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Lab Resource: Stem Cell Line

Generation of human erythroblast-derived iPSC line using episomal reprogramming system

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ABSTRACT

Peripheral blood mononuclear cells were isolated and cultured to a pure pro-EBL population and reprogrammed using episomal plasmids. The pluripotency of transgene-free induced pluripotent stem cell (iPSC) line was verified by the expression of pluripotency-associated markers and by *in vitro* spontaneous differentiation towards the 3 germ layers. The iPSC line showed normal karyotype. Peripheral blood is a non-invasive easy accessible cell source and combined with EBL outgrowth *in vitro*, a routine process obtaining sufficient amount of homogenous cells can be obtained within a week. Using episomal delivery, pro-EBLs can be reprogrammed in a transgene-free, cost effective system.

Resource Table

Unique stem cell line identifier	SANi003-A
Alternative name(s) of stem cell line	MEE630.cl5
Institution	Sanquin, Amsterdam, The Netherlands
Contact information of distributor	e.vandenakker@sanquin.nl
Type of cell line	iPSC
Origin	peripheral blood (mobilized) mononuclear cells-derived EBL
Additional origin info	Age: 51 Sex: Female
Cell source	Erythroblast
Method of reprogramming	Episomal, Oct4, Klf4, Sox2, c-Myc, Lin28
Genetic modification	NO
Type of modification	–
Associated disease	–
Gene/locus	–
Method of modification	–
Name of transgene or resistance	–
Inducible/constitutive system	–
Date archived/stock date	November 2015
Cell line repository/bank	–
Ethical approval	Informed consent was given in accordance with the Declaration of Helsinki and Dutch national and Sanquin internal ethic boards.

Resource utility

Besides normal iPSC applications, SANi003-A may also be useful to study the persistent somatic epigenetic marks after re-programming in conjunction with iPSC from other somatic origin.

Resource details

Peripheral blood was collected from a healthy donor and a pure CD71^{high}/CD235a^{low} erythroblast population was expanded and cultured (van den Akker et al., 2010; Heideveld et al., 2015) (Fig. 1A).

To generate episomal iPSC lines two plasmids pEB-C5 (Chou et al., 2011, # 28213) and pEB-Tg (Chou et al., 2011, # 28220) were nucleofected into EBLs. iPSC-like colonies started to appear on day 9–10 and were individually picked 20–26 days post-transfection. Eight stable lines were maintained and stored while two lines were further analyzed at each passage for their episomal plasmid elimination. Both iPSC lines were transgene-free by passage 4 confirmed with transgene-specific PCR (Fig. 1B) and SANi003-A line was chosen for further pluripotency characterization (Fig. 1C, scale bar 400 μm).

The pluripotency of transgene-free SANi003-A line was confirmed by alkaline phosphatase staining (Fig. 1E, scale bar 400 μm) and by FACS for SSEA4, OCT4, SOX2, TRA-1-60 and TRA-1-81 markers (Fig. 1D).

Karyotype analysis of the SANi003-A iPSC line was performed using COBRA-FISH, proving normal diploid 46, XY karyotype, without any detectable abnormalities (Fig. 1F).

The *in vitro* spontaneous differentiation potential of the iPSC line towards the three germ layers was demonstrated by the expression of ectodermal (βIII-TUBULIN), mesodermal (BRACHYURY) and endodermal

