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Lab Resource: Single Cell Line

# Establishment of an induced pluripotent stem cell line DHMi005-A from a healthy male proband

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#### ABSTRACT

We generated an induced pluripotent stem cell (iPSC) line from a healthy male 29-year-old proband. Adipose fibroblasts (AFs) were reprogrammed using Sendai virus. Generated iPSCs showed typical stem cell morphology. From passage 9 on, iPSCs were free of virus. Pluripotency in the iPSCs was verified and spontaneous differentiation showed expression of all three germ layers. Karyotyping indicated no anomalies for the generated iPSCs. Many patient-specific iPSCs are generated from subcutaneous fat fibroblasts obtained during surgical procedure. The described control iPSC line was generated equally and therefore serves as an ideal control for adipose-fibroblast-based patient-specific iPSC lines in disease modeling.

#### (continued) Resource Table Gene/locus N/A Unique stem cell line identifier DHMi005-A https://hpscreg.eu/user/ Date archived/stock date December 2021 cellline/edit/DHMi005-A https://hpscreg.eu/user/cellline/edit/ Cell line repository/bank Alternative name(s) of stem cell line Control L DHMi005-A Institution Department of Cardiovascular Surgery, Ethical approval Ethical committee of the Medical Faculty Institute Insure, German Heart Center of the Technical University of Munich Munich 5943/13 Contact information of distributor Dr. rer. nat. Martina Dreßen. dressen@dhm.mhn.de Type of cell line iPSC Human Origin Additional origin info required for Age: 29 years human ESC or iPSC Sex: male 1. Resource utility Ethnicity: Caucasian Cell Source Adipose fibroblasts Clonality Clonal The extent of initial cell source influencing iPSC reprogramming and CytoTune-iPSTM-iPS 2.0 Sendai Method of reprogramming subsequent differentiation is uncertain. Therefore, it is advantageous to Reprogramming (Invitrogen, use control iPSCs with the same origin as the patient's iPSCs. The ThermoFisher Scientific) generated adipose-fibroblast-based iPSCs serve as ideal control for dis-Genetic Modification NO ease modeling since many patient-specific iPSCs are generated from Type of Genetic Modification N/A Evidence of the reprogramming Sendai-footprinting, RT-PCR (passage 9) adipose fibroblasts. transgene loss (including genomic copy if applicable) 2. Resource details Associated disease N/A (continued on next column) The established cell line described here was generated from

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Fig. 1. Characterization of the iPSC control line DHMi005-A from a male healthy proband generated from adipose fibroblasts.

#### Table 1

#### Characterization and validation.

Classification	Test	Result	Data
Morphology Phenotype	Photography Bright field Qualitative analysis (Immunocytochemistry)	Normal Positive staining for SOX2, TRA1-81	Fig. 1A Fig. 1D
	Quantitative analysis (Flow cytometry, RT- qPCR)	94,90% cells positive for cell surface marker TRA1-81 and 82,7% cells positive for cell surface marker TRA1-60 Transcript expression of pluripotency markers OCT4, SOX2, NANOG, REX1	Fig. 1E Fig. 1C, F
Genotype	Karyotype (G-banding) and resolution	46XY	Fig. 1G
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	Tested 16 sites (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL, D5S818, FGA), all matched	Submitted in archive with journal
Mutation analysis (IF	Sequencing Southern Blot OR WGS	N/A N/A	
APPLICABLE) Microbiology and virology	Mycoplasma	Mycoplasma testing by RT- PCR	Fig. 1H
Differentiation potential	Embryoid body formation	SOX17 and AFP (endoderm), HAND1 and NKX2.5 (mesoderm), OTX1 and KRT14 (actoderm)	Fig. 1I
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) with $\beta$ -actin as reference gene	Endoderm: SOX17, AFP; Ectoderm: OTX1, KRT14; Mesoderm: HAND1, NKX2.5	Fig. 1I qRT- PCR with reference gene β-actin
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	not shown but available with author
Genotype additional info	Blood group genotyping		not shown but available
(OPTIONAL)	HLA tissue typing		with author not shown but available with author

fibroblasts cultured from subcutaneous fat (AFs) using the nonintegrative Sendai virus (Resource Table). Clones were manually picked and characterized. The iPSCs showed typical stem cell morphology without differentiating areas (Fig. 1A). At passage 9 (p9) after reprogramming, the Sendai virus was absent (Fig. 1B). Expression of endogenous reprogramming factors and pluripotency markers (Fig. 1C) was validated. Pluripotency was verified by immunocytochemistry for SOX2 and TRA1-81 (Fig. 1D) and by flow cytometry for TRA1-81 and TRA1-60 (Fig. 1E). Expression levels of the pluripotency markers OCT4, SOX2, NANOG, and REX1 confirmed pluripotency in the generated iPSCs, in line with other iPSCs and in contrast to the patientspecific fibroblasts (Fig. 1F). STR analysis was performed in DNA samples isolated from the patient's fibroblasts and the iPSCs. This analysis clearly confirmed the identity of the cell line (submitted in archive with journal). Twelve well-spread metaphases were analysed and karvotyped. Representative normal karyogram of iPSCs is shown in Fig. 1G. The cell line tested negative for mycoplasma (Fig. 1H). Spontaneous differentiation induced by embryoid body formation (Moretti et al., 2010) generated cells of all three germ layers (endoderm SOX17, AFP; mesoderm HAND1, NKX2.5; and ectoderm OTX1, KRT14, Fig. 1I).

#### 3. Materials and methods

#### 3.1. Reprogramming and iPSC culture

Proband's adipose fibroblasts were cultured in high glucose DMEM (Gibco), containing 10% fetal-calf-serum (ThermoFisher Scientific), 1% sodium-pyruvate (Gibco) and 1% antibiotics (PanReac AppliChem). Reprogramming was done using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (A16517, Invitrogen, ThermoFisher Scientific). Outgrowing iPSC colonies were manually picked and cultured in TeSR<sup>TM</sup>E8<sup>TM</sup> (StemCell Technologies) on Matrigel-coated plates (8.7 µg/cm<sup>2</sup>, Corning) at 37 °C and 5% CO<sub>2</sub>. iPSCs were passaged every 4–6 days at a 1:6 ratio using ReLeSR (StemCell Technologies) supplemented with ROCK inhibitor (10 µM, StemCell Technologies).

#### 3.2. Pluripotency marker expression

iPSCs (p21) were cultured on Matrigel-coated chamber slides for 2 days. Cells were fixed for 10 min at -20 °C with acetone (SOX2) or methanol (TRA1-81). After washing (1x PBS) and permeabilization with PBS/0.1% Triton-X-100 (1x PBS-T, 10 min), cells were blocked with 1x PBS-T containing 5% goat serum for 30 min. After washing three times with 1x PBS-T, cells were incubated with the primary antibodies (Anti-SOX2 rabbit polyclonal IgG antibody, Anti-TRA1-81 mouse monoclonal IgM antibody) diluted in 1x PBS-T containing 1.5% goat serum overnight at 4 °C. Cells were washed three times with 1x PBS-T and incubated with the appropriate secondary antibodies diluted in 1x PBS-T containing 1.5% goat serum for 1 h in the dark at 4 °C. After three washing steps with PBS and one washing with aq. bidest., slides were air dried and embedded with mounting medium containing DAPI (abcam). Images were taken with an Axiovert 200 M (Zeiss) using the Carl Zeiss<sup>TM</sup> Axio Vision Rel. 4.8.2. Software (Zeiss). All steps were performed at room temperature for 5 min unless otherwise described. Primary and secondary antibodies are indicated in Table 2.

