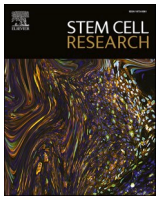
Stem Cell Research 69 (2023) 103109

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr



Lab Resource: Multiple Cell Lines

Generation of iPSC lines from peripheral blood mononuclear cells from five healthy donors

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A B S T R A C T

We describe the generation and characterisation of five 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, and pluripotency was validated by flow cytometry and immunofluorescence of pluripotency markers, and their differentiation 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	MCRIi002-A MCRIi003-A MCRIi007-A MCRIi008-A MCRIi009-A
Alternative name(s) of stem cell lines	PB002 (MCRIi002-A) PB003 (MCRIi003-A) PB007 (MCRIi007-A) PB008 (MCRIi008-A) PB009 (MCRIi009-A)
Institution	Murdoch Children's Research Institute
Contact information of distributor	Dr. Katerina Vlahos Katerina. Vlahos@mcri.edu.au
Type of cell lines	iPSC
Origin	Human
Additional origin info required	PB002 (MCRIi002-A) Age: 35 Sex: Female Ethnicity if known: Caucasian PB003 (MCRIi003-A) Age: 56 Sex: Female Ethnicity if known: Caucasian PB007 (MCRIi007-A) Age: 40 Sex: Female Ethnicity if known: Caucasian PB008 (MCRIi008-A) Age: 38 Sex: Male Ethnicity if known: Caucasian PB009 (MCRIi009-A) Age: 51 Sex: Male Ethnicity if known: Caucasian
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal, manually isolated
Method of reprogramming	Transgene free Sendai virus
Genetic Modification	NO
Type of Genetic Modification	n/a
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	n/a
Gene/locus	n/a
Date archived/stock date	May 2016
Cell line repository/bank	https://hpscreg.eu/cell-line/MCRIi002-A https://hpscreg.eu/cell-line/MCRIi003-A https://hpscreg.eu/cell-line/MCRIi007-A https://hpscreg.eu/cell-line/MCRIi008-A https://hpscreg.eu/cell-line/

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https://doi.org/10.1016/j.scr.2023.103109
Received 3 April 2023; Accepted 1 May 2023
Available online 3 May 2023

