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Lab Resource: Multiple Cell Lines

Generation of iPSC lines from peripheral blood mononuclear cells from 5 healthy adults

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Abstract: We describe the generation and characterization of 5 human induced pluripotent stem cell (iPSC) lines derived from peripheral blood mononuclear cells (PBMCs) of healthy adult individuals. The PBMCs were reprogrammed using non-integrating Sendai viruses containing the reprogramming factors POU5F1 (OCT4), SOX2, KLF4 and MYC. The iPSC lines exhibited a normal karyotype, expressed pluripotency markers and differentiated into cells representative of the three embryonic germ layers. These iPSC lines can be used as controls in studying disease mechanisms.

Resource table

Unique stem cell lines identifier	MCRIi001-A MCRIi004-A MCRIi005-A MCRIi006-A MCRIi010-A
Alternative names of stem cell lines	PB001 (MCRIi001-A) PB004 (MCRIi004-A) PB005 (MCRIi005-A) PB006 (MCRIi006-A) PB010 (MCRIi010-A)
Institution	Murdoch Children's Research Institute
Contact information of distributor	Dr. Katerina Vlahos katerina.vlahos@mcri.edu.au
Type of cell lines	iPSCs
Origin	Human
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Transgene free Sendai virus
Multiline rationale	Control non-isogenic iPSC lines
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	May 2016
Cell line repository/bank	n/a
Ethical approval	RCH Human Research Ethics Committee 35121A

Resource utility

Integration-free induced pluripotent stem cell (iPSC) lines from the blood of healthy adult individuals cells can serve as controls for drug development and disease modeling studies.

Resource details

Five human iPSC lines were derived from PBMCs using Sendai virus carrying the reprogramming factors POU5F1 (OCT4), SOX2, KLF4 and MYC (Table 1). Each line formed colonies comprising tightly packed cells with a high nucleus to cytoplasm ratio and prominent nucleoli (Fig. 1A). Expression of the pluripotency marker POU5F1 (OCT4) was verified by immunofluorescence staining (Fig. 1A) whilst flow cytometry was used to assess expression of TRA1–81, SSEA-4 and CD9 (Fig. 1B). Mouse embryonic fibroblasts (MEFS) negative for these pluripotent markers were present in the cultures, and were thus utilised

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
PB001 (MCRIi001-A)	PB001	Male	61	Caucasian	n/a	n/a
PB004 (MCRIi004-A)	PB004	Female	33	Caucasian	n/a	n/a
PB005 (MCRIi005-A)	PB005	Female	45	Caucasian	n/a	n/a
PB006 (MCRIi006-A)	PB006	Male	34	Caucasian	n/a	n/a
PB010 (MCRIi010-A)	PB010	Male	22	Caucasian	n/a	n/a

in gating. Over 90% of viable cells analysed expressed TRA1–81 and SSEA-4, and > 80% were CD9-positive (Fig. 1B). The Sendai virus is non-integrating and loss of the reprogramming genes from the cells was confirmed by RT-PCR analysis after 10 passages (Fig. 1C). Directed differentiation of the iPSC lines resulted in the expression of markers consistent with development of the three germ layers. Co-expression of EPCAM, CXCR4 and KIT, at Day 4 of differentiation, marked nascent endoderm(Cheng et al., 2012) (Fig. 1D). During formation of mesoderm, epithelial markers of pluripotent stem cells (such as *E*-CADHERIN and EPCAM)(Evseenko et al., 2010; Ng et al., 2005) are transiently co-expressed with mesodermal genes (such as CD13)(Skelton et al., 2016). Thus, co-expression of EPCAM and CD13, at Day 5 of differentiation, marks early mesoderm (Fig. 1E). MAP2 expression, at Day 35 of differentiation, indicated the appearance of cortical neurons, a derivative of ectoderm(Shi et al., 2012) (Fig. 1F). In addition, all iPSC lines showed a normal molecular karyotype (at 0.5 Mb resolution) and an identical genotype to their corresponding PBMC sample when analysed by array comparative genomic hybridisation (SNP array), confirming that no major perturbations in genomic integrity occurred during reprogramming (Table 2).