#### 3.3. Short tandem repeat (STR)

Genomic DNA of iPSCs (p28) and proband's fibroblasts (p3) was sent to Eurofins Genomics (Ebersberg, Germany). Genetic characteristics were determined by PCR-single-locus-technology. Sixteen independent markers, which are given in Table 1 were investigated.

#### 3.4. In vitro differentiation potential

Expression of endodermal (SOX17, AFP), mesodermal (HAND1,

#### Table 2

AntibodyDilutionCampary Cat #RHDPluripotency Markers (ICC)Anti-SOS2 rabbit polyclonal IgG antibody, Anti-TRA1-81 mouse monoclonal IgM antibody1.75Mcex Millipore Cott# MAB4381AB 177638 MAB4381Secondary antibodies (ICC)Goart Anti-Habbit IgG H&L (Alexa Fluor® 488) antibody, IgM H&L (Alexa Fluor® 488) antibody, Anti-TRA1-81 mouse monoclonal IgM antibody, Anti-TRA1-81 mouse monoclonal IgM antibody, Anti-TRA1-81 mouse monoclonal IgM antibody, II:001:00Mcex Millipore Cott# MAB4381AB 2501490 AB 2601490Secondary antibodies (FACS)Anti-TRA1-81 mouse monoclonal IgM antibody, Anti-TRA1-81 mouse monoclonal IgM antibody, B B 10565983 A B 10565983 A B 2050375Secondary antibodies (FACS)Alexa Fluor® (47 Mouse anti-Human TRA-1-60 Antigen Goart Anti-Mouse IgM nu chain (Alexa Fluor® 647) antibody B B isociences Cat# 560590 A B 2050375B 20565983 A B 2050375 A B 2050375 A B 2050375Sendai-Foorprinting (qRT-PCR)SeVSize of handS' cacAaccaccaccaccaccaccaccaccaccaccaccacc		Antibodies used for immunocytochemistry/flow-cytometry				
Pluripotency Markers (ICC)         Anti SOX2 rabbit polycomal IgG antibody, Anti-TRA1-81 mouse monoclonal IgM antibody         1.75 monoclonal IgM antibody.         Absen Car# ab157385, Mered Millipore Car# Mark981         Absen Car# ab157385, Mark981         Absen Car# ab157385, Mark981         Absen Car# ab157385, Mark981         Absen Car# ab157385, Mark981         Absen Car# ab1577, Absen Car# ab15077, Absen Car# ab15077, Ab2500, Absen Car# ab15077, Absen Car# ab15077, Ab150, Absen Car# ab150, Ab150, Ab150, Absen Car# ab150, Ab150, Absen Car# ab150, Ab150,		Antibody	Dilution	Company Cat #	RRID	
manoclonal igM antibodyinfoMath allingMath allingMath all modeSecondary antibodies (ICC)Gat Anti-Rabibit IgG H&L (Alexa Fluor® 488) antibody, Goat Anti-Moue1:500Alexan Cat# ab 150212Ale 2801496Pluripotency Markers (FACS)Anti-TRA1-81 mouse anti-Human TBA-1-60 Antigen1:500Alexan Cat# ab 150221Ale 2801497Secondary antibodies (FACS)Face Fluor® 647 muce anti-Human TBA-1-60 Antigen1:50Alexan Cat# ab 150123Ale 2801497Secondary antibodies (FACS)TragetFrace Fluor® 647 muce anti-Human TBA-1-60 Antigen1:50Alexan Cat# ab 150123Ale 28054983Secondary antibodies (FACS)TragetFraceSize of bloc (Cat Cat Cat Cat Cat Cat Cat Cat Cat Cat	Pluripotency Markers (ICC)	Anti-SOX2 rabbit polyclonal IgG antibody, Anti-TRA1-81 mouse	1:75	Abcam Cat# ab137385,	AB_2814892	
Secondary antibodies (ICC)     Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody, Goat Anti-Mouse     1:50     Alcam Cat# ab150077, AB 250356       Pluripotency Markers (FACS)     Anti-TRA1-in lonses monoclonal IgM antibody,     1:100     Merck Nillpore Cat#     AB 250356       Secondary antibodies (FACS)     Alexa Fluor® 488) antibody,     1:100     Merck Nillpore Cat#     AB 250356       Secondary antibodies (FACS)     Alexa Fluor® 488) antibody,     1:100     Merck Nillpore Cat#     AB 250356       Secondary antibodies (FACS)     Alexa Fluor® 488) antibody,     1:100     Merck Nillpore Cat#     AB 250356       Secondary antibodies (FACS)     Alexa Fluor® 488) antibody,     1:100     Merck Nillpore Cat#     AB 250356       Secondary antibodies (FACS)     Target     Secondary antibodies (FACS)     Secondary antibodies (FACS)     Alexa Cat# ab15012     AB 260356       Sendai-Footprinting (qRT-PCR)     SeV     Secondary antibodies (FACS)     Secondary antibodies (FACS)     Secondary Alexa Cat# ab15012     AD 260376       Pluripotency Markers (qRT-PCR)     SeV     Secondary Alexa Cat# ab15012     Secondary Alexa Cat# ab15012     AD 260376       Pluripotency Markers (qRT-PCR)     Secondary Alexa Cat# ab15012     AD 260376       Pluripotency Markers (qRT-PCR) <t< td=""><td></td><td>monoclonal IgM antibody</td><td>1:75</td><td>Merck Millipore Cat#</td><td>AB_177638</td></t<>		monoclonal IgM antibody	1:75	Merck Millipore Cat#	AB_177638	
