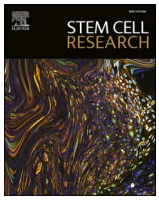
Stem Cell Research 81 (2024) 103606

Contents lists available at ScienceDirect

Stem Cell Research

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



Lab Resource: Single Cell Line

Generation of the CTRL EiPS J9 mR6F-8 iPSC line derived from healthy human outgrowth blood endothelial cells (BOECs) using mRNA reprogramming methodology

Silvia Selvitella [a,b,c], Marta Pérez Franco [a,b,c], Begoña Aran [a,b,c], Anna Veiga [a,b,c], Bernd Kuebler [a,b,c,*]

[a] Barcelona Stem Cell Bank, Spain
[b] Regenerative Medicine Program, Institut d'Investigació Biomèdica, IDIBELL, Spain
[c] P-CMR[C]-Program of Translation of Regenerative Medicine in Catalonia, Spain

A B S T R A C T

mRNA reprogramming is a technology for generating iPSCs with high efficiency and safety. However, it is not suitable for reprogramming non-adherent cells, the primary cell type in blood. An alternative is to obtain adherent cells from blood for this technology. To validate this approach, we generated a human iPSC line from blood outgrowth endothelial cells (BOECs) using mRNA-based reprogramming. The resulting line, CTRL EiPS J9 mR6F-8, meets iPSC criteria, including an undifferentiated state, pluripotency, genome integrity. This line is available upon request.

Resource Table

Unique stem cell line identifier	*ESi111-A/Spanish Stem Cell Bank*
Alternative name(s) of stem cell line	*Ctrl EiPS J9 mR6F-8*
Institution	*Barcelona Stem Cell Bank, Regenerative Medicine Programme, Institute for Biomedical Investigation of Bellvitge (IDIBELL), ĹHospitalet de Llobregat, Barcelona, Spain.*
Contact information of distributor	*aveiga@idibell.cat*
Type of cell line	*iPSC*
Origin	*Human*
Additional origin info required for human ESC or iPSC	*Age: 51* *Sex: Male*
Cell Source	*Blood Outgrowth Endothelial Cells (BOECs)*
Clonality	*Clonal*
Method of reprogramming	*mRNA*
Genetic Modification	*NO*
Type of Genetic Modification	*N/A*

(*continued on next column*)

Resource Table (*continued*)

Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*N/A*
Associated disease	*Healthy*
Gene/locus	N/A
Date archived/ stock date	*2023*
Cell line repository/ bank	*Human Pluripotent Stem Cells Registry* https://hpscreg.eu/cell-line/ESi111-A *Spanish Stem Cell Bank (Banco nacional de lineas celulares (BNLC))* https://www.isciii.es/QueHacemos/Servicios/BIOBANCOS/BNLC/Paginas/LineasiPS.aspx
Ethical approval	*Ethics Committee of Clinical Research of The Center of Regenerative Medicine of Barcelona. Approval number 12/2008/ Catalan Authority Departament of Salut. Meeting of the Committee for Monitoring and Control of the Donation and Use of Human Cells and Tissue. Reference Number 100/83/1*

1. Resource utility

Validation and optimization of the mRNA-based reprogramming

* Corresponding author at: Barcelona Stem Cell Bank, Spain.
E-mail address: bkuebler@idibell.cat (B. Kuebler).

https://doi.org/10.1016/j.scr.2024.103606
Received 12 September 2024; Accepted 30 October 2024
Available online 9 November 2024

technology on blood outgrowth endothelial cells starting from a human whole blood sample to increase the reprogramming options of the Barcelona Stem Cell Bank iPSC generation service.

2. Resource details

Blood outgrowth endothelial cells (BOECs) were derived from a 72 mL whole blood sample obtained from a 51-year-old healthy male after informed consent, following the protocol of Ormiston et al. (2015). Briefly, peripheral blood mononuclear cells (PBMCs) were isolated by density gradient and cultured for 25 days in EGM-2 medium until the appearance of cobblestone colonies. These cells were characterized by immunocytochemistry with antibodies against the endothelial markers Vimentin and VEGFC.

For reprogramming, BOECs were transfected on 8 consecutive days with an RNA mixture of synthetic, non-modified reprogramming factors (OCT4, SOX2, KLF4, cMYC, NANOG, and LIN28), immune evasion mRNA (E3, K3, B18), and mature, double-stranded microRNA clusters from the 302/367 cluster (Poleganov et al., 2015). The first colonies with iPSC appearance began to emerge on day 10. Finally, ten clones were obtained, cultured until the third passage, and later cryopreserved.