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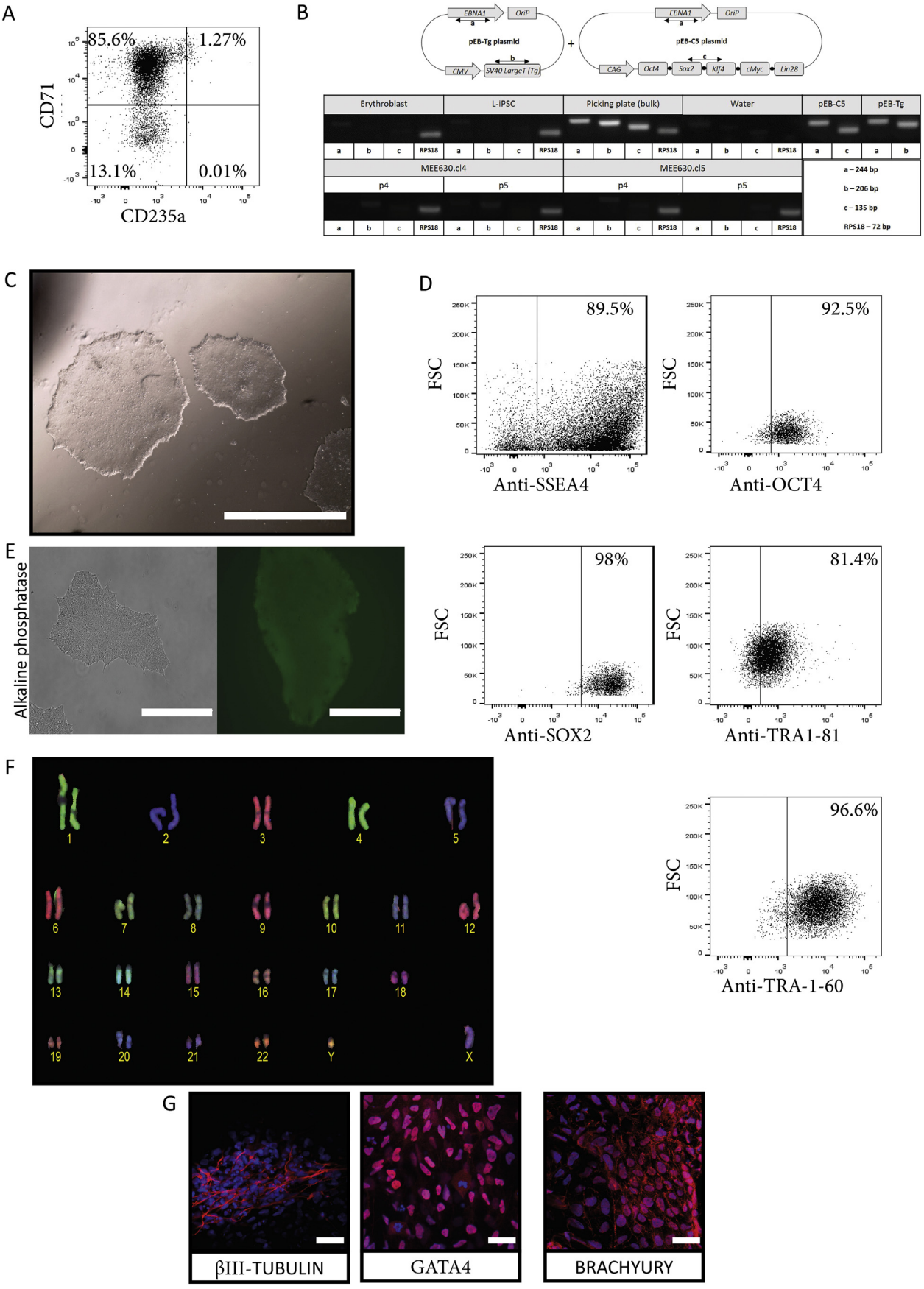

Fig. 1. Characterization of SANi003-A iPSC line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 C
Phenotype	Immunocytochemisty	Alkaline phosphatase positive	Fig. 1 E
	Flow cytometry	OCT3/4: 92.5% TRA-1-81: 81.4% TRA-1-60: 96.6% SSEA-4: 89.5% SOX2: 98%	Fig. 1 D
Genotype	COBRA-FISH	46XY, 5–8 Mb resolution	Fig. 1 F
Identity	Microsatellite PCR (mPCR)	Not performed	
	STR analysis	Performed on iPSC	
Mutation analysis	Sequencing	–	
	Southern Blot OR WGS	–	
Microbiology and virology	Mycoplasma	Negative by MycoAlert Mycoplasma detection kit (Lonza # LT07-118)	not shown but available with author
Differentiation potential	Embryoid body formation	Embryoid body formation to all three germ layers	Fig. 1G
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	not shown but available with author
Genotype additional info	Blood group genotyping	MLPA	not shown but available with author
	HLA tissue typing	Unknown	

(GATA4) markers (Fig. 1G, blue dapi, red corresponding markers, scale bar 40 μm) using Immunocytochemistry staining.

Materials and methods

Experimental procedures

All chemicals were purchased from Sigma-Aldrich (Munich, Germany) and culture reagents from Thermo Fisher Scientific (Waltham, Massachusetts, USA), unless otherwise specified.

Cell culture

Cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2. The iPSCs were cultured on Matrigel (BD Biosciences) in essential-8 medium (E8).