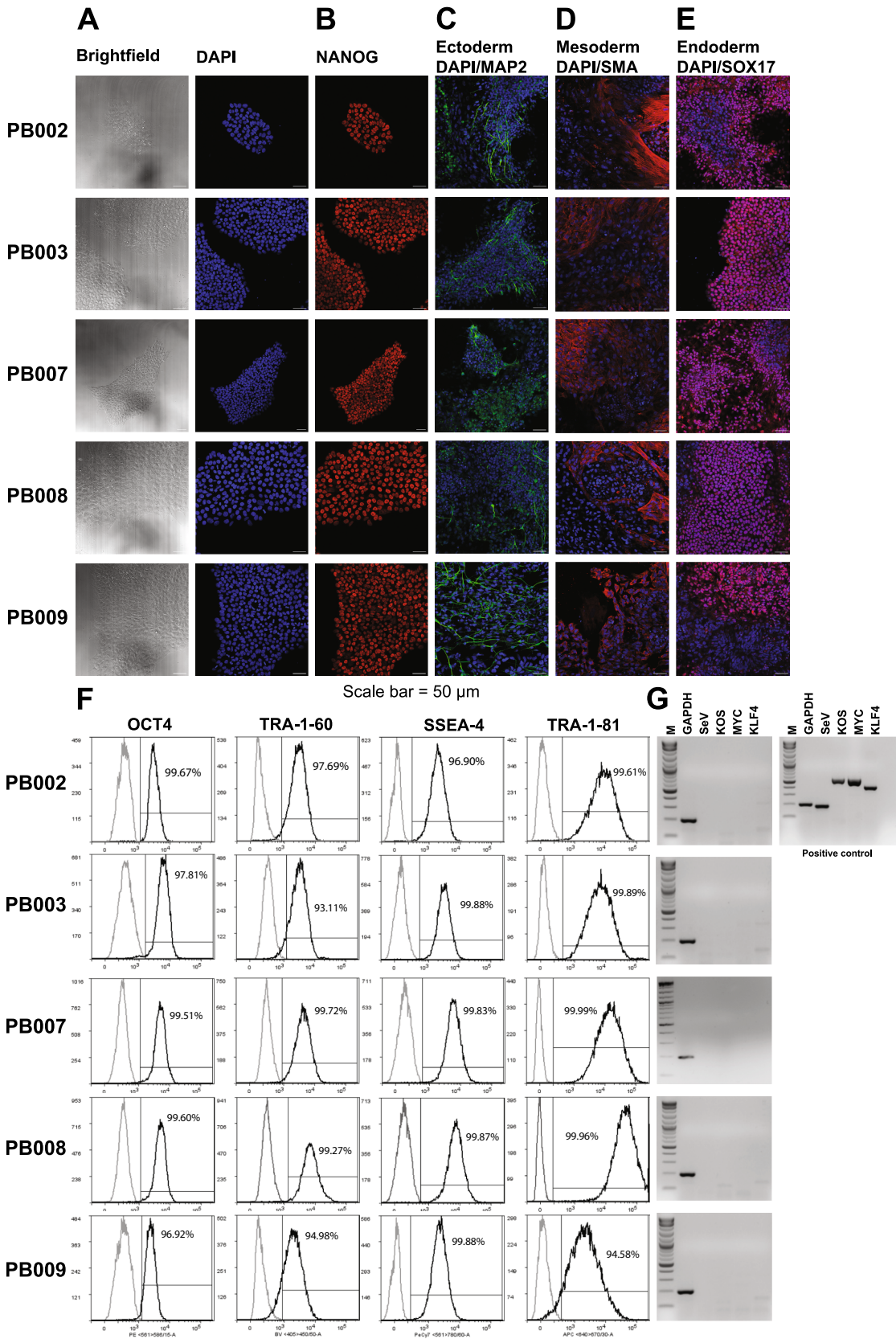
A
Brightfield
DAPI
B
NANOG
C
Ectoderm
DAPI/MAP2
D
Mesoderm
DAPI/SMA
E
Endoderm
DAPI/SOX17
PB002
PB003
PB007
PB008
PB009
Scale bar = 50 μm
F
OCT4
TRA-1-60
SSEA-4
TRA-1-81
99.67%
97.69%
96.90%
99.61%
97.81%
93.11%
99.88%
99.89%
99.51%
99.72%
99.83%
99.99%
99.60%
99.27%
99.87%
99.96%
96.92%
94.98%
99.88%
94.58%
G
M
GAPDH
SeV
KOS
MYC
KLF4
Positive control

Fig. 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis (Immunofluorescence)	Nanog	Fig. 1B
	Quantitative analysis (Flow Cytometry)	Oct3/4: >96% Tra 1–60: >93% SSEA-4: >96% Tra 1–81: >94%	Fig. 1F
Genotype	Karyotype (SNP array) and resolution	PB002 (MCRIi002-A): arr(1–22,X)x2 PB003 (MCRIi003-A): arr(1–22,X)x2 PB007 (MCRIi007-A): arr(1–22,X)x2 PB008 (MCRIi008-A): arr(1–22)x2,(XY)x1 PB009 (MCRIi009-A): arr(1–22)x2,(XY)x1 Resolution 0.50 Mb	Supplementary A
Identity	Genetic analysis	SNPduo comparative analysis performed to compare parental and both derived clones	Supplementary A
		Identical genotypes (>99.9%) for the entire genome, indicating derived cell lines are from the same individual	Supplementary A
Mutation analysis (IF APPLICABLE)	Sequencing	n/a	–
	Southern Blot OR WGS	n/a	–
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR. Negative	Supplementary A
Differentiation potential	Embryoid body formation (Immunofluorescence)	Expression of genes in embryoid bodies: smooth muscle actin, microtubule-associated protein 2, SRY-box transcription factor 17	Fig. 1 panel C, D and E
List of recommended germ layer markers	Expression of these markers demonstrated at protein (IF) levels	Ectoderm: MAP2 Endoderm: SOX17 Mesoderm: A-SMA	IF with specific antibodies (Table 2)
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Data with author
Genotype additional info (OPTIONAL)	Blood group genotyping	n/a	–
	HLA tissue typing	n/a	–