One clone was selected for further characterization (Table 1). The morphology of the colonies was the caracteristic one of IPSC line, compacted groups of cells forming well-defined borders (Fig. 1.A). The complete characterization included karyotyping, evaluation of the undifferentiated state, three lineage differentiation capacity, and STR analysis (Identity). The line obtained was named Ctrl EiPS J9 mR6F-8, presented a normal karyotype (46, XY) (Fig. 1B) and was positive for the markers of undifferentiated state SOX2, NANOG, OCT-4, SSEA-3, SSEA-4, TRA-1–60, and TRA-1–81 in immunocytochemistry assays (Fig. 1.C and D). Furthermore, it demonstrated the capacity to differentiate into the three germ layers after embryoid body formation and differentiation, showing positive markers for endoderm (AFP and FOXA2), mesoderm (GATA4 and ASMA), and ectoderm (TUJ1 and GFAP)(Fig. 1.E). Additionally, all the STR markers analyzed for the blood sample and the derived IPSC line matched, and it was negative for mycoplasma presence. The characterization results met the ISSCR Standards for Human Stem Cells Use in Research recommendations (ISSCR, 2023). After its characterization, the line was registered in the hPSCreg and in the BNLC in Spain. The line is available for third parties under request.

3. Materials and methods

3.1. Derivation, characterization, and reprogramming of BOECs

Peripheral blood mononuclear cells (PBMCs) were isolated using Lymphocyte-H gradient (Cedarlane) centrifugation. The collected PBMCs were resuspended in EGM-2 Medium, (EBM-2 basal medium (Lonza) supplemented with EGM-2 singleQuots (Lonza)), with 10 % FBS (Hyclone), counted, and seeded in plates previously coated with 50ug/ml rat collagen I (Corning) at a density of 9.53 million cells/cm^2. Colonies of BOECs appeared between days 20 and 25. After reaching 80–90 % confluency, BOECs were passaged using 0.05 % Trypsin/EDTA (ThermoFisher Scientific) and seeded at 7 million cells/cm^2. For characterization, immunocytochemistry was performed with antibodies against Vimentin and VEGFC at passage 2.

BOECs between passages 2 and 4 were seeded on 23 μg/cm^2 Matrigel-coated six-well dishes (Corning) at a density of 7.5×10^4 cells/cm^2. Using a non-modified (NM)-RNA cocktail (StemRNATM 3rd Gen Reprogramming Kit, Stemgent) mixed with Lipofectamine RNAiMAX (Invitrogen), daily transfections were performed for eight days following the manufacturer's instructions. On day 10, the medium was changed to mTeSR-1 (Stemcell Technologies) for maintenance. The first colonies emerged between days 11 and 12. One clone was selected for further characterization.

3.2. Cell culture

iPSCs were maintained in mTeSR-1 medium (Stemcell Technologies) at 37 °C and 5 % CO_2, with daily medium changes. Every 6–7 days, when the cells reached 80 % confluency, they were passaged. Briefly, colonies were disaggregated into clumps by incubation with 0.5 mM EDTA (ThermoFisher) for 2 min at 37ºC and then recovered by flashing. One-sixth of the volume containing the clumps was transferred to a new well previously coated with 23 μg/cm^2 Matrigel containing 2 mL of fresh mTeSR-1 without using any survival promoter.

3.3. Immunocytochemistry

iPSCs growing in slideflasks were fixed in 4 % paraformaldehyde (PFA) (Sigma) for 20 min at room temperature (RT). Three washing steps were performed with 1× TBS for 5 min each, followed by incubation in blocking/permeabilization buffer (1× TBS + 0.5 % Triton + 3 % Donkey Serum and 3 % Goat Serum) for 60 min at RT. Primary antibodies (Table 2) were diluted with TBS++ (1X TBS + 0.1 % Triton + 3

