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Lab Resource: Multiple Cell Lines

Generation of three isogenic human induced pluripotent stem cell lines from normal neonate skin fibroblasts

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A B S T R A C T

Characterized human induced pluripotent stem cell lines are important for basic research. Here, we report the establishment of three isogenic human induced pluripotent stem cell (hiPSC) lines generated from normal neonate male skin fibroblasts. Pluripotency was induced using the integration free Sendai virus reprogramming method. The pluripotency, identity, quality, and safety of the lines were confirmed to establish characterized human induced pluripotent stem cell lines to be used as normal control cell lines in future studies.

Resource table:

Unique stem cell lines identifier	UTUi001-A, UTUi001-B, UTUi001-C
Alternative name(s) of stem cell lines	TUR1 (UTUi001-A), TUR2 (UTUi001-B), TUR3 (UTUi001-C)
Institution	Institute of Biomedicine, University of Turku
Contact information of distributor	Elisa Närvä, elisa.narva@utu.fi
Type of cell lines	hiPSC
Origin	human
Additional origin info required	Age: Neonate, Sex: Male
Cell Source	BJ CRL-2522 (ATCC) foreskin fibroblasts
Clonality	mixed
Method of reprogramming	Sendai-virus
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	–
Gene/locus	N/A
Date archived/stock date	October 10, 2023
Cell line repository/bank	https://hpscreg.eu/cell-line/UTUi001-A, https://hpscreg.eu/cell-line/UTUi001-B, https://hpscreg.eu/cell-line/UTUi001-C
Ethical approval	Ethics Committee for Human Sciences at the University of Turku (6/2023)

1. Resource utility

Human induced pluripotent stem cell lines and reprogramming technology are of vital importance for future medicine. TUR1-3 cell lines described here are the first human induced pluripotent stem cell lines produced locally and will provide an important cell source to be used in future studies.

2. Resource details

Normal human foreskin fibroblasts were reprogrammed into human induced pluripotent stem cells (hiPSCs) using a non-integrated Sendai virus based reprogramming method (Fusaki et al., 2009; Takahashi et al., 2007; Takahashi & Yamanaka, 2006; Yu et al., 2007). Isogenic cell lines were reprogrammed in independent transduction plates and enriched from individual manually picked colonies 24 days after transduction.

Resulting isogenic hiPSC lines TUR1, TUR2, and TUR3 showed typical pluripotent cell morphology (Fig. 1A) and expression of pluripotency markers TRA-1-60 and NANOG detected by immunofluorescence staining on single cell level (Fig. 1B). Further, the resulting hiPSC populations were over 99 % positive for the pluripotency markers TRA-1-60, and SSEA-4 whereas expression of the differentiation marker SSEA-1 remained below 1 % in all cell lines analysed with flow cytometry (Fig. 1C).

The pluripotency of the cells was confirmed as the ability of the cells to form embryonic bodies (EBs) (Fig. 1D). The expression of germ layers (endo-, ecto-, meso- and trophectoderm) in EBs was first tested with RT-PCR (Fig. 1E) and further validated by immunofluorescence staining of

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the EB ice sections (Fig. 1F).

The microbiology and virology of the cell lines were tested. All the cell lines were mycoplasma negative (Fig. 1G). In addition, the generated hiPSC lines were virus-free at passage 20 (Fig. 1H).

All generated hiPSCs had a normal male karyotype (48, XY) (Fig. 1I). In addition, based on STR analysis, the hiPSC lines and the original human foreskin fibroblast cell line had identical SNP profiles to confirm the identity. STR analysis also confirmed a normal male karyotype (48, XY) based on the primer pair AMEL, which is specific for male karyotype. Collectively, the TUR 1–3 cell lines fulfilled all the hallmarks of pluripotent stem cells (Fig. 1, Table 1).