Juripotency Markers (FACS)Juripotence ABS) mithody1:500Abcam Cat* ab150121Ab 2801490Pluripotency Markers (FACS)Anti-TRA1-81 mouse monoclonal IgM antibody,1:100Morch Millipore Cat*AB_10565983Secondary antibodies (FACS)Alexa Fluor® 647 Mouse anti-Human TRA-1-60 Antigen1:20BD Biosciences Cat# 560850AB_10565983Secondary antibodies (FACS)Primers	Secondary antibodies (ICC)	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody, Goat Anti-Mouse	1:500	Abcam Cat $\#$ ab150077.	AB 2630356	
Pluripotency Markers (PACS)         Anti-TRA1-81 mouse monocional IgM antibody,         1:100         Merck Millipote Cat# MAB431         AB_177638           Secondary antibodies (PACS)         Alexa Fluor® 647 Mouse anti-Human TRA-1-60 Antigen cold Anti-Mouse [qM nu chain (Alexa Fluor® 647) antibody         1:2000         Abcam Cat# ab150123         AB_10555983 AB_10555983           Secondary antibodies (PACS)         Terret         Forward/Reverse primer (5'-3')         Secondary antibodies (PACS)         Secondary antibodies (PACS)         AB_10555983           Sendai-Footprinting (qRT-PCR)         SeV         Size of band         Forward/Reverse primer (5'-3')         S-CACACACACAGAGATTAAACGAGATTATACAGATATATCCAGAGAGATTAAACGAGAGATTAAACGAGATTAAACGAGATTAAACGAGATTAAACGAGATTAAACGAGATTAAACGAGATTAAACGAGATTAAACGAGT		IgM H&L (Alexa Fluor® 488) antibody	1:500	Abcam Cat# ab150121	AB 2801490	
Secondary antibodies (FACS)     Alexa Fluor® 647 Mouse anti-fuman TRA-1-60 Antigen Goat Anti-Mouse (pM nu chain (Alexa Fluor® 647) antibody     155 12000     BD Bose Cenes Cat# 560850 Abcam Cat# ab150123     AB 10656983 AB 2993175       Secondary antibodies (FACS)     Target     522 of band     Forward/Reverse primer (5'-3')       Sendai-Footprinting (qRT-PCR)     SeV     181 bp     5'-GGATCACTAGGTGATATCGAGC-3' 5'-ACCAGACAGAGAGATATCTATCA 3'       Pluripotency Markers (qRT-PCR)     OC74 (endogenous)     96 bp     5'-CCAAACGGCGAGAAGATGA-3' 5'-CCAAAGGGCTACAGTGCA-3'       Pluripotency Markers (qRT-PCR)     OC74 (endogenous)     191 bp     5'-ACCAGAGCTCACATCTCCAG-3' 5'-ACCAGAGCTCCACAGTCCACAGTG-3'       REX1     105 bp     5'-ACCAGACTCCCAGCAGCTACTCCCAG-3' 5'-ACCAGGCTCCAAGTCCCAG-4' 5'-ACCGGGGTTCTCCCACGTCTAGGCAGTG-3'       Germ layer Endoderm     ARD01     105 bp     5'-ACCTGCCAGTCTCAGGGTG-3' 5'-ACCCCAGGTTCAGGCTG-3' 5'-ACCCCAGGTTCAGGCTG-3'       Mesoderm     HAND1     72 bp     5'-ACCTCCCACGCTCAGGTG-3' 5'-CGCCCAGGTTCTCCCAGGTGC-3' 5'-CGCCCAGGTTCGCCCCCCCCCAG-3' 5'-CGCCCCAGGTCGGGGTG-3' 5'-CGCCCCAGGTCGGGGTG-3' 5'-CGCCCCAGGTCGGGGTG-3' 5'-CGCCCCAGGCTGGGGGTG-3' 5'-CGCCCCAGGCTGGGGGTG-3' 5'-CGCCCCCCCCCCCAGGTGGGGGGGGG-3' 5'-CGCCCCCCCCCCCCCCGGGGTG-3' 5'-CGCCCCCCCCCCCCCCCC-3' 5'-CGCCCCCCCCCCCCCCCCC-3' 5'-CGCCCCCCCCCCCCCCCCCC-3' 5'-CGCCCCCCCCCCCCCCCCCCC-3' 5'-CGCCCCCCCCCCCCCCCCCCCCC-3' 5'-CGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	Pluripotency Markers (FACS)	Anti-TRA1-81 mouse monoclonal IgM antibody,	1:100	Merck Millipore Cat# MAB4381	AB_177638	
Secondary antibodies (FACS)     Goat Anti-Mouse igM mu chain (Alexa Fluor® 647) antibody     12000     Abcam Cat# ab150123     Al 22893175       Primers       Target     Size of 5'-ACCAGACCACAGGGTATATCGAGC-3' 5'-ACCAGACGCACAGGGTATATCGAGC-3' 5'-ACCAGAGGCACTACGAGGGTATATCGAGC-3' 5'-ACCAGAGGCATACGAGGGTATATCGAGC-3' 5'-ACCAGAGGCATACGAGGGTATATCGAGC-3' 5'-ACCAGAGGCATACGAGGGTATATCGAGC-3' 5'-CACAGGGCATGCAGGGTATATCGAGC-3' 5'-CACAGGGCATGCAGGGTATATCGAGC-3' 5'-CACAGGGCATGCAGGGTATATCGAGC-3' 5'-CACAGGGCATGCAGGGTATATCGAGC-3' 5'-CACAGGGCATGCAGGGTATATCGAGC-3' 5'-CACAGGGCATGCAGGGTATATCGAGGT-3' 5'-CACAGGGCATGCAGGGTA-3' 5'-CACAGGGCATGCAGGGTA-3' 5'-CACAGGGCATGCAGGGTA-3' 5'-CACCAGGGCATGCAGGGTA-3' 5'-CACCAGGGCAGGCAGGTC-3' ADANOG     191 bp     5'-AGCGGAAGTCCACAGGCTA-3' 5'-CACCAGGGCAGGCAGGTC-3' 5'-CACCAGGCCAGGCAGTCA-3' 5'-CACCAGGCCAGGCAGTCA-3' 5'-CAGCCCCAAGTCCAGCGCAGC-3' 5'-CAGCCCCAAGTCCAAGTCCACGCAGC-3' 5'-CAGCCCCAAGTCCAAGTCCACGCAGGAG-3' 5'-CAGCCCCAAGTCCAAGTCCACGCAGGAG-3' 5'-CACCCCCCAAGTCCAAGTCCACGCAGGAG-3' 5'-CACCCCCAAGTCCAAGTCCACGCAGGAGGAGGAGTCA-3' 5'-CACCCCCAAGTCCAAGTCCACGCAGGAGGAGGAGTCA-3' 5'-CACCCCCAAGTCCAAGTCCAAGGCAGGAGGAGGAGTCA-3' 5'-CACCCCCAAGTCCAAGTCCACGCAGGAGGAGGAGGAGGAGGAGTCA-3' 5'-CACCCCCAAGTCCAAGTCCAAGGCAGGAGGAGGAGGAGGAGGAGTCA-3' 5'-CACCCCCAAGTCCAAGTCCAAGGCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG		Alexa Fluor® 647 Mouse anti-Human TRA-1–60 Antigen	1:5	BD Biosciences Cat# 560850	AB 10565983	
initialinitialSendai-Footprinting (qRT-PCR)SevSie of Social CACACGGGATACGGATACGGAGAGAGATAGGAGAGAGATAGGAGAGAGA	Secondary antibodies (FACS)	Goat Anti-Mouse IgM mu chain (Alexa Fluor® 647) antibody	1:2000	Abcam Cat# ab150123	AB_2893175	