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	*Normal*	Fig. 1 *Panel A*
Phenotype	Qualitative analysis-*Immunocytochemistry*	*Positive for undifferentiated state markers: Oct4, Nanog, Sox2, Tra 1–60, SSEA-3, SSEA-4, Tra-1–80.*	Fig. 1 *panel D*
	Quantitative analysis: *Mean Fluorescence Intensity*	*Expression of undifferentiated state markers: Oct4, Nanog, Sox2, Tra 1–60, SSEA-3, SSEA-4, Tra-1–80.*	Fig. 1 *panel C*
Genotype	Karyotype (G-banding) and resolution	*46XY, Resolution: 500*	Fig. 1 *panel B*
Identity	STR analysis	*STR Profiling was performed. 16 sites tested, all matched.*	*Submitted in archive with journal*
Mutation analysis (IF APPLICABLE)	Sequencing	*N/A*	
	Southern Blot OR WGS	*N/A*	
Microbiology and virology	Mycoplasma	*Mycoplasma testing by RT-PCR. Negative*	
Differentiation potential	Embryoid body formation	*Three germ layers formation. All markers were positive.*	Fig. 1 *panel E*
List of recommended germ layer markers	**Expression of these markers has to be demonstrated at protein levels**	*Ectoderm: TUJ1 and GFAP. Endoderm: FOXA2 and AFP. Mesoderm: ASMA and GATA4.*	Fig. 1 *panel E*
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*	