Materials and methods

Cell processing

Blood samples collected from healthy adults were diluted 1:2 (vol:vol) in PBS/2% FBS and layered over Lymphoprep (StemCell Technologies) in SepMate™-15 tubes (StemCell Technologies). PBMCs were isolated by centrifugation at 1200 rcf for 10 min, transferred to a fresh tube, washed with PBS/2% FBS and centrifuged at 300 rcf for 10 min.

iPSC generation

PBMCs were cultured for 7 days in StemSpan™ SFEM II (StemCell Technologies) supplemented with StemSpan™ erythroid expansion supplement (StemCell Technologies) prior to reprogramming with Cytotune™-iPS 2.0 Sendai Reprograming kit (ThermoFisher Scientific). Transduced cells were plated on culture dishes seeded with irradiated mouse embryonic fibroblasts (MEFs), and maintained in Knockout DMEM/20%Knockout serum replacer (ThermoFisher Scientific) supplemented with 50 ng/mL of FGF2 (Costa et al., 2008). iPSC colonies were mechanically isolated and further expanded as described previously (Costa et al., 2008).

Detection of Sendai virus genome and transgenes

After 10 passages, iPSC lines were tested for elimination of Sendai virus. Total RNA was extracted and transcribed into cDNA using the Tetro cDNA synthesis kit (Bioline). RT-PCR was performed using the listed primers according to the manufacturer's instructions (Table 3). Positive control RNA was derived from cells harvested immediately after Sendai virus transduction.

Immunofluorescence (IF)

iPSCs were cultured on 12-well culture plates or glass slides for IF analysis. Cells were fixed with 4% Paraformaldehyde for 10 min at room temperature, permeabilized in 0.2%Triton™-X-100 (Sigma) for 10 min, blocked in 20% Goat Serum (Life Technologies) for 60 min. Cells were then incubated with primary antibodies for 2 h, followed by secondary antibodies for 2 h at room temperature (Table 3). Subsequently, nuclei were stained with DAPI (VectorLabs) and images were captured with an LSM 780 confocal microscope running Zen Black software and an Axio Observer.Z1 microscope with an Axiocam 506 mono camera running Zen Blue software (Carl-Zeiss).

Flow cytometry (FACS) analysis

Flow cytometry was performed as described previously (Costa et al., 2008; Ng et al., 2008). Harvested iPSCs were incubated with directly conjugated antibodies for 15 min on ice (Table 3). Dead cells were identified by propidium iodide (Sigma) staining. Samples were analysed using an LSR Fortessa (BD Bioscience).

Differentiation

iPSCs were differentiated into definitive endoderm as monolayer cultures(Loh et al., 2014) and analysed by flow cytometry after 4 days. For mesoderm differentiation, spin embryoid bodies were formed in APEL medium(Ng et al., 2008) supplemented with 15 ng/mL BMP4, 25 ng/mL VEGF, 25 ng/mL SCF, 3 ng/mL ACTIVIN A, 10 ng/mL FGF2 and 0.5 μM CHIR-99021 and analysed by flow cytometry after 5 days. To assess ectodermal potential, iPSCs were differentiated into cortical neurons using the DUAL SMAD inhibition protocol(Shi et al., 2012) and analysed for the presence of MAP2-expressing neurons after 35 days.

Karyotype analysis

Karyotypes were analysed using the Human CytoSNP-12 (PB001,PB004,PB005) and Infinium CoreExome-24 (PB006, PB010) SNP arrays. Data was compared to the human reference sequence hg19/GRCh37 (Feb 2009). PBMCs were analysed using the Infinium GSA-24 SNP array and this data compared to the corresponding iPSC lines using SNPduo comparative analysis (http://pevsnerlab.kennedykrieger.org/SNPduo/). No differences were detected between the original PBMC sample and its corresponding iPSC line.

Mycoplasma detection

Absence of mycoplasma contamination was confirmed by PCR by the commercial service provider, Cerberus Sciences (Adelaide, Australia).