IndexTargetSize of bandProrad/Reverse primer (5'-3) bandSendai-Footprinting (qRT-PCR)SeV8.18.1SeCAGACAGGGGAAGAGATTAGAGAGATGAGAGAGATGAGAGAGATGAGAGAGAGAGATGA		Primers				
Sendai-Footprinting (qRT-PCR)       SeV       181 bp       S-GACTACATAGGACATATCGAGC-3'         Sendai-Footprinting (qRT-PCR)       SeV       181 bp       S-GACTACGACAAGGATTAGCAGC-3'         Pluripotency Markers (qRT-PCR)       ACTB (β-actin)       96 bp       S-CCAACGGCGACAGGGATAG-3'         SOX2 (endogenous)       191 bp       S-GACCAGGCTTACCAGGGATAG-3'         NANOG       191 bp       S-GACAGGCTTACCAGGGATAG-3'         NANOG       193 bp       S-GCAGCGGCTACCGAGGTG-3'         REXI       105 bp       S-GAGTGTCTCCAGAGGTG-3'         Germ layer       SOX17       81 bp       S-GCGCCGAGTGTGCAGCAGAG-3'         Endoderm       SOX17       81 bp       S-GCGCCAAGGTGGGCAGAGGGTGA-3'         Mesoderm       HAND1       2 bp       S-GCGCCTACTCAGGGGTGAG-3'         NKX2.5       NKX2.5       S-GATGCCCCAAGGTGGGAGG-3'       S-GCGCCCAAGGTGGGAGG-3'         S-GGATGCCCCAAGGTGGAGGAGGGTGAG-3'       S-GGATGCCCCCAGGTGGAG-3'       S-GGATGCCCCAGGGGGTGAG-3'         Germ layer       NF       S-GCCCCCAGGTGGCAGG-3'       S-GCCCCCCCCCCCCCGAGGGGGGAGG-3'         Germ layer       S-GGATGCCCCAAGGTGGCGAGG-3'       S-GGAGGCGTCCCAGGGGGGGGGGGGGGGGAG-3'       S-GGAGGCGTCCCCCCCGGGGGGGGAG-3'         Mesoderm       HAND1       S-GCCCCCCAGGTGGCGGGGGGGGG-3'       S-GGCCCCCCAAGGTGGCCGAGGGGGGG-3'       S-GGCCCCCC		Target	Size of	Forward/Reverse primer (5'-3')		
Sendai-Pootprinting (qRT-PCR)     SeV     181 bp     5-GGATCACTAGGTGATATGGACG's 5-CGAGCAGGGGAGAGATTAGAGATTGTATC 3       Pluripotency Markers (qRT-PCR)     ACTB (b-actin)     60 bp     5-CGAAGCGGGAGGGATAG-3' 5-CGAGGGGTGAGGGTGAG'S 5-CGAGGGGTGAGGGTGAG'S 5-CGAGGGGTGAGGGTGAG'S 5-GGGGTGTTGCCCAGGGGTGAG'S 5-GCGGGTGTTGCCCAGGGGTGAG'S 5-GCGGGTGTTGCCCAGGGGTGAG'S 5-GCGGGTGTTGCCCAGGGGTGAG'S 5-GCGGGTGTTGCCCAGGGGGTGAG'S 5-GCGGGTGTTGCCCAGGGGGTGAG'S 5-GGTGTTGCCCTTGGGAGGGG'S 5-GGTGTTGCCCTTGGGAGGGTGAG'S 5-GGTGTTGCCCTGCGCAGGGGTGAG'S 5-GGTGGTGCCCAGGGGTGAG'S 5-GGTGGTGCGCCAGGGGGTGAG'S 5-GGTGGTGGCGAGGGGTGGG'S 5-GGGCGCAGGGGGGGGGGGG'S 5-GGGCGCAGGGGGGGGGGG'S 5-GGGCGCAGGGGGGGGGG'S 5-GGGCGCAGGGGGGGGGG'S 5-GGGCGCAGGGGGGGGGGG'S 5-GGGCGCAGGGGGGGGGGGG'S 5-GGGCGCAGGGGGGGGGGG'S 5-GGGCGCAGGGGGGGGGGG'S 5-GGGCGCGAGGGGGGGGGGGGG'S 5-GGGCGCGAGGGGGGGGGGGGGGGGGGGGGGGGGGGGG'S 5-GGGCGCGAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG			band			
S-ACCAGACAGAGTTTAAGAGATATGTATC:         Buripotency Markers (qRT-PCR)       ACTB (b-actin)       96 bp       5-CCAAGCGGGAAGAGTGA-3' 5-CCAAGCGGGTACGGGATAG-3' 5-CCAAGCGGGTACGGGATAG-3' 5-CCAAGGGGTTCGTGGG-3'         Buripotency Markers (qRT-PCR)       OC74 (endogenous)       191 bp       5-AGCAGGGGTGCGGG-3' 5-CCACGGGGTTGCCGGT-3'         Buripotency Markers (qRT-PCR)       SOX2 (endogenous)       191 bp       5-AGCAGGGTGCCGGA-3' 5-CCACGGGTTGCCACGTG-3'         Buripotency Markers (qRT-PCR)       SOX2 (endogenous)       193 bp       5-TGCGTGTAGCGACGGG-3' 5-CGCGGGTTGCCACGTG-3'         Buripotency Markers (qRT-PCR)       REX1       105 bp       5-AGTAGTGCTACAGGTGC-3' 5-CGGCGCTTGGACGGCGAG-3' 5-CGGCGCGTGGCGACGGGA-3' 5-CGGCGCGTTGGACGAGAG-3' 5-CGGCGCGTGGCGACGGA-3' 5-CGACGCTCCACGGGGTGA-3' 5-CGACGCTCCACGGGGTGAC-3' 5-CGACGCTCCACGGGGTGAC-3' 5-CGACGCTCCACGGGGGTGC-3' 5-CGACGCTCCACGGGGTGC-3' 5-CGACGCTCCACGGGGGTGC-3' 5-CGACGCGGAGGGGA-3' 5-CGACGCGGAGGGGA-3' 5-CGACGCGGAGGGGAGTG-3' 5-CGACGTCGCCACGCGGGGTGA-3' 5-CGACGTCGCCCACGCGGGGTGA-3' 5-CGACGCGGAGGGGAGTGA-3' 5-CGACGCGGAGGGGAGTGA-3' 5-CGACGCGGAGGGGAGTGA-3' 5-CGACGCGGAGGGGAGTGA-3' 5-CCACGCGGGAGGGGAGTGA-3' 5-CCACGCCGGAGGGGAGTGA-3' 5-CCACGCGGGAGGGGAGTGA-3' 5-CCACGCGGGAGGGGAGGGGAGTGA-3' 5-CCACGCGGGGAGGGGAGGGGAGGGGA-3' 5-CCACGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	Sendai-Footprinting (qRT-PCR)	SeV	181 bp	5'-GGATCACTAGGTGATATCGAGC-3' 5'-ACCAGACAAGAGTTTAAGAGATATGTATC- 3' 5'-CCAACCGCGAGAAGATGA-3' 5'-CCAACCGCGAGAGAGATGA-3'		