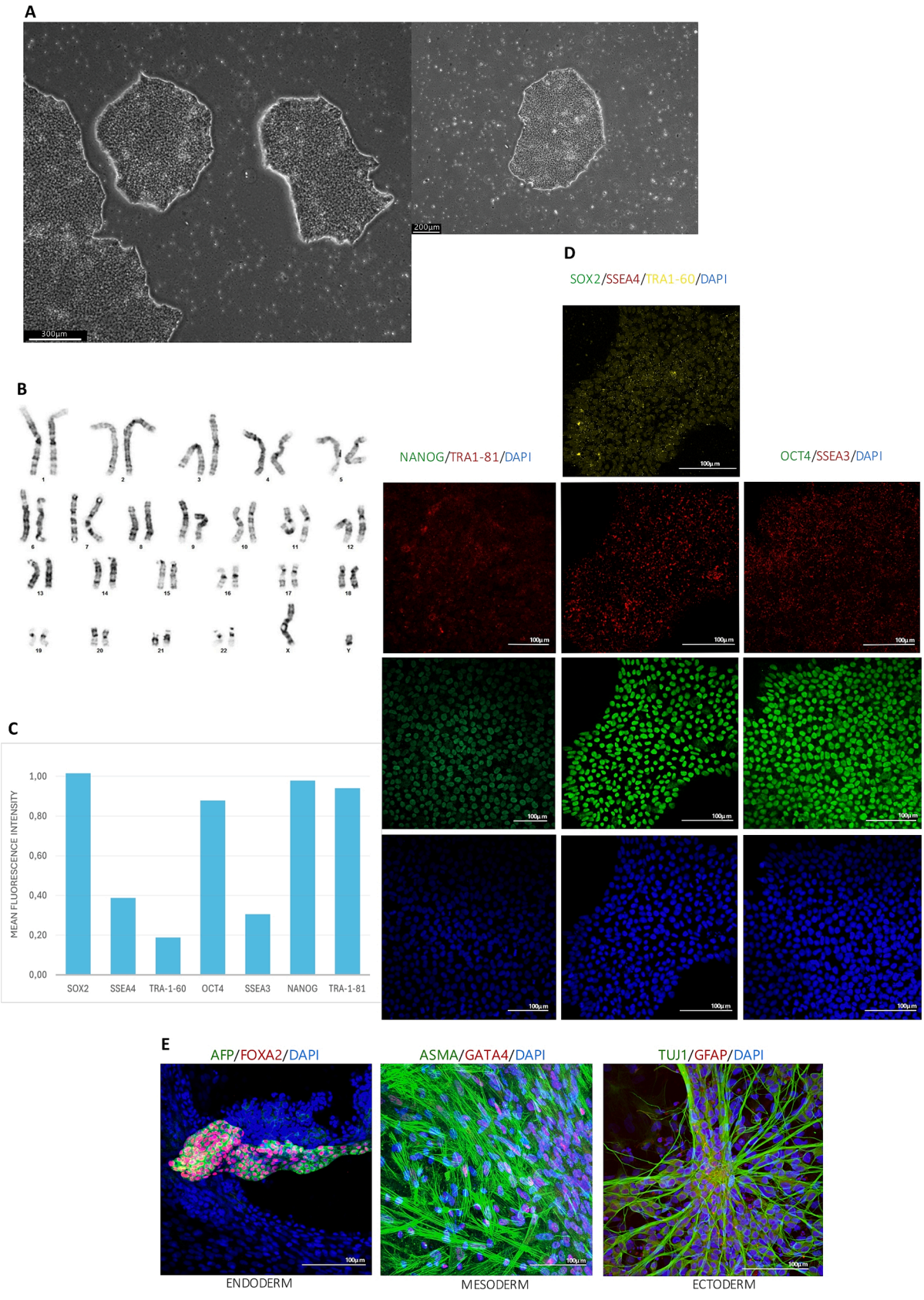

A
300µm
200µm
B
C
MEAN FLUORESCENCE INTENSITY
1,00
0,80
0,60
0,40
0,20
0,00
SOX2
SSEA4
TRA-1-60
OCT4
SSEA3
NANOG
TRA-1-81
D
SOX2/SSEA4/TRA1-60/DAPI
NANOG/TRA1-81/DAPI
OCT4/SSEA3/DAPI
100µm
E
AFP/FOXA2/DAPI
ASMA/GATA4/DAPI
TUJ1/GFAP/DAPI
ENDODERM
MESODERM
ECTODERM

Fig. 1.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
BOECs Markers	Mouse anti-Vimentin	1:100	Sigma-Aldrich, V5255	AB_477625
	Mouse anti-VEGFC	1:100	mAbcam, 63221	AB_10676125
Undifferentiate State Markers	*Mouse anti-OCT4*	*1:2*	*Santa Cruz, sc-5279*	*AB_628051*
	Goat anti-Nanog	*1:5*	*R&D Systems, AF1997*	AB_355097
	Rabbit anti-SOX2	*1:100*	*Thermo Fisher, PA1-16968*	AB_2195781
	Rat anti-SSEA3	*1:200*	*Thermo Fisher, MA1-020*	AB_2536682
	Mouse anti-SSEA4	*1:200*	*Thermo Fisher, MA1-021*	AB_2536687
	Mouse anti-TRA-1–60	*1:100*	*Millipore, MAB4360*	AB_10550257
	Mouse anti-TRA-1–81	*1:100*	*Millipore, MAB4381*	AB_177638
Differentiation Markers	*Mouse anti-TUJ1*	*1:500*	Covance, MMS-435P	AB_2313773
	Rabbit anti-GFAP	*1:500*	Agilent, Z0334	AB_10013382
	Mouse anti-ASMA	*1:400*	Sigma-Aldrich, A5228	AB_262054
	Rabbit anti-GATA4	*1:25*	Santa cruz, sc-9053	AB_2247396
	Rabbit anti-AFP	*1:200*	Agilent, A0008	AB_2650473
	Goat anti-FOXA2	*1:50*	R&D Systems, AF2400	AB_2294104
Secondary antibodies	*AF488 goat anti-mouse*	*1:200*	*Jackson, 115-546-071*	*AB_2338865*
	Cy3 goat anti-Rat IgM	*1:200*	*Jackson, 112-165-020*	*AB_2338243*
	Cy2 AF488 donkey anti-rabbit	*1:200*	*Jackson, 711-545-152*	*AB_2313584*
	Cy3 goat anti-mouse IgG	*1:200*	*Jackson, 115-165-071*	AB_2338687
	Cy5 DyLight649 goat anti-mouse	*1:200*	*Jackson, 115-495-075*	AB_2338809
	Cy2 AF488 donkey anti-goat	*1:200*	*Jackson, 705-545-147*	AB_2336933
	Cy3 donkey anti-mouse IgM	*1:200*	*Jackson, 715-165-140*	AB_2340812
	Cy3 donkey anti-goat	*1:200*	*Jackson, 705-165-147*	AB_2307351
	Cy2 AF488 Donkey anti-mouse IgG	*1:200*	*Jackson, 715-545-151*	AB_2341099
	Cy3 donkey anti-rabbit	*1:200*	*Jackson, 711-165-152*	AB_2307443
	Cy3 Goat anti-mouse IgM	*1:200*	*Jackson, 115-165-075*	AB_2338689
	Cy2 Goat anti-mouse IgG	*1:200*	*Jackson, 115-225-071*	AB_2338742

% Donkey Serum and 3 % Goat Serum), added to cells and incubated for 72 h at 4 °C in a humid chamber. After washing three times with TBS++ for 5 min each, cells were incubated with diluted secondary antibodies (Table 2) for 2 h at 37 °C. Nuclei were stained with DAPI (Invitrogen), adding a 1:10000 dilution for 10 min at RT. Images were taken using a Leica TSC SPE/SP5 microscope or Zeiss LSM 980 Airyscan2 inverted confocal microscope.