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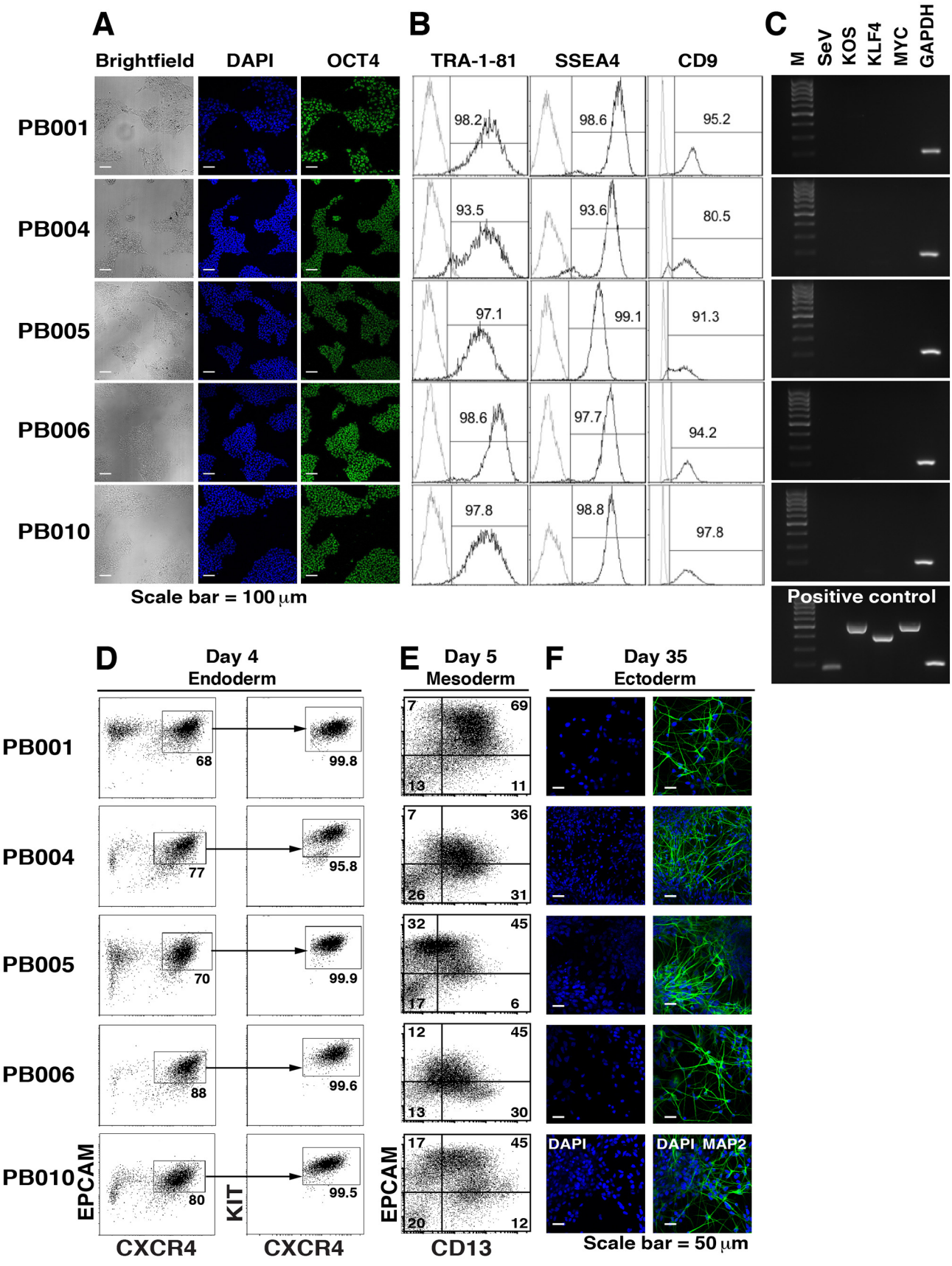
A
Brightfield
DAPI
OCT4
PB001
PB004
PB005
PB006
PB010
Scale bar = 100 μm
B
TRA-1-81
SSEA4
CD9
98.2
98.6
95.2
93.5
93.6
80.5
97.1
99.1
91.3
98.6
97.7
94.2
97.8
98.8
97.8
C
M
SeV
KOS
KLF4
MYC
GAPDH
Positive control
D
Day 4
Endoderm
68
99.8
77
95.8
70
99.9
88
99.6
80
99.5
EPCAM
KIT
CXCR4
CXCR4
E
Day 5
Mesoderm
7
69
13
11
7
36
26
31
32
45
17
6
12
45
13
30
17
45
20
12
EPCAM
CD13
F
Day 35
Ectoderm
DAPI
DAPI MAP2
Scale bar = 50 μm