(*continued*)

	MCRIi009-A
Ethical approval	RCH Human Research Ethics Committee 35121A

1. Resource utility

Integration-free induced pluripotent stem cell (iPSC) lines derived from the blood of healthy adult individuals can serve as controls for drug development and disease modelling studies. Further gene-editing extends their utility as isogenic control iPSC lines.

2. Resource details

The cell lines described herein were generated concurrently with previously published iPSCs (Vlahos et al., 2019) which have since been distributed and used by the wider scientific community (Sim et al., 2021). Blood samples were collected from healthy adult individuals and peripheral blood mononuclear cells (PBMCs) were isolated. Five human iPSC lines were derived from PBMCs using Sendai virus carrying the reprogramming factors POU5F1 (OCT4), SOX2, KLF4 and MYC. 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 NANOG was verified by immunofluorescence staining and microscopy (Fig. 1B) whereas flow cytometry was used to assess expression of POU5F1 (OCT4), TRA-1–60, TRA1-81 and SSEA-4. Over 90% of viable cells analysed expressed POU5F1 (OCT4), TRA-1–60, TRA1-81 and SSEA-4 (Fig. 1F). The Sendai virus is non-integrating and loss of the reprogramming genes from the cells was confirmed by RT-PCR analysis after 12 passages (Fig. 1G). All iPSC lines were able to form embryoid bodies *in vitro*, which expressed markers consistent with development of all three germ layers. Specifically, immunofluorescence staining identified the ectodermal marker MAP2 (Fig. 1C), the mesodermal marker SMA (Fig. 1D) and the endodermal marker SOX17 (Fig. 1E). In addition, all iPSC lines showed a normal molecular karyotype (with 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 (Supplementary A). Furthermore, all iPSC lines were mycoplasma free. All characterisation and validation analyses are summarised in Table 1.

3. 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 g for 10 min, transferred to a fresh tube, washed with PBS + 2% FBS and centrifuged at 300 g for 10 min.

iPSC generation: PBMCs were cultured for 7–8 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 supplemented with 50 ng/mL of FGF2 (Costa et al., 2007). iPSC colonies were mechanically isolated for further expansion as described previously (Costa et al., 2007). Once established, iPSC lines were adapted to feeder

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	PE anti-human Oct3/4 antibody	1:100	Thermo Fisher Scientific Cat# 12–5841-80	RRID: AB_914364
Pluripotency Markers	BV421 anti-human TRA-1–60 antibody	1:100	BD Biosciences Cat# 562,711	RRID: AB_2737738
Pluripotency Markers	PE/Cy7 anti-human SSEA-4 antibody	1:100	BioLegend Cat# 330,420	RRID: AB_2629631
Pluripotency Markers	Alexa Fluor 647 anti-human TRA-1–81 antibody	1:100	BioLegend Cat# 330,706	RRID: AB_1089242
Pluripotency Markers	Rabbit anti-human Nanog monoclonal antibody	1:200	Cell Signaling Technology Cat# 4903	RRID: AB_10559205
Differentiation Markers	Monoclonal Mouse Anti Human Smooth Muscle Actin antibody	1:25	Agilent Cat# M0851	RRID: AB_2223500
Differentiation Markers	MAP2 antibody - Neuronal Marker	1:5000	Abcam Cat# ab5392	RRID: AB_2138153
Differentiation Markers	Human SOX17 Affinity Purified Polyclonal Ab antibody	1:100	R and D Systems Cat# AF1924	RRID: AB_355060
Secondary antibodies	Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:1000	Thermo Fisher Scientific Cat# A10042	RRID: AB_2534017
Secondary antibodies	Donkey Anti-Mouse IgG (H + L) Polyclonal Antibody, Alexa Fluor 647	1:1000	Molecular Probes Cat# A-31571	RRID: AB_162542
Secondary antibodies	Goat Anti-Chicken IgG (H + L) Antibody, Alexa Fluor 488	1:1000	Molecular Probes Cat# A-11039	RRID: AB_142924
Secondary antibodies	Donkey anti-Goat IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 633	1:1000	Thermo Fisher Scientific Cat# A-21082	RRID: AB_2535739

Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Sendai Reprogramming Vector (RT-PCR)	SeV	181 bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
Sendai Reprogramming Vector (RT-PCR)	KOS (KLF4, OCT3/4, SOX2)	528 bp	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG
Sendai Reprogramming Vector (RT-PCR)	KLF4	410 bp	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA
Sendai Reprogramming Vector (RT-PCR)	MYC	532 bp	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG
House-Keeping Gene (RT-PCR)	GAPDH	197 bp	GGAGCGAGATCCCTCCAAAAT/ GGCTGTTGTCATACTTCTCATGG

free culture on plates coated with Matrigel (Corning) in Essential 8 (E8) medium (ThermoFisher Scientific).

Detection of Sendai virus genome and transgenes: After 12 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 according to the manufacturer's instructions (Table 2). Positive control RNA was derived from cells immediately post Sendai virus transduction.

Embryoid Body (EB) formation: Differentiation of iPSCs into germ layer-specific cells was performed using the EB-based method (Ng et al., 2008). Breifly, iPSCs were seeded in ultra-low adherence 96 well plates and cultured in EB medium containing E8 media (ThermoFisher Scientific) and 0.5% polyvinyl alcohol (PVA) (Sigma). After 24 h, the cells were cultured in E8 medium and media was changed every 2 days for 2 weeks. After 2 weeks, the EBs were plated onto Matrigel-coated glass-bottom plate and cultured in E8 medium for 2 weeks.

Immunofluorescence (IF): 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 at 4 °C overnight, followed by secondary antibodies for 2 h at room temperature (Table 2). Subsequently, nuclei were stained with DAPI (Vector-Labs) and images were captured with an LSM 780 confocal microscope and analysed using Zen Black software or an Axio Observer.Z1 microscope with an Axiocam 506 mono camera using Zen Blue software (Carl-Zeiss).

Flow Cytometry analysis: Dissociated iPSCs were incubated with directly conjugated antibodies for 30 min on ice (Table 2). Cells were fixed/permeabilized prior to intracellular Oct4 staining using eBioscience Transcription Factor Staining Buffer Set (ThermoFisher Scientific). Samples were analysed using an LSR Fortessa (BD Bioscience).

Molecular karyotype analysis: Karyotypes were analysed using the Infinium CoreExome-24 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 (https://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) (Supplementary A).

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.

Data availability

Data will be made available on request.

Acknowledgements

The Murdoch Children's Research Institute iPSC Core Facility was funded by a generous donation from the Stafford Fox Medical Research Foundation and is currently supported by Phenomics Australia (PA), supported by the Australian Government through the National Collaborative Research Infrastructure Strategy program. The facility is also supported by the Novo Nordisk Foundation reNEW Centre for Stem Cell Medicine (NNF21CC0073729).

This study was funded in part by Australian National Health & Medical Research Council research fellowships awarded to A.G.E. (GNT1117596) and E.G.S. (GNT1079004). Additional infrastructure funding to the Murdoch Children's Research Institute was provided by the Australian Government National Health and Medical Research Council Independent Institute Infrastructure Support Scheme and the Victorian Government's Operational Infrastructure Support Program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103109.

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