3.4. Karyotype determination

To evaluate genomic integrity, 20 metaphases were analyzed by G-banding karyotype at passage 8. When cells reached 70 % of confluency in a 100 mm plate, they were treated for 3 h with colcemid (Gibco), then trypsinized, incubated in hypotonic solution(KCl, GIBCO), and fixed in Carnoy solution (75 % methanol, 25 % acetic acid). The sample was maintained at −20 °C until shipment to the Genetic-Cytogenetic lab of Sant Joan de Déu Hospital in Barcelona, Spain, for karyotyping.

3.5. Immunocytochemistry of undifferentiated state and three germ layer differentiation assay

To confirm the undifferentiated state, immunocytochemistry was performed at passage 6, with antibodies against SOX2, OCT4, NANOG, TRA-1–81, TRA-1–60, SSEA3, and SSEA4. Cells were seeded in slide flasks, and after colonies reached approximately 1 mm in diameter, they were fixed for fluorescence staining. For quantification, the mean intensity of the images was analyzed using IMAGE J. The results were normalize by DAPI mean intensity.

To assess pluripotency, two-thirds of a 90 % confluent 100 mm plate at passage 6 were seeded in a 96-well plate not treated for cell-culture by centrifugation at 800g for 10 min. After 24 h in culture, the formed embryoid bodies (EBs) were transferred to a 60 mm bacterial culture plate, and 24 h later the EBs were seeded in Matrigel-coated slide flasks containing differentiation media for the three germ layers: Ectoderm (N2/B27 medium: 50 % DMEM-F12, 50 % Neurobasal, 1 % N2, 0.5 % B27, 1 % Glutamax, 1 % Penicillin-Streptomycin), Endoderm (EB medium: Knockout DMEM (GIBCO), 10 % FBS, 1 % NEAA, 1 % Glutamax, 0.1 % β-mercaptoethanol, 1 % Penicillin-Streptomycin), Mesoderm (EB medium supplemented with 0.5 mM ascorbic acid). The medium was changed every 48 h for 3 weeks for Endoderm and Mesoderm, and 4 weeks for Ectoderm, and incubated at 37 °C and 5 % CO_2. To analyze differentiation, immunocytochemistry was performed using antibodies against TUJ1 and GFAP for ectoderm, AFP and FOXA2 for endoderm, and GATA4 and ASMA for mesoderm.

3.6. Authentication and mycoplasma testing

To confirm identity, genomic DNA was extracted from the blood sample and the generated IPSC line and a STR analysis was performed on both. 20 markers were used and analyzed for matching. At passage 5, cultures were tested for mycoplasma contamination by PCR using the Venor®GeM Classic kit (Minerva Biolabs) and they were negative.

CRediT authorship contribution statement

Silvia Selvitella: . **Marta Pérez Franco:** Investigation, Methodology, Writing – review & editing. **Begoña Aran:** Project administration, Supervision, Writing – review & editing. **Anna Veiga:** Project administration, Resources, Supervision, Writing – review & editing. **Bernd Kuebler:** Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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.

Acknowledgements

This study has been funded by Instituto de Salud Carlos III (ISCIII), Spain; through the project PT23/00092. Co-funded by the European Union. We thank CERCA Programme, Spain / Generalitat de Catalunya, Spain; for institutional support.

References

Ormiston, M.L., Toshner, M.R., Kiskin, F.N., Huang, C.J., Groves, E., Morrell, N.W., Rana, A.A., 2015. Generation and culture of blood outgrowth endothelial cells from human peripheral blood. J. Visualized Exp. 106.

Poleganov, M.A., Eminli, S., Beissert, T., Herz, S., Moon, J.I., Goldmann, J., Sahin, U., 2015. Efficient reprogramming of human fibroblasts and blood-derived endothelial progenitor cells using nonmodified RNA for reprogramming and immune evasion. Human Gene Ther. 26 (11), 751–766.

The International Society for Stem Cell Research (ISSCR), 2023. Standards for Human Stem Cell Use in Research. https://isscr.org/standards-document.