3. Materials and methods

3.1. Reprogramming

BJ CRL-2522 (ATCC) fibroblasts were cultured in KnockOut DMEM/F-12 (Thermo Fisher Scientific, 12660012), 20 % FBS (Sigma, F7524-500ML), 2 mM glutamine (Euroclone, ECB3004D), 25 U/ml penicillin-streptomycin (Gibco, 15140-122). Fibroblasts were transduced using

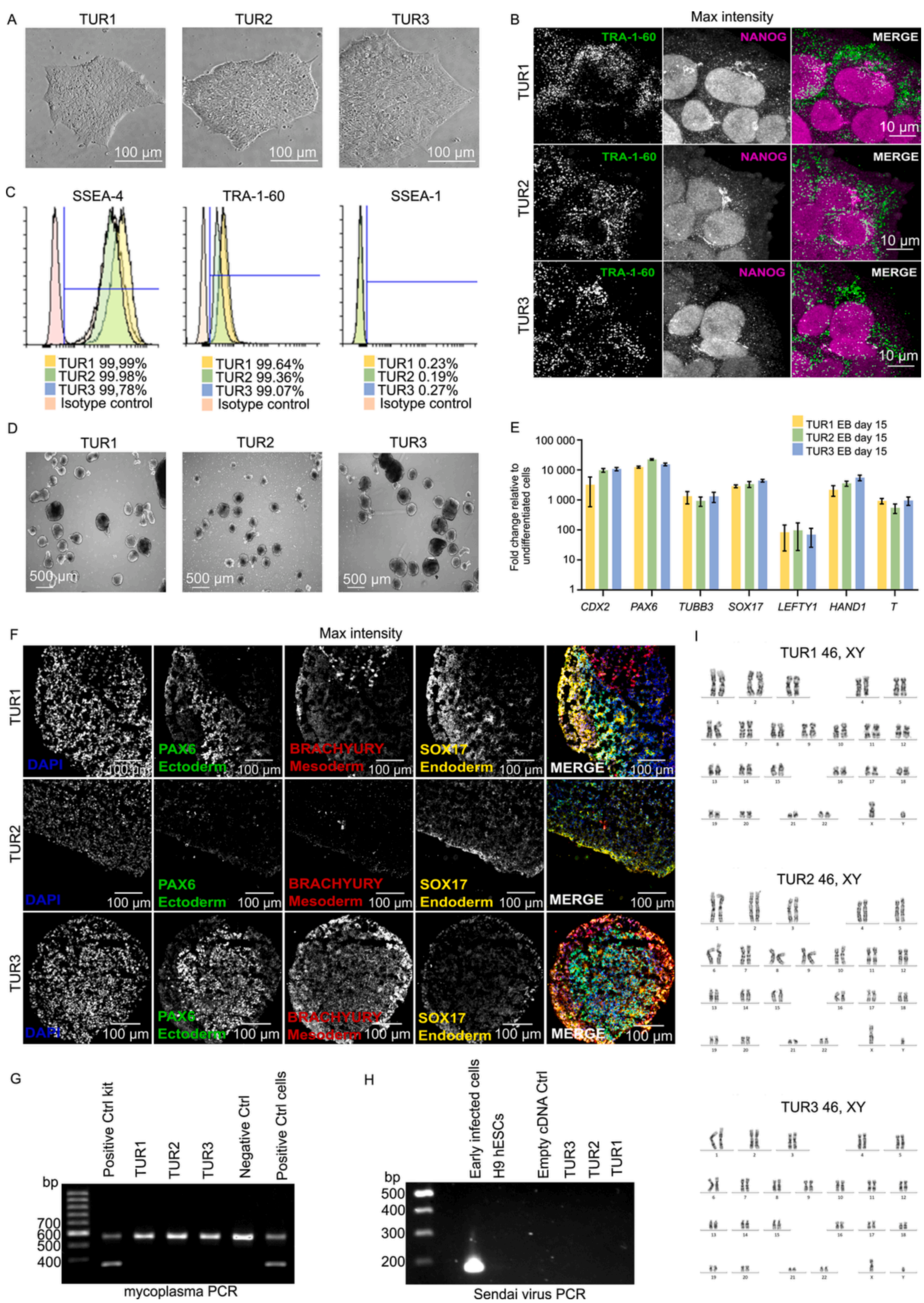

Fig. 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	compact colonies with a well-defined edge	Fig. 1 panel A
Phenotype	Qualitative analysis (*Immunocytochemistry*)	Positive expression of pluripotency markers TRA-1–60, and NANOG	Fig. 1 panel B
	Quantitative analysis (*Flow cytometry*)	Expression of pluripotency markers SSEA-4 and TRA-1–60>99 %. Expression of differentiation marker SSEA-1 <1 %	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46, XY Resolution: 10 megabases	Fig. 1 panel I
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	16 loci (AMEL, CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX and vWA) tested and all matched	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	MycoplasmaSendai virus	Mycoplasma test by PCR was negativePCR for Sendai virus RNA was negative	Fig. 1 panel G Fig. 1 panel H
Differentiation potential	Embryoid body formation and trilineage expression determined by immunofluorescence and RT-PCR	Embryonic bodies formed and expressed endoderm markers SOX17 and LEFTY1, mesoderm markers BRACHYURY and HAND1 and ectoderm markers PAX6 and TUBB3. Also, trophectoderm marker CDX2 was positive.	Fig. 1 panel D, E and F
List of recommended germ layer markers	**Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer**	Embryonic bodies formed and expressed endoderm markers SOX17 and LEFTY1, mesoderm markers BRACHYURY and HAND1 and ectoderm markers PAX6 and TUBB3. Also, trophectoderm marker CDX2 was positive.	Fig. 1 panel D, E and F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, A16517) according to the manufacturer's instructions. 