ACTB (β-actin)       96 bp       5'-CCAACGGGAAGAGTGA-3'         Pluripotency Markers (qRT-PCR)       OC74 (endogenous)       148 bp       5'-GGGATGGGGTACGGGGTAG-3'         SOX2 (endogenous)       191 bp       5'-AGCGAGGTTACTGTGGG-3'       5'-GGAAGGGATGACGTGT-3'         NANOG       193 bp       5'-AGCGAGTTTGCACAGTGCACGGT-3'         ExX1       105 bp       5'-AGTGTTGCACAGTCGCAGAGGATG-3'         Germ layer       5'-GTTTGTAGAGATTCCACAGTCGAGAGGAGAGA-3'         Endoderm       SOX17       81 bp         APP       90 bp       5'-GTGCCACGCGGAGAGAGA-3'         S'-GTGCCACGTCCAGGGTTGAGGAAGA-3'       5'-GTGCCCACCTCACGATCGAGGAGA-3'         Mesoderm       APP       90 bp       5'-GGCCAGGGTGAGGAGAG-3'         KX2.5       S'-AGTCCAGGCTCAGGGTGAGGAGAG-3'       5'-GGCCAGGCTCAGGGTGAG-3'         KX2.5       102 bp       5'-AGTCCAGGCTGCAGGGTG-3'         KX2.5       102 bp       5'-GCACCAGGGGAGG-3'         Fetoderm       CTX1       125 bp       5'-GGCTGAGGCTACAGG'         House-keeping gene (qRT-PCR)       ACTB (β-actin)       125 bp       5'-GCCACCGCGGAGATGA-3'         House-keeping gene (qRT-PCR)       ACTB (β-actin)       96 bp       5'-CCCACCGCGGAGATAG-3'         Fordogenous reprogramming factory (qRT-PCR)       C/H2 (endogenous)       131 bp						
ACIB (I-actin)       96 bp       5-4CAACGCGAACAACIGA-3' F-CCAACGGCTACGGGATAG-3'         Pluripotency Markers (qRT-PCR)       OC74 (endogenous)       148 bp       5-GGCATGCGCTACGGGATAG-3'         SOX2 (endogenous)       191 bp       5'-AGCCAGGGTTACAGGATAG-3'         NANOG       193 bp       5'-AGCCAGCACTCCACAGGATG-3'         REXI       105 bp       5'-AGCTGCATCCAGGATCCAGGATG-3'         REXI       105 bp       5'-AGTGTTGCATCAGGATCCAGGATG-3'         Germ layer       5'-AGTGTCTCCACAGGATGCAGCAG-3'         Endoderm       SOX17       81 bp       5'-AGCTGCAGGATGGAGAGA-3'         Mesoderm       AFP       5'-GGCCCAAGGTCGAGGGTGA-G'       5'-GGCCCAAGGTCGAGGGGTGA-G'         Mesoderm       HAND1       72 bp       5'-GGCCCAAGGTCGAGGGTGA-G'       5'-GGCCCAGGCTCACGGGGTAG-G'         KKX2.5       NKX2.5       102 bp       5'-GGCTGCCAGGCGCACG-G'       5'-GGCCAGGCCAAGGCGAGTCG-3'         F-Goderm       71       5'-GGCCAGGCGCACGCGGCTAC-3'       5'-GGCCAGGCCAGGCGCACGGAGGATG-3'       5'-GGCCAGGCCAGGCGCGCGGCG-3'         Mesoderm       HAND1       12 bp       5'-GGCCAGGCGCAGGCGGAGGAGAG-3'       5'-GGCCAGGCGCCAGGGGAGG-3'       5'-GGCCAGGCGCGCGCGGGAGGAGGAGGAGGAGGAGGAGGAG						
Pluripotency Markers (qRT-PCR) OCT4 (endogenous) 148 bp 5'-GGATGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG		ACTB (β-actin)	96 bp			
Printpotency Markers (QLPFCM)       OCP (endogenous)       146 bp       5-GGACACAGGGTTGACGGGTG-3'         SOX2 (endogenous)       191 bp       5'-AGCAGGGTTGACGGGTG-3'         SOX2 (endogenous)       193 bp       5'-ACCGGGGTTTTCTCCAAGGCTG-3'         NANOG       193 bp       5'-AGCAGGGTTGACGACTG-3'         SOX2 (endogenous)       193 bp       5'-AGCGGGTTTTTCGCATGCGACTG-3'         REX1       105 bp       5'-AGTGGCTCACGGCTG-3'         Germ layer       5'-GTGTGCCTTCTGGACGAGGAG-3'       5'-TGTGCCCTCTCCACGAGGAG-3'         AFP       90 bp       5'-AGCCGGGGTTGAG-3'         Mesoderm       HAND1       72 bp       5'-AGCCAGGCTGAGGGG-3'         KX2.5       5'-GTGCCTTCACTGCGCGAGGAGG-3'       5'-GGAGGAAAACCTTCGGGGT-3'         KKX2.5       102 bp       5'-GTGCTCTCCAGGCGAGG-3'         S'-GGCCTGAGATTGCTA-3'       5'-GGCCTGCAGGCTGCA-3'         KRT14       5' 5'-GGCCTGCCTGCCGGGAGTCA-3'         House-keeping gene (qRT-PCR)       ACTB ([-actin)         House-keeping gene (qRT-PCR)       ACTB ([-actin)         Fadogenous       101 bp       5'-CCCCGGGGAGCAGCAGGA'         S'-CCCCGGGGAGGACGA-3'       5'-CCCCCGGGAGGCACGCGACTGCTG-3'         Fadogenous reprogramming factors (qRT-PCR)       ACTB ([-actin)       101 bp       5'-CCCCCGAGGGCTCTGCA-3' <td>Divinotency Markers (aPT DCP)</td> <td>OCTA (andogenous)</td> <td>149 hp</td> <td colspan="2" rowspan="3">5 -CCAGAGGGGTACAGGGATAG-5 5'-GGGATGGCGTACTGTGGG-3' 5'-GCACCAGGGGTGACGGTG-3' 5'-AGCAGACTTCACATGTCCCAG-3'</td>	Divinotency Markers (aPT DCP)	OCTA (andogenous)	149 hp	5 -CCAGAGGGGTACAGGGATAG-5 5'-GGGATGGCGTACTGTGGG-3' 5'-GCACCAGGGGTGACGGTG-3' 5'-AGCAGACTTCACATGTCCCAG-3'		
SOX2 (endogenous)       191 bp       5'-AGCAGACTTCACATGTCCCAG-3' 5'-AGCGGGTTTTCTCACATGTCCCAG-3' 5'-AGCGGGTTTTCTCCATGCTGCTG-3' 5'-GGTTGTTTGCATTGGAGCATGCGACTG-3' 5'-GGTGGTCTCCCACAGTCCGACGGCG-3' 5'-GTGGCCTCTTGGAGCATGC-3' 5'-GTGGCCTCTCGCACGGCGG-3' 5'-GTGGCCTCTCGCAGGGTT-3'         Germ layer       81 bp       5'-AGCCGGGTTAGCGAAGA-3' 5'-CTGCCCACGTCGGCGAGGAG-3' 5'-CTGCCCACGTCAGGAGAG-3' 5'-CTGCCCAGGTCAGCAGGAG-3' 5'-CTGCCCAGGTCAGCAGAG-3' 5'-CTGCCCAGGTCAGCAGGAG-3' 5'-CTGCCCAGGTCAGGGAGGAG-3' 5'-CTGCCCAGGTCAGGGAGGAG-3' 5'-CTGCCCAGGTCAGGGAGGAG-3' 5'-CTGCCTCCCCAGGTCAGG-3' 5'-CTGCCTCCCCAGGTCTAG-3' 5'-CTGCCTCCCCAGGTCGCGAG-3' 5'-CTGCTCTCCCAGGTCGCGAGGAGGAG-3' 5'-CTGCCTCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCCGGGTCG-3' 5'-CTGCCTCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCCGGGGTCG-3' 5'-CTGCCTCCCCCCCGGGTCG-3' 5'-CTGCCTCCCCCCCCGGGTCG-3' 5'-CTGCCTCCCCCCCCGGGTCG-3' 5'-CTGCCTCCCCCCCCCCGGGTCG-3' 5'-CTGCCCCCCCCCCCCCCCCCGGTCG-3' 5'-CTGCCCCCCCCCCCCCCCGGGTCG-3' 5'-CTGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	Fullpotency Markers (qrt1-FCrt)	OC14 (endogenous)	140 DP			
