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Lab Resource: Multiple Cell Lines

Generation of human induced pluripotent stem cell (iPSC) lines (UUMCBi001-A, UUMCBi002-A) from two healthy donors

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A B S T R A C T

Availability of numerous high-quality iPSC lines is needed to overcome donor-associated variability caused by genetic background effects. We generated two human iPSC lines from dermal fibroblasts of two healthy females using Sendai virus reprogramming. Quality assessment of the iPSC lines confirmed the expression of pluripotency markers, trilineage differentiation capacity and absence of exogenous expression of reprogramming factors. Both iPSC lines were genetically stable with a genotype that matched the fibroblast lines of donors. These iPSC lines add to available reference lines as a resource for disease modeling of polygenic and multifactorial diseases, for evaluation of differentiation protocols and toxicology screening.

1. Resource Table

Unique stem cell lines identifier	**UUMCBi001-A** **UUMCBi002-A**
Alternative names of stem cell lines	**JLB1c10 (UUMCBi001-A)** **JLB2c2 (UUMCBi002-A)**
Institution	**Uppsala University**
Contact information of distributor	**Joey Lau, Joey.Lau@mcb.uu.se**
Type of cell lines	**iPSC**
Origin	**human**
Cell Source	**fibroblasts**
Clonality	**Clonal**
Method of reprogramming	**Sendai virus**
Multiline rationale	**Control lines generated simultaneously**
Gene modification	**NO**
Type of modification	**N/A**
Associated disease	**N/A**
Gene/locus	**N/A**
Method of modification	**N/A**
Name of transgene or resistance	**N/A**
Inducible/constitutive system	**N/A**
Date archived/stock date	**December 2019**
Cell line repository/bank	https://hpscreg.eu/cell-line/UUMCBi001-A https://hpscreg.eu/cell-line/UUMCBi002-A
Ethical approval	**Obtained from Regional ethics committee, Uppsala, September 7, 2016. Registration number: 2016/209**

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
JLB1c10 (UUMCBi001-A)	JLB1c10	Female	35	Caucasian	N/A	healthy
JLB2c2 (UUMCBi002-A)	JLB2c2	Female	19	Caucasian	N/A	healthy

2. Resource utility

The application of patient-derived induced pluripotent stem cells (iPSC) in biomedical research is dependent on high-quality reference iPSC lines. The iPSC lines from two healthy females increase the number of well-characterized control lines as a resource for disease modeling and genome editing, evaluation of cell differentiation protocols and toxicology screening. Studies based on several iPSC lines of different biological origins reduce the risk for genetic background effects.

3. Resource details

The implementation of induced pluripotent stem cell (iPSC) technology [1] has opened up novel avenues in biomedical research during the last decade. The iPSC has proven a useful tool for studies on cell differentiation, for tissue regeneration, disease modeling, drug development and cell therapy. All these studies require access to high quality reference iPSC lines derived from healthy individuals. Freely available high quality control iPSC lines are also needed to validate observations and to overcome donor specific variability. A large number of genetically independent reference lines are essential in disease modeling of polygenic diseases and for toxicology screening [2].

To this end, we obtained skin punch biopsies from two healthy females (JLB1 and JLB2; Table 1) after written informed consent. Primary dermal fibroblasts were reprogrammed using integration-free Sendai virus. One clone derived from each donor (JLB1c10 and JLB2c2) was selected, expanded and characterized (Table 2). Clonal expansion of the iPSC lines was performed on LN521 coated dishes and both cultures displayed typical iPSC morphology with tightly packed colonies of small, round cells with a large nucleus-to-cytoplasm ratio having light, clear borders using Bright-field microscopy (Fig. 1A; scale bar 100 μm).

Both iPSC lines expressed the pluripotency markers NANOG, SOX2, TRA-1-60 and SSEA4 confirmed by flow cytometry (Fig. 1B; scale bar 100 μm) and immunocytochemistry (Fig. 1C), respectively. The analyses demonstrated that 97.7% (JLB1c10) and 96.7% (JLB2c2) of cells were double positive for TRA-1-60 and SSEA4, respectively. Cell line authentication was investigated using a set of 16 polymorphic short tandem repeats (STRs). All STRs of the two iPSC lines matched their respective parental fibroblast line (data available upon request).