7 days after the transduction, the cells were plated to Matrigel-coated (12 µg/ml) (Corning, 354277) plates and medium was changed to Essential 8™ medium (Gibco, A1517001), 25 U/ml penicillin-streptomycin followed by daily medium change. 24 days after the transduction, single colonies were picked and expanded at 37 °C, 5 % CO_2 with 0.5 mM EDTA (Invitrogen, 15575–038) in PBS (ratio 1:3–1:4 every 2–3 days) without ROCK inhibitor.

3.2. *Karyotyping*

HiPSCs (p9) were treated with 200 ng/ml colcemid (Roche Diagnostics, 10295892001) in E8 medium for 2 h at 37 °C. Harvested single cells were treated with 0.075 M KCl 25 min at 37 °C and fixed (methanol: acetic acid 3:1). G-banding was performed by Giemsa-staining. 20 metaphases analyzed per cell line with ZEIZZ Imager Z2 -microscope and MetaSystems CoolCube 1-MetaSystems Metafer 4 program.

3.3. *Immunofluorescence staining*

The hiPSCs (p7) or frozen sectioned EBs were fixed with 4 % paraformaldehyde for 15 min at 37 °C in E8 and permeabilized with 0.05 % TRITON x100 (Sigma-Aldrich, 9036–19-5) in 1x PBS for 20 min at RT. Samples were stained with primary antibodies in 30 % horse serum for 24 h at 4 °C followed by secondary antibodies and DAPI in 30 % horse serum for 1 h at RT (Table 2). The imaging was carried out using 3i CSU-W1 Spinning disk confocal microscopy.

3.4. *Flow cytometry*

Cells (p20) were dissociated into single cells and fixed with 4 % paraformaldehyde. Cells were stained with primary antibodies and isotype control for 24 h at 4 °C followed by fluorescent conjugated secondary antibody staining for 1 h at 4 °C (Table 2). Samples were analyzed using BD LSRFortessa Blues flow cytometry and Flowing software.

3.5. *In vitro differentiation by EMBRYOID bodies formation*

EBs were grown in E8 from detached cells (p26) for 15 days using Low Attachment Surface plates (Corning, 3471) at 37 °C, 5 % CO_2. 20 % FBS was added for final three days.