First CategoriesFirst PS'ACCGGGTTTICTCCATGCTGT-3' S'-GGTTGTTTGCACGCTCGACGTGT-3' S'-GGTTGTTTGCACGCTCGACGGT-3' S'-GGTTGTTTGGAAGCATCGACGGT-3' S'-GGTGGTTCGCCACGCCACGCA-3' S'-GGCCGAGTTGAGGCAAGA-3' S'-TCTGGCCCTCCTCACGGGTAG-3' S'-GGCCGAGTTGAGGCAAGA-3' S'-TCTGGCCCCCCCCCACGAG-3'Germ layer		SOX2 (endogenous)	191 bp			
NANOG193 bp5'-EGCTTTGAAGCATCGACTGT-3' 5'-GGTTGTTCGCCTTTGGACTG - 3' 5'-GGTGGTTGGCCTTCTGGAAGCATG - 3' 5'-GGTGGTTGGCCTCTGGAAGGTG - 3' 5'-GGGCGTGGCAGGGGGGGGGAGGAGGGGGGGGGGGGGGGG		(	P	5'-ACCGGGTTTTCTCCATGCTGT-3'		
REX1       105 bp       5'-GGTTGTTGGCTTTGGGACTG -3' 5'-AGTAGTGCTCACAGAGCAG'3' 5'-GTGGCCTCTCTGAAGGTCAAGAG'3' 5'-GTGCCTCTCTGAAGGTTGAAGCAAGA-3' 5'-GTGCCTCTCTCACGAAGA-3' 5'-GTGCCTCCTCCACGAAGA-3' 5'-GTGCCTCACGAAGAG-3' 5'-GTGCCTCACGAAGAG-3' 5'-GGAGGAAAACTTGTGCTC-G-3         Mesoderm       AFP       90 bp       5'-AGTCCAAGGCAGGAGGAG-3' 5'-GGAGGAAAACCTTCGTG-3' 5'-GGAGGAAAACCTTCGTGG-3' 5'-GGAGGAAAACCTTCGTGGC-3' 5'-GGAGGAAAACCTTCGTGGC-3' 5'-GTGTCTTCCCAGGTCGAGCAGAGA' 5'-GTGTCTTCCCAGGTCGACG-3' 5'-GTGTCTTCCCAGGTCGACG-3' 5'-GTGTCTTCCCAGGTCCACC-3' 5'-GTGTCTTCCCAGGTCCACC-3' 5'-GTGTCTTCCCAGGTCCACC-3' 5'-GTGTCTTCCCCAGGTCAAGAGGGGATGA-3' 5'-GTGTCTTCCCCCGGAATG-3' 5'-GTGGCTGGGGGGATGA-3' 5'-CTGCTGGGGGGGATGA-3' 5'-CTGCTGGGGGGGATGA-3' 5'-CTGCTGGGGGGATGA-3' 5'-CTGCTGGGGGGATGA-3' 5'-CTGCTCGCCCCCTTCCCAGTT-3' 5'-CTGCTGGGGGGATGA-3' 5'-CTGCTGGGGGGATGA-3' 5'-CTGCTCGCCCCCCTTCCCCAGTT-3' 5'-CTGCTGGGGGGATGA-3' 5'-CTGCTCGCCCCCCTTCCCCAGTT-3' 5'-CTGCTCGCCCCCCTTGCGGGATGA-3' 5'-CTGCTCGCCCCCCTTCCCCAGTT-3' 5'-CTGCTCGCCCCCCTTCCCCAGTT-3' 5'-CTGCTCGCCCCCCTTCCCCAGTT-3' 5'-CTGCTCCAGCCCCCCTTCCCCAGTT-3' 5'-CTGCTCCAGCCCCCCTTCCCCAGTT-3' 5'-CTGCTCCAGCCCCCCTTCCCCAGT-3' 5'-CTGCTCCAGCCCCCCTTCCCCAGT-3' 5'-CTGCTCCAGCCCCCCTTCCCAGT-3' 5'-CTGCTCCAGCCCCCCTTCCCAGT-3' 5'-CTGCTCCAGCCCCCCTTCCCCAGT-3' 5'-CTGCTCCAGCCCCCCTTCCCCAGT-3' 5'-CTGCTCCAGCCCCCCCTTCCCCAGT-3' 5'-CTGCTCCAGCCCCCCTTCCCCAGT-3' 5'-CTGCTCCAGCCCCCCCTTCCCAGT-3' 5'-CTGCTCCAGCCCCCCTTCCCAGT-3' 5'-CTGCTCCAGCCCCCCCCTTCCCAGT-3' 5'-CTGCTCCAGCCCCCCCCTCCCCAGT-3' 5'-CTGCTCCAGCCCCCCCTTCCCAGT-3' 5'-CTGCTCCAGCCCCCCCCTCCCCCCCCCCCCCCCCCCCCC		NANOG	193 bp	5'-TGCTTTGAAGCATCCGACTGT-3'		
REX1       105 bp       5'.AGTAGTGCTCAACAGTCAGCAGGA' 5'.TGTGCCTCTTTGAAGGTTT-3'         Germ layer       5         Endoderm       81 bp       5'.AGCCGAGTTGAGCAAGA-3' 5'.TCTGCCTCCTCACGAGAG-3'         AFP       90 bp       5'.GTGCCAAGCTCAGGGTGTAG-3 5'.CAGCCTCAAGTTGTTCCTCTG-3         Mesoderm       HAND1       72 bp       5'.AGCAGGAGAGAG-3' 5'.CGAGGTGAG-3' 5'.CGAGCTCAAGCTGGCTGAG-3' 5'.CGAGCTCAAGCTGGCTGCT-3'         Mesoderm       HAND1       72 bp       5'.AGCGGAGAGAG-3' 5'.CGAGCTCAAGCTGGCTGCT-3' 7'.CTTATCCACGTGCCTAAGC-3' 5'.CGCACTGGAGGAGTGG-3' 5'.CTGTCTTCCACGCGGAGTCTA-3'         Ectoderm       NKX2.5       102 bp       5'.TCTTCATCCACGTGCCTA-3GC-3' 5'.CGCACTGGAGGAGTCTA-3'         Ectoderm       07 X1       5'.GGCACTGGAGAGGATTG-3' 5'.CGCACTGGAGGAGTCTA-3'         Mesoeleeping gene (qRT-PCR)       KRT14       85 bp       5'.CACCACTGCGGAGATGA-3' 5'.CTTCTGTGGCACCACTTGGG-3' 5'.CTTCTGTGGCACCACTTGGG-3' 5'.CTGCTCAGGAGGATGA-3' 5'.CTGCTCAGGAGGATAG-3' 5'.CTGCTCAGGAGATGA-3' 5'.CTGCTCAGCACTGCGGATAG-3' 5'.CTGCTCAGCACTTCCTCAAG-3'         Factors (qRT-PCR)       KLF4 (endogenous)       133 bp       5'.CTTCTGGCGCACTTCTGA-3' 5'.CTGCTCAGCACTTCTGA-3'         Factors (qRT-PCR)       -MYC (endogenous)       101 bp       5'.CACCACCTGCGCACTTCTGA-3'				5'-GGTTGTTTGCCTTTGGGACTG -3'		