Fig. 1.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Qualitative analysis (Immunofluorescence)	POU5F1 (OCT4)	Fig. 1A
	Quantitative analysis (Flow cytometry)	Tra 1–81: > 93% SSEA-4: > 93% CD9: > 80%	Fig. 1B
Genotype	SNP array (resolution 0.50 Mb)	PB001 (MCRIi001-A): arr(1 − 22)x2,(XY)x1 PB004 (MCRIi004-A): arr(1–22,X)x2 PB005 (MCRIi005-A): arr(1–22,X)x2 PB006 (MCRIi006-A): arr(1–22)x2,(XY)x1 PB010 (MCRIi010-A): arr(1–22)x2,(XY)x1	Submitted in archive with journal
Identity	Genetic analysis	SNPduo comparative analysis performed to compare parental and both derived clones	Submitted in archive with journal
		Identical genotypes (> 99.9%) for the entire genome, indicating derived cell lines are from the same individual	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	n/a	n/a
	Southern Blot OR WGS	n/a	n/a
Microbiology and virology	Mycoplasma	Negative	Data with author
Differentiation potential	Directed differentiation (Flow cytometry; Immunofluorescence)	Endoderm: EPCAM (CD326); CXCR4 (CD184); KIT (CD117) Mesoderm: EPCAM (CD326); CD13 Ectoderm: MAP2	Fig. 1D, E, F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Data with author
Genotype additional info (OPTIONAL)	Blood group genotyping	n/a	n/a
	HLA tissue typing	n/a	n/a

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4 Monoclonal Antibody	1:400	Cell Signaling Technology Cat# 2840, RRID:AB_2167691
Pluripotency Markers	Mouse Anti-CD9 Monoclonal Antibody, FITC Conjugated, Clone M-L13	1:10	BD Biosciences Cat# 555371, RRID:AB_395773
Pluripotency Markers	PE/Cy7 anti-human SSEA-4 antibody	1:100	BioLegend Cat# 330420, RRID:AB_2629631
Pluripotency Markers	Alexa Fluor 647 anti-human TRA-1-81 antibody	1:100	BioLegend Cat# 330706, RRID:AB_1089242
Differentiation Markers	PE conjugated anti-human CD326 (EPCAM) antibody, Clone EBA-1	1:30	BD Biosciences Cat# 347198, RRID:AB_400262
Differentiation Markers	PE/Cy7 conjugated anti-human CD184 (CXCR4) antibody	1:100	BioLegend Cat# 306513, RRID:AB_2089652
Differentiation Markers	APC conjugated anti-human CD117 (KIT) antibody	1:50	BD Biosciences Cat# 341096, RRID:AB_400563
Differentiation Markers	PE/Cy7 conjugated anti-human CD13 antibody	1:100	BioLegend Cat# 301711, RRID:AB_10900061
Differentiation Markers	BV 421 conjugated anti-human CD326 (EPCAM) antibody	1:30	BioLegend Cat# 324219, RRID:AB_11124342
Differentiation Markers	Unconjugated anti-bovine MAP2 (2a/2b) antibody, Clone AP-20	1:400	Sigma-Aldrich Cat# M1406, RRID:AB_477171
Secondary antibodies	Goat anti-Rabbit IgG (H + L), Cross Absorbed Secondary Antibody, Alexa Fluor 488	1:400	Thermo Fisher Scientific Cat# A-11008, RRID:AB_143165
Secondary antibodies	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11029, RRID:AB_2534088

Primers

	Target	Forward/Reverse primer (5′-3′)
Sendai Reprogramming Vector (RT-PCR)	SeV	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
Sendai Reprogramming Vector (RT-PCR)	KOS (KLF4, OCT3/4, SOX2)	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG
Sendai Reprogramming Vector (RT-PCR)	KLF4	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA
Sendai Reprogramming Vector (RT-PCR)	MYC	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG
House-Keeping Gene (RT-PCR)	GAPDH	GGAGCGAGATCCCTCCAAAAT/GGCTGTTGTCATACTTCTCATGG

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