3.6. *RT-PCR*

RNA was isolated with NucleoSpin® Tissue RNA kit (Macherey-Nagel, 740971.50) and RNA quality was determined with the Nanodrop One device. RNA was treated with DNase I Amplification Grade Kit (18068015, Invitrogen) followed by cDNA synthesis using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem, 4368814). RT-PCR was performed in triplicate reactions using TaqMan Fast Advanced Master Mix (2X) (Applied Biosystems, 4444557) and primers listed in Table 2. RT-PCR was performed on CFX96 Real-Time system thermocycler (Bio Rad): 2 min at 50 °C, 20 s at 95 °C, 40 cycles of 95 °C for 3 s, 60 °C for 30 s. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method relative to the housekeeping gene.

3.7. *Mycoplasma detection*

Mycoplasma detection was performed using the LookOut® Mycoplasma PCR Detection Kit (Sigma-Aldrich, MP0035-1KT) (p7).

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	*Mouse Alexa Fluor 647 anti-human TRA-1–60,* *Goat anti-hNanog,* Mouse anti-hSSEA-4	*1:50* *1:50* *1:100*	*BD Biosciences Cat # 560850,* *R&D System* *Cat # AF1997,* *Abcam* *Cat # ab16287*	*RRID: AB_10565983* *RRID: AB_355097* *RRID: AB_778073*
Differentiation Markers	*Mouse anti-hSSEA-1,* *Mouse anti-hPax6,* *Mouse anti-hBrachyury,* *Rabbit anti-hSOX17*	*1:100* *1:100* *1:20* *1:200*	*Abcam* *Cat # AB16285,* *Santa Cruz Biotechnology Cat # sc-81649,* *R&D System* *Cat # AF2085,* *NovusBio Cat # NBP1-80362*	*RRID: AB_870663* *RRID: AB_1127044* *RRID: AB_2200235* *RRID: AB_11054502*
Secondary antibodies	*488 Donkey anti-mouse IgG,* *555 Donkey anti-goat IgG,* *Alexa Fluor 647 Donkey anti-rabbit IgG*	*1:400* *1:400* *1:400*	*Invitrogen* *Cat # A21202,* *Invitrogen* *Cat # A21432,* *ThermoFisher Cat # A31573*	*RRID: AB_141607* *RRID: AB_2535853* *RRID: AB_2536183*
	Primers			
	Target	Size of band	Forward/Reverse primer (5′-3′)	
Sendai Virus (PCR)	*SeV*	*200 bp*	*GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC*	
House-keeping Gene (qPCR)	*GAPDH*	*157 bp*	*Assay ID: Hs02786624g1, ThermoFisher Scientific*	
Differentiation marker (qPCR)	*CDX2*	*81 bp*	*Assay ID: Hs01078080_m1, ThermoFisher Scitific*	
Differentiation marker (qPCR)	*HAND1*	*65 bp*	*Assay ID: Hs02330376_s1, ThrmoFisher Scientific*	
Differentiation marker (qPCR)	*LEFTY1*	*136 bp*	*Assay ID: Hs00764128_s1, ThermoFisher Scietific*	
Differentiation marker (qPCR)	*PAX6*	*86 bp*	*Assay ID: Hs01088114_m1, ThermoFisher Scietific*	
Differentiation marker (qPCR)	*SOX17*	*149 bp*	*Assay ID: Hs00751752_s1, ThermoFisher Scientific*	
Differentiation marker (qPCR)	*T*	*132 bp*	*Assay ID: Hs00610080_m1, ThermoFisher Scientific*	
Differentiation marker (qPCR)	*TUBB3*	*134 bp*	*Assay ID: Hs00801390_s1, ThermoFisher Scientific*	

3.8. Sendai virus detection

The cells were tested for the presence of Sendai virus RNA by PCR (p20) using DreamTaq Green PCR master mix kit (Thermo Scientific, K1081) according to the manufactureŕs instructions. RNA extraction and cDNA synthesis are described above. Primers are listed in Table 2.

3.9. STR-analysis

DNA was isolated using DNA NucleoSpin® Tissue Kit (Macherey-Nagel, 740952.50). STR analysis of 16 loci (Table 1) was performed by Eurofins.

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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