Gern layer         Endoderm       SOX17         AFP       90 bp       5'-AGGCCAAGCTCACGGAGTGAG-AAG-3' 5'-CAGCCTCAAGTTGTTCCTCTG-3         Mesoderm       HAND1       5'-AGGCCAAGAAGACGCGAAGG-3' 5'-GGAGGAAAACCTTCGTGGTG-3' 5'-GGAGGAAAACCTTCGTGGT-3'         Mesoderm       NKX2.5       102 bp       5'-AACTCAAGGTGCA-3' 5'-CGGCTCAAGCTCACGCGACGC-3' 5'-CTGTCTTCTCCAGGCTCA-3' 5'-CTGTCTTCTCCAGGCTCA-3' 5'-CTGTCTTCTCCAGGCTCA-3' 5'-CGCACTGGAAGAACCTTCGT-3'         Ectoderm       0TX1       5'-GGACGAAAACCTTCCCCAGT-3' 5'-CGCACTGGAGGAGAGCTTCTT-3' 5'-GCCACTGCTGCTCCCCCCAGT-3' 5'-CGCACTGGCGAAGGATTG-3' 5'-CGCACTGGCGGAATGA-3' 5'-CTGCTCTCCTCCCCCCAGTT-3' 5'-ATGACCTTGGTGGCGGATTG-3' 5'-ATGACCTTGGTGGCGGATTG-3' 5'-ATGACCTTGGTGGCGGATTG-3' 5'-ATGACCTTGGTGCGCGAATGA-3' 5'-CCAACGCCCAGCAGGAAGACTGA-3' 5'-CCAACGCCCAGCGAAGAACGATGA-3' 5'-CCAACGCCCAGCGAAGAACGATGA-3' 5'-CCAACGCCCAGCGAAGAACGATGA-3' 5'-CCGCACGCGCAACAGGGATAG-3' 5'-CTGCTCAGCACCTCTCGCAGCACGCACGGATAG-3' 5'-CTGCTCAGCACCTCTCGCAGCACGCACGCACGGATAG-3' 5'-CTGCTCAGCACCTCCTCGCAGCACGCACGCACGGATAG-3' 5'-CTGCTCAGCACCTCTCCCAGGATGC-3' 5'-CTGCTCAGCACCTCCCCAGGATGA-3' 5'-CTGCTCAGCACCTCCCCAGGATGA-3' 5'-CTGCTCAGCACCTCCTCGCAGCACGGATGA-3' 5'-CTGCTCAGCACCTCCTCGCAGCACGCACGGATGA-3' 5'-CTGCTCAGCACCTCCTCGCAGCACGCACGCACGCACGCAC		REX1	105 bp	5'-AGTAGTGCTCACAGTCCAGCAG-3'		
Germ layer       SOX17       81 bp       S'-ACGCCGAGTTGAGCAAGA-3'         Endoderm       AFP       90 bp       S'-GTGCCAAGCTCAGGGTGTAG-3'         Mesoderm       HAND1       72 bp       S'-AACTCAAGAAGACGCGGATGG-3'         NKX2.5       102 bp       S'-GAGGCAAAGCTCGTGCT-3'         Mesoderm       07X1       102 bp       S'-GATCAACCTGCGCGACGC-3'         KRT14       125 bp       S'-GACCTGCGGAGAGGACGTC-3'         House-keeping gene (qRT-PCR)       ACTB (β-actin)       96 bp       S'-CCACCGGCGAAGAGACG-3'         Endogenous reprogramming factors (qRT-PCR)       KLF4 (endogenous)       133 bp       S'-TCTTCGTGCACCCACTTGGG-3'         Functional control of the contro				5'-TGTGCCCTTCTTGAAGGTTI	-3′	
Inductionof bpof bp<of bp	Germ layer Endoderm	SOX17	81 hn	5'-ACGCCGAGTTGAGCAAGA-3'		
AFP90 bp5'-GTGCCAAGCTCAGGGTGTAG-3' 5'-CAGCCTCAAGTTGTTCCTCTG-3MesodermHAND172 bp5'-AACTCAAGAAGCCGATGG-3' 5'-GAGGAAAACCTTCGTGCT.3'MKX2.5NKX2.55'5'-CTGTCTACCACGTGCCTACAGC-3' 5'-CTGTCTTCCCAGCGTCCACC-3EctodermOTX1125 bp5'-GATCAACCTGCCGGAGTCTA-3' 5'-CGCACTGGAGAGAGGACTTCTT-3'KRT145'5'-CACCTCTCCTCCCCACC-3House-keeping gene (qRT-PCR)ACTB (β-actin)85 bp5'-CCACCGGAGAGAGTGA-3' 5'-CCACGGGGTACAGGGATTA-3' 5'-CCACGGGGAAGAGGATGA-3'Endogenous reprogramming factors (qRT-PCR)KLF4 (endogenous)133 bp5'-TCTCGTGCACCCACTTGGG-3' 5'-CTGCTCAGCACCTTCGTCCACCACTTGGG-3' 5'-CTGCTCAGCACTTCGTGCACCCACTTGGG-3' 5'-CTGCTCAGCACTTCGTCCACCACTTGGG-3' 5'-CTGCTCAGCACTTCGTCCACCACTTGGG-3' 5'-CTGCTCAGCACTTCCTCAAG-3'	Endoucini	50,17	01 bp	5'-TCTGCCTCCTCCACGAAG-3'		
MesodermHAND172 bp5'-CAGCCTCAAGATGTTCCTCTG-3MesodermHAND172 bp5'-AACTCAAGAAGGCGGATGG-3' 5'-GGAGGAAAACCTTCGTGCT.3'MKX2.5102 bp5'-TTCTATCCACGTGCCTCAGC-3 5'-CTGTCTTCCCAGCTCCACC-3 5'-CTGTCTTCCCAGCTCCACC-3 5'-CTGCTTCCCCGGAGTCTA.3' 5'-CGCACTGGAGAGGACTTCTT-3'Ectoderm0TX1125 bp5'-CACCTGCCGCGAGTCTA.3' 5'-CGCACTGGAGAGGACTTCTT-3'House-keeping gene (qRT-PCR)ACTB (β-actin)85 bp5'-CACCTCTCCTCCCCAGGATGA-3' 5'-CCAACAGGGATAG-3'Endogenous reprogramming factors (qRT-PCR)KLF4 (endogenous)133 bp5'-TCTTCGTGCACCCACTTGGG-3' 5'-CTGCTCAGCACTTCCTCAAG-3'C-MYC (endogenous)101 bp5'-CACCAGCAGCGACTCTGA-3'		AFP	90 bp	5'-GTGCCAAGCTCAGGGTGTAG-3		
Mesoderm       HAND1       72 bp       5'-AACTCAAGAAGGCGGATGG-3'         NKX2.5       5'-GGAGGAAAACCTTCGTGCT-3'       5'-GGAGGAAAACCTTCGTGCCTACAGC-3'         Ectoderm       07X1       102 bp       5'-TTCTATCCACGTGCCGAGGCTCA-3'         KRT14       125 bp       5'-GACCTCGGGAGGACTTCTT-3'         House-keeping gene (qRT-PCR)       ACTB (β-actin)       5'-CGACGGAGGCGTACGGGATGA-3'         Endogenous reprogramming factors (qRT-PCR)       KLF4 (endogenous)       96 bp       5'-CCAACCGGCGACACTTGGG-3'         Factors (qRT-PCR)       KLF4 (endogenous)       133 bp       5'-TCTTCGTGCACCCACTTGGG-3'         Factors (qRT-PCR)       -WYC (endogenous)       101 bp       5'-CACCAGCGGAGCTCTCTGA'			· · ·	5'-CAGCCTCAAGTTGTTCCTCTG-3		
Find a construction of the constru	Mesoderm	HAND1	72 bp	5'-AACTCAAGAAGGCGGATGG-3' 5'-GGAGGAAAACCTTCGTGCT-3'		
NKX2.5       102 bp       5'.TTCTATCCACGTGCCTACAGC.3 5'.CTGTCTTCTCCAGCTCACGC.3         Ectoderm       0TX1       125 bp       5'.GATCAACCTGCCGGAGTCTA.3' 5'.CGCACTGGAGAGGACTTCT-3' 5'.CGCACTGGGGAGGAGTCT-3' 5'.CGCACTGGGGAGGCTT-3'         House-keeping gene (qRT-PCR)       ACTB (β-actin)       96 bp       5'.CCACCGCGGAGATGA-3' 5'.CCCAGGGGGATGA-3' 5'.CCGCAGGGGGTACAGGGATGA-3'         Endogenous reprogramming factors (qRT-PCR)       KLF4 (endogenous)       133 bp       5'.TCTTCGTGCACCCACTTGGG-3' 5'.CTGCTCAGCACTTCCTCAAG-3'         C-MYC (endogenous)       101 bp       5'.CACAGCGGAGCTCTGA-3'			-			