The endogenous expression of markers corresponding to the pluripotent and undifferentiated state was analyzed using scorecards and showed a pattern comparable to the marker expression in a reference set of 23 pluripotent stem cell lines (Fig. 1D; [3]). Additionally, scorecards confirmed the loss of exogenous expression of the four reprogramming factors, as the Sendai virus could not be detected (ΔCt values $\geq$ 40). The differentiation potential of the JLB1c10 iPSC and JLB2c2 iPSC lines was assessed by embryoid body (EB) differentiation for 25 days. Both iPSC lines showed spontaneous differentiation into the three germ layers ectoderm, mesoderm and endoderm, as confirmed by scorecards (Fig. 1D).

The CytoScan™HD DNA array was used to investigate genomic integrity. No acquired chromosomal aberrations could be detected at a resolution of $>$ 1 Mb (Supplementary File 2; [4]). Additionally, no trace of mycoplasma infection was detected in either the JLB1c10 iPSC line or the JLB2c2 iPSC line using MycoAlert™ Mycoplasma detection kit (Supplementary File 1).

The two iPSC lines presented herein offer a useful resource as control lines in various primary studies and for validation purposes.

4. Materials and methods

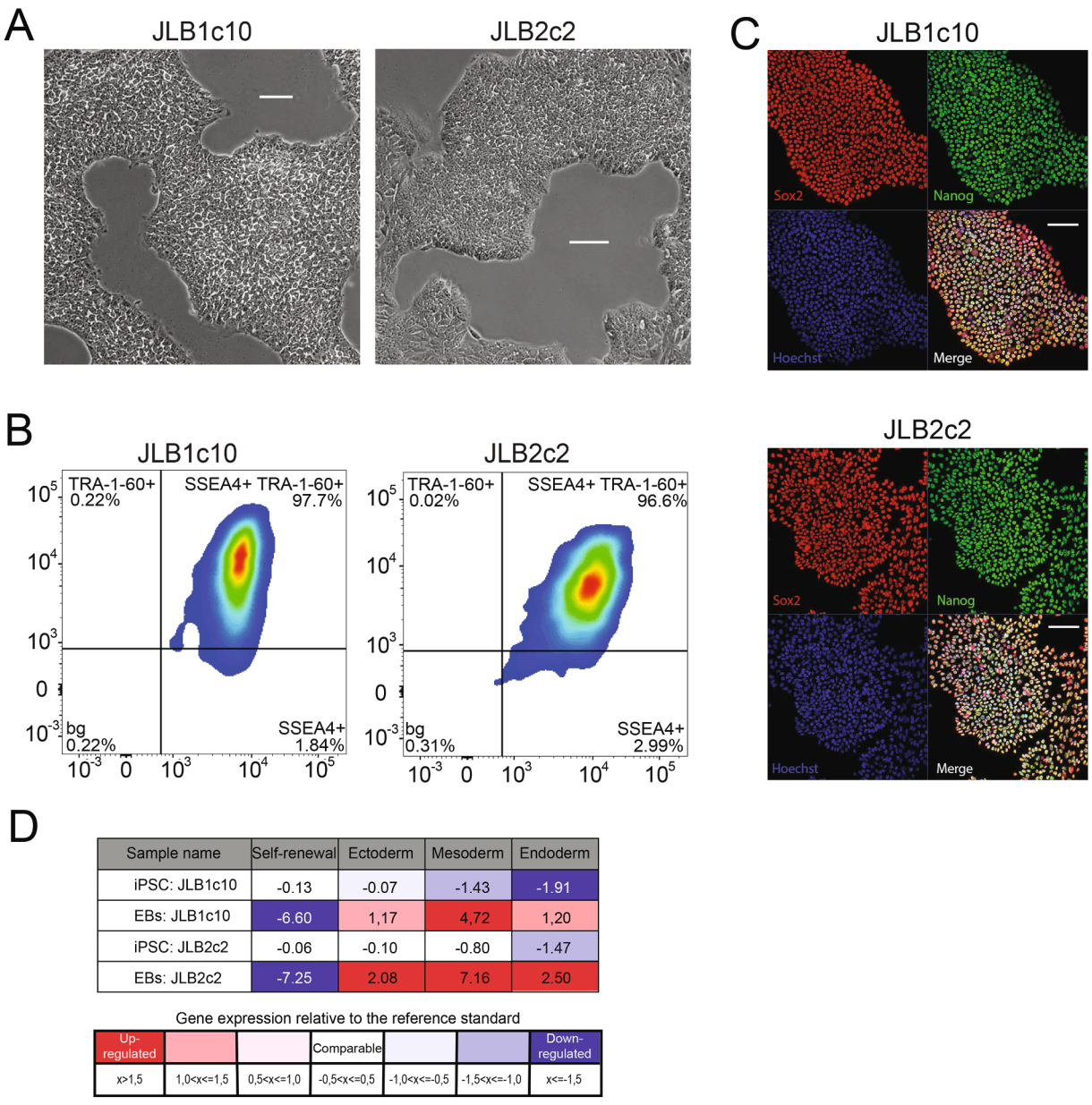
4.1. Culture conditions

Fibroblasts were cultured in DMEM (Sigma, cat no: D5796), 10% fetal bovine serum (ThermoFisher Scientific, cat no: 10500056), 2 mM GlutaMAX™ (ThermoFisher Scientific, cat no: 35050038) and 1% penicillin/streptomycin (ThermoFisher Scientific, cat no: 15140122) in a humidified atmosphere with 5% CO_2 at 37 °C and passaged using TrypLE™ Express (ThermoFisher Scientific, cat no: 12604039) and Defined Trypsin Inhibitor (ThermoFisher Scientific, cat no: R007100).

IPSCs were cultured in Essential-8™ medium (ThermoFisher Scientific, cat no: A1517001), on human Laminin-521 (Biolamina, cat no: LN-

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	All iPSC lines appear normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	All iPSC lines are positive for pluripotency markers NANOG and SOX2	Fig. 1 panel C
	Qualitative analysis (scorecards)	All iPSC lines are pluripotent and undifferentiated compared to a reference set of 23 hPSC lines	Fig. 1 panel D
