTTTGTTTGGTGGTTTTTTTATATAGA	18
	reverse primer	ACCATTCCACAATCATAATAAAATACTTT	19
	pyrosequencing primer	ACCAATAACAATAAACAAAATTT	20

ABHD12B after 3'LTR-1 (pos.3)	forward primer	GTTGTGGAGTTATTTAGATTTGGGTTTA	21
	reverse primer	CTTTTCCTACCATACATAACACTTTAAC	22
	pyrosequencing primer	TTTTTTATTAAGGGGTTGG	23
ABHD12B after 3'LTR-2 (pos.4)	forward primer	TTTTTTTTTTGAAGGTGAGGGAAAGTAGTT	24
	reverse primer	AACCTATAAATCTCCATTTCTCTCATCTC	25
	pyrosequencing primer	TGGTAGGAATGGGGT	26
C4orf51 5'LTR (pos.1,2)	forward primer	GGATAATTTGAAAATGTTTTTGGTTAAGG	27
	reverse primer	ATAATTCTTCAATTACTTCAAACCATCTA	28
	pyrosequencing primer	GGTTTTTGAGTTTAAGTTAAG	29
C4orf51 3'LTR (pos.1,2,3)	forward primer	TTTTTTTTTTGGTTTATTTTGGTTTAAAAG	30
	reverse primer	ACAAACCATATCTCAAATAAAAAATTTCAT	31
	pyrosequencing primer	ATATAAAATTTGTTTGGTGG	32

[0053] [Table 12]

Region	Sequence	SEQ ID NO
HHLA1 3'LTR-1	TGTGAAAGTCCTCTTCCTGGCTCATCCTGGCTCAAAAAGC ACCCCCACTGAGCACCTTGAGACCCCCACTCCTGCCCGCC AGAGAACAAACCCCCTTTGACTGTAATTTTCCTTTACCTA CCCAAATCCTATAAAACGGCCCCACCCTTATCTCCCTTCA CTGACTCTCTTTTCGGACTCAGCCCGCCTGCACCCAGGTG AAATAAACAGCTTTATTGCTCACACAAAGCCTGTTTGGTG GTCTCTTCACACGGACGCACATGAAATTTAGTTGTATCCA TAAGGCATATGGAGGAGACTAATTCCTCTTCCAAAGACAT GTACCAGAGTCCTGGAGAGG	33
HHLA1 3'LTR-2	AAAGCCTGTTTGGTGGTCTCTTCACACGGACGCACATGAA ATTTAGTTGTATCCATAAGGCATATGGAGGAGACTAATTC CTCT	34

ABHD12B before 5'LTR and 5'LTR	TGTGTACCAATGTATGGTCAATTTTGGCAAATTTTCCATAT GCTTGAAAAGAATGTGTTCTGCTGTTTTTCATGCAGTGTTC TATGTACGTCGATTGAATCGGGATTATTAACCATGCTTAA ATTTGTCAGGCCTCTGAGCCCAAGCCAAGCCATCGCATCC CCTGTGACTTGCAGGTATCTGCCCAGATGGCCTG	35
ABHD12B 3'LTR	AAGCCTGTTTGGTGGTCTCTTCACACAGACGCGCATGAAA AAATTTTGTCTATTGTTACTGGTTTTTTGGACTGCTTGCTT TTTCAGTTACTCAAAGAGGATTATTAAAGTACCTCATCAT GATTGTGGAATGGT	36
ABHD12B after 3'LTR-1	GCTGTGGAGCCACTCAGACTTGGGTTCAAATCTGTCCTTG GCCACATACCCTTTGTGACCTTGGTAAATTGTTTCTCCCTA AGTTTTCCCATTTTTTTACCAAGGGGTTGGCGAAGACCAC TGCACAGGGTTGTTGTGAAGACTGAATTAAGTAAGATAAT GTATGTAAAGTACCCAGCTGCTAGTAAGCACTAGACAAA TACTTGTTCCTTTCCGTCCCTCTTTCTGTTACAAATTAGGC TAAAGTGTTATGTATGGCAGGAAAAG	37
ABHD12B after 3'LTR-2	CTTCTTTCTTGAAGGTGAGGGAAAGCAGTTAGGAAACAG AGCGAGGAACAGGTGAATGTTAACTCAGACCCCTGGCAG GAATGGGGCTGTTCTACGTTATAAACTGCCTGAGAGTTAA TAGAGGACTTCCACACAAGTCTTTCGCACTCGTTATTCTTT TAAATCCTCACAGCAACTCTCTGAGTTTGTCATCATTGCTT CCACTTAGAGATGAGAGAAATGGAGACCTATAGGTT	38
C4orf51 5'LTR	GGATAATTTGAAAATGCCCTTGGCCAAGGGGAAGCTCCA CCAGTCAGTTGGGGGAGCTTAGAATTTTATTTTTGGTTTA CAAGTTCATTATATATTTTGGATATTAACTCCTTGTCAGGC CTCTGAGCCCAAGCCAAGCCATCGCATCCCCTGTGACTTG CACATATACGCCCAGATGGCCTGAAGTAACTGAAGAATC AC	39
C4orf51 3'LTR	TCCTTTTCCTGGCTCATCCTGGCTCAAAAGCACCCCCACT GAGCACCTTGCGACCCCCACTCCTGCCCGCCAGAGAACA AACCCCCTTTGACTGTAATTTTCCTTTACCTACCCAAATCC TATAAAACGGCCCCACCCTTAACTCCCTTCACTGACTCTC TTTTCGGACTCAGCCCACCTGTACCCAGGTGATTAAAAGC TTTATTGCTCACACAAAACCTGTTTGGTGGTCTCTTCACAC GGACGCGCATGAAACTCCTTATCTGAGATATGGTTTGC	40