Ectoderm       OTX1       125 bp       5'-GATCAACCTGCCGGAGTCTA-3'         KRT14       5'-CGCACTGGAGAGGACTTCT-3'       5'-CGCACTGGAGAGGACTTCT-3'         House-keeping gene (qRT-PCR)       ACTB (β-actin)       5'-ATGACCTGGCGGAGTGCA-3'         Endogenous reprogramming factors (qRT-PCR)       KLF4 (endogenous)       96 bp       5'-CCACGCGGAGACTGC-3'         Endogenous reprogramming factors (qRT-PCR)       KLF4 (endogenous)       133 bp       5'-TCTTCGTGCACCCACTTGGG-3'         F-CMYC (endogenous)       010 bp       5'-CCACAGGGGACTCTGA'		NKX2.5	102 bp	5'-TTCTATCCACGTGCCTACAG	-C-Ś	
Ectoderm     OTX1     125 bp     5'-GATCAACCTGCCGGAGTCTA-3' 5'-CCACTGGAGAGGACTTCTA-3' 5'-CCACTGGAGAGGACTTCT-3' 5'-CCACTCGCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTC				5'-CTGTCTTCTCCAGCTCCACC-3		
KRT14       85 bp       5'-CGCACTGGAGAGGACTTCTT-3'         House-keeping gene (qRT-PCR)       ACTB (β-actin)       5'-ATGACCTTGGTGGGGATTT-3'         House-keeping gene (qRT-PCR)       ACTB (β-actin)       96 bp       5'-CCACCGCGAGAAGGATGA-3'         Endogenous reprogramming factors (qRT-PCR)       KLF4 (endogenous)       133 bp       5'-TCTTCGTGCACCCACTTGGG-3'         c-MYC (endogenous)       101 bp       5'-CACCAGGGGACTCTGA-3'       5'-CACCAGCACCACCTTGGG-3'	Ectoderm	OTX1	125 bp	5'-GATCAACCTGCCGGAGTCTA-3' 5'-CGCACTGGAGAGGACTTCTT-3'		
KRT14     85 bp     5'-CACCTCTCCTCCTCCCCCCAGTT-3' 5'-ATGACCTTGGTGGGGATTT-3' 5'-CCACCGGGGGATGC-3'       House-keeping gene (qRT-PCR)     ACTB (β-actin)     96 bp     5'-CCACCGGGGATGC-3' 5'-CCAGGGGGTACG-3'       Endogenous reprogramming factors (qRT-PCR)     KLF4 (endogenous)     133 bp     5'-TCTTCGTGCACCCACTTGGG-3' 5'-CTGCTCAGGCACTTCCTCAAG-3'       c-MYC (endogenous)     101 bp     5'-CACCAGCGGGCACTCTGA-3' F'-CACCCAGCACTCCGCACTTCGTC-3'						
House-keeping gene (qRT-PCR)       ACTB (β-actin)       96 bp       5'-CCAACGCGAGAAGATGA-3'         Endogenous reprogramming factors (qRT-PCR)       KLF4 (endogenous)       133 bp       5'-CTTCGTGCAGCACTTGGG-3'         c-MYC (endogenous)       010 bp       5'-CAACGCGAGCACTCTGA-3'		KRT14	85 bp	5'-CACCTCTCCTCCTCCCAGTT-3'		
House-keeping gene (qRT-PCR)     ACTB (β-actin)     96 bp     5'-CCAACCGCGAGAGAGTGA-3'       Endogenous reprogramming factors (qRT-PCR)     KLF4 (endogenous)     133 bp     5'-TCTGGTGCACCACTTGGG-3'       c-MYC (endogenous)     101 bp     5'-CACCAGCAGCGATCTGA-3'				5'-ATGACCTTGGTGCGGATTT-	3′	
Endogenous reprogramming KLF4 (endogenous) 133 bp 5'-CCTCGTGCACCCACTTGGG-3' factors (qRT-PCR) 5'-CTTCGTGCACCCACTTGGG-3' c-MYC (endogenous) 101 bp 5'-CACCAGCAGCGACTCGA-3'	House-keeping gene (qRT-PCR)	ACTB (β-actin)	96 bp	5'-CCAACCGCGAGAAGATGA-3	3' 	
Endogenous reprogramming     KLF4 (endogenous)     133 bp     5-ICTICGTGCACCCACTTGGG-3'       factors (qRT-PCR)     5'-CTGCTACGCACTTCGCA3'     5'-CTGCTCAGCACTTCGCA3'       c-MYC (endogenous)     101 bp     5'-CACCAGCAGCGACTCTGA-3'	Pada and a second second second		100 h -	5-CCAGAGGCGTACAGGGATA	G-3'	
<i>c-MYC</i> (endogenous) 5 - CIGCLAGCACITCCTCA-3 5 - CIGCLAGCACITCCA-3 5 - CIGCLAGCACITCCA-3 5 - CIGCLAGCACITCCA-3 5 - CIGCLAGCACITCTCA-3 5 - CIGCLAGCACITCCA-3 5 - CIGCLAGCACITCA-3 5 - CIGCLAGCACITCA-3 5 - CIGCLAGCACITCA-3 5 - CIGCLAGCACITCA-3 5 - CIGCLAGCACITC	Endogenous reprogramming	KLF4 (endogenous)	133 Dp	5-IUTICGIGCACCCACTIGGC	r-3 2.2/	
C-MTC (endogenous) 101 bp 5 -CACCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC	IACIOIS (UKI-PCK)	e MVC (ondogonous)	101 bp		r-0 /	
		c-mic (curroscuous)		5'-CAUCAGUAGUGAUIUIGA-3 5'-CATCCACACTTTTCC-3'		

*NKX2.5*), and ectodermal (*OTX1, KRT14*) markers was measured in iPSCs and on day 21 after induction of embryoid body formation (Moretti et al., 2010). Differentiation potential was calculated as fold gene expression on day 21 compared to iPSCs with  $\beta$ -actin as house-keeping gene. RNA extraction, cDNA production and RT-PCR was performed as described in the Supplementary Material. Used primers are listed in Table 2.

#### 3.5. Karyotype analysis

Treatment of iPSC culture (p22) and karyotype analysis was done according to standard conditions (Bangs and Donlon, 2005), with the following specified slight modifications. iPSCs were incubated with Colcemid (40 ng/mL) for 2 h. Cells were detached using 0.5 mM PBS/ EDTA (Gibco).

#### 3.6. Mycoplasma detection

Supernatant of dense iPSC culture (p18) was collected after 24 h and Mycoplasma Detection Kit Venor®GeM Classic (MB Minerva Biolabs) was used according to the manufacturers instructions.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102662.

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