	Quantitative analysis (Flow Cytometry)	All iPSC lines are positive for cell surface markers TRA-1-60 (JLB1c10 97.9%; JLB2c2 96.6%) and SSEA4 (JLB1c10 99.5%; JLB2c2 99.6%)	Fig. 1 panel B
Genotype	CytoScan™HD Array (resolution $>$ 1 Mb)	No acquired genomic aberration detected	Supplementary File 2
Identity	STR analysis (AmpFLSTR™ Identifier™ PCR Amplification Kit)	DNA profiling for 16 polymorphic sites, all matched	Available with the authors
Microbiology and virology	Mycoplasma testing using luminescence (MicoAlert Mycoplasma Detection Kit, Lonza)	Negative	Supplementary File 1
Differentiation potential	Embryoid body formation and differentiation followed by Scorecard analysis	Spontaneous *in vitro* differentiation for four weeks. The expression of all three germ layers was detected.	Fig. 1 panel D
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	



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Sample name	Self-renewal	Ectoderm	Mesoderm	Endoderm
iPSC: JLB1c10	-0.13	-0.07	-1.43	-1.91
EBs: JLB1c10	-6.60	1,17	4,72	1,20
iPSC: JLB2c2	-0.06	-0.10	-0.80	-1.47
EBs: JLB2c2	-7.25	2.08	7.16	2.50

Gene expression relative to the reference standard

Up-regulated			Comparable			Down-regulated
x>1,5	1,0<x<=1,5	0,5<x<=1,0	-0,5<x<=0,5	-1,0<x<=-0,5	-1,5<x<=-1,0	x<=-1,5

Fig. 1. Characterization of the human iPSC lines UUMCBi001-A and UUMCBi002-A from two healthy donors.

521) coated plates (5%CO2, 37 °C) and passaged with TrypLE™ Express and Defined Trypsin Inhibitor when 70–80% confluent.

For embryoid body formation iPSCs were dissociated with Accutase (Sigma, cat no: A6964), seeded into a Costar Ultra-Low Cluster 96 well round bottom plate (Corning, cat no: 7007), and cultured in DMEM/F12 medium supplemented with 25% KOSR, 3% FBS, 1% 2 mM Glutamax, 1% NEAA, 1% P/S and 10 μM Rho-kinase inhibitor Y27632 (Stem Cell Technologies, cat no: 72304). Formed embryoid bodies were transferred to non-adherent culture plates and further differentiated for 25 days.

4.2. Reprogramming

Fibroblasts were reprogrammed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific, cat no: A16517), expressing the four Yamanaka factors hKlf4, hc-Myc, hSox2, hOct3/4. HiPSC colonies were manually picked for passage 1 to human Laminin-521 coated dishes and clonally expanded (see Culture conditions).