"Underline CG sequence" is analyzed for DNA-methylated state.

[0054] From the above result, clone exhibiting differentiation resistance can be sorted by the recognition of the expression of C4orf51, HHLA1 and ABHD12B. Similarly, clone ex-

hibiting differentiation resistance can be sorted by the recognition of the DNA-methylated state in LTR7 region or neighborhood thereof located in C4orf51, HHLA1, and ABHD12B gene bodies.

[0055] All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

Industrial Applicability

[0056] The invention of the present application can be used in the fields of producing re-generative medicine materials.

Claims

[Claim 1]

A method for screening for a human induced pluripotent stem cell line exhibiting no differentiation resistance, comprising the steps of:

(i) measuring expression of at least one large intergenic non-coding RNA (lincRNA) or mRNA selected from group A and/or group B,

(ii) selecting a human induced pluripotent stem cell line in which the lincRNA or mRNA selected from group A expresses or the lincRNA or mRNA selected from group B does not express,

wherein the lincRNA or mRNA selected from group A is selected from the group consisting of

(A20) DLX5,

(A50) MEIS2,

(A53) lincRNA:chr17:21434064-21435857 reverse strand, and

(A58) GREB1L,

wherein the lincRNA or mRNA selected from Group B is selected from the group consisting of

(B4) C4orf51,

(B9) C7orf57,

(B10) lincRNA:chr7:124873114-124899839 reverse strand,

(B12) OC90,

(B13) lincRNA:chr8:133071643-133092468 reverse strand,

(B14) lincRNA:chr8:133073732-133075753 reverse strand,

(B15) HHLA1,

(B16) lincRNA:chr8:133076031-133093351 reverse strand,

(B17) lincRNA:chr8:133090096-133097869 reverse strand,

(B22) ABHD12B,

(B23) lincRNA:chr18:54721302-54731677 reverse strand,

(B27) ZNF541,

(B28) TBX1,

(B29) CXorf61, and

(B30) DB090170 TESTI4 Homo sapiens cDNA clone TESTI4038997 5', mRNA sequence [DB090170].

[Claim 2]

The method according to claim 1, wherein the lincRNA or mRNA selected

from Group B is selected from the group consisting of:
(B4) C4orf51,
(B15) HHLA1, and
(B22) ABHD12B.

[Claim 3]

A method for screening for a human induced pluripotent stem cell line exhibiting no differentiation resistance, comprising the following steps:
(i) measuring DNA-methylated state of LTR region located in at least one gene selected from group of (B4) C4orf51, (B15) HHLA1, and (B22) ABHD12B, and
(ii) selecting a human induced pluripotent stem cell line in which the LTR7 region is in a DNA-methylated state.