4.3. Cell authentication

Cell authentication on DNA from fibroblasts and iPSC lines was performed using AmpFLSTR™ Identifiler™ PCR Amplification Kit, ThermoFisher Scientific (cat no: 4322288) and amplified products were separated on a SeqStudio Genetic Analyzer, Applied Biosystems. Fragment lengths were analyzed using GeneMapper™ Software v5.

4.4. Genome integrity

Genome integrity was analyzed on DNA from the iPSC lines at Uppsala University Hospital, Clinical Genetics unit, Uppsala, using the CytoScan™HD Array (ThermoFisher Scientific, cat no: 901835).

4.5. RT-PCR

RNA was isolated using the miRNeasy micro kit (Qiagen, cat no: 217084). cDNA was synthesized using a High Capacity cDNA Synthesis kit (ThermoFisher Scientific, cat no: 4368814) from 1 μg of total RNA.

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse IgG anti-NANOG	1:200	Millipore Cat# MABD24, RRID: AB_11203826
Pluripotency Markers	Mouse IgG anti-SSEA4	1:100	Thermo Fisher Scientific Cat# 41-4000, RRID: AB_2533506
Pluripotency Markers	Mouse IgM anti-TRA-1-60	1:100	Thermo Fisher Scientific Cat# 41-1000, RRID: AB_2533494
Pluripotency Markers	Goat IgG anti-SOX2	1:500	R and D Systems Cat# AF2018, RRID: AB_355110
Secondary Antibodies	AF488 donkey anti-mouse IgG	1:300	(Jackson ImmunoResearch Labs Cat# 715-545-151, RRID: AB_2341099
Secondary Antibodies	AF594 donkey anti-goat IgG	1:300	Jackson ImmunoResearch Labs Cat# 705-585-147, RRID: AB_2340433
Secondary Antibodies	AF488 Goat anti-mouse IgM	1:1000	Thermo Fisher Scientific Cat# A-11001, RRID: AB_2534069
Secondary Antibodies	AF555 Goat anti-mouse IgG	1:1000	Thermo Fisher Scientific Cat# A-21426, RRID: AB_2535847

4.6. Immunofluorescence

Cells were fixed in 4% formaldehyde for 5 min and pre-incubated in 1xPBS, 3% Donkey serum, 0.3% TritonX100. Primary antibodies (mouse anti-NANOG, goat anti-SOX2; Table 3) were diluted in pre-incubation buffer and incubated at 4 °C overnight. Secondary antibodies (donkey anti-mouse AF488, donkey anti-goat AF594; Table 3) were incubated at room temperature for 60 min. Nuclear marker, DAPI (1 μg/ml; Sigma cat no: D8417) was incubated for 10 min at room temperature and specimens were mounted onto microscope slides using fluorescence mounting medium (DAKO, cat no: S3023). Specimens were imaged using a Zeiss LSM microscope 780.

4.7. Flow cytometry

IPSCs were harvested with accutase (Sigma cat no: A6964) and washed in 1%BSA/1xPBS. Primary antibodies (mouse-IgG anti-SSEA4, mouse-IgM anti TRA-1-60; Table 3) were incubated at room temperature for 30 min. Secondary antibodies (goat anti-mouse IgM AF488, goat anti-mouse IgG AF555; Table 3) were incubated for 20 min at room temperature. Cells were analyzed on a LSR-FORTESSA (BD).

4.8. Scorecard assay

RNA was extracted (see RT-PCR) and quality was assessed by Agilent BioAnalyzer. Samples were run on TaqMan® hPSC Scorecard™ Panel (ThermoFisher Scientific, cat no: A15872/A15870) following the manufacturer's protocol [3]. Scorecards were analyzed with the company's software at https://apps.thermofisher.com/hPSCscorecard/home.htm.

4.9. Mycoplasma

Cell culture supernatants from the iPSC lines were analyzed for mycoplasma using the MycoAlert™ Mycoplasma Detection kit (Lonza, cat no: LT07-318).

Ethical approval

Both tissue donors for the study have signed written informed consent and the experimental protocol underwent revision and approval by the regional ethical committee of Uppsala, Sweden (Dnr 2016/209).

Declaration of Competing Interest

None.

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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.2020.102114.

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