Isolation of primary cell source and reprogramming of EBLs

Peripheral blood (mobilized)-derived EBLs were cultured as described previously (van den Akker et al., 2010). Briefly, mononuclear cells were isolated by ficoll gradient and cultured in expansion medium. The emergence of pure pro-EBL population was confirmed by FACS and 2×10^6 EBLs were nucleofected with 8 μg pEB-C5 (Chou et al., 2011, # 28213) and 2 μg pEB-Tg (Chou et al., 2011, # 28220) plasmids using the Human CD34 cell nucleofector Kit (Lonza). Two days post-transfection the cells were seeded onto irrMEF in expansion medium supplemented with 50 μg/ml Ascorbic Acid (0.5×10^6 EBLs/60 mm dish). From day 5 post-transfection half amount of media was changed to HESC-medium (DMEM/F12, 20% Knockout Serum Replacement, 1% 100× MEM Non-Essential Amino Acid, 0.1 mM β-me, 0.5% P/S, 10 ng/ml bFGF) + 50 μg/ml Ascorbic Acid. From day 7 onward culture was fully changed to HESC-medium + 50 μg/ml Ascorbic Acid. The appeared ES-like colonies were manually picked (Day 20–26) and cultured as iPSCs thereafter (Table 1).

Genomic PCR

gDNA was isolated by DNeasy Blood & Tissue Kit (Qiagen). Transgene-specific genomic PCR was used to confirm the elimination of episomal plasmids from the iPSCs. PCR primers are listed in Table 2. PCR reactions were performed using GoTaq G2 Hot Start Green Master Mix (Promega). Positive controls: gDNA from picking plate, plasmid DNA. Negative control: gDNA from EBL, gDNA from Lentiviral iPSC (L-iPSC). Housekeeping: RPS18.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Differentiation markers	Mouse Neuronal Class III B-TUBULIN	1:2000	Covanc, # MMS-435P, AB_2313773
	Rabbit anti-Brachyury	1:50	Santa Cruz, #sc-20,109, AB_2255702
	Mouse anti-GATA4	1:50	Santa Cruz, #sc-25,310, AB_627667
	Alkaline phosphates	1:500	Thermo Fisher Scientific, #A14353, No RRID
Secondary antibodies	Donkey anti-Mouse IgG (H + L) Secondary Antibody	1:2000	Thermo Fisher Scientific, A-21202, AB_141607
	Goat anti-Rabbit IgG (H+L) Secondary Antibody	1:2000	Thermo Fisher Scientific, A-11008, AB_143165
Pluripotency markers flow cytometry	TRA-1-81	1:100	Stem Cell Technologies, # 60065AZ.1, AB_1118559
	TRA-1-60	1:100	Stem Cell Technologies, # 60064AD, AB_1118557
	OCT4	1:10	R&D Systems, IC1759A-100, AB_1152112
	SOX2	1:10	R&D Systems, IC2018P-100, AB_357273
	SSEA4	1:10	R&D Systems, FAB1435C, AB_1208043
Erythroid-specific markers	CD71	1:200	MACS, 130-091-727, AB_615103
	CD235a	1:200	DAKO, F087001, AB_578691

Primers		
	Target	Forward/Reverse primer (5′-3′)
Episomal plasmid (a) (genomic PCR)	Epstein-Barr virus nuclear antigen 1 (EBNA1)	TTTAATACGATTGAGGGCGTCT/GGTTTTGAAGGATGCGATTAAG
Episomal plasmid (b) (genomic PCR)	SV40 Large T (Tg)	GCCAGGTGGGTTAAAGGAGC/GGTACTTATAGTGGCTGGGCTGT
Episomal plasmid (c) (genomic PCR)	Sox2-klf4	CCATTAACGGCACACTGCCCCTGT/AGGACGGGAGCAGAGCGTCGCTGA
Housekeeping gene (genomic PCR)	RPS18	GGACAACAAGCTCCGTGAAGA/CAGAAGTGACGCAGCCCTCTA

Karyotyping

Karyotype analysis was performed using COBRA-FISH as described elsewhere (Szuhai and Tanke, 2006). 20 metaphase spreads for each sample were analyzed.

In vitro spontaneous differentiation

iPSC cells were harvested using ReLeSR (Stem Cell Technologies) and colony clumps were transferred to an ultra-low attachment dish (Corning) in E8 medium. On day 5 embryoid bodies (EB) were plated on 0.1% gelatin coated coverslips in a 24 wells plate and medium was changed to; 1% Pen/Strep, 20% FBS, 1% 100× MEM Non-essential amino acid solution, 0.1 mM β-mercaptoethanol in DMEM. On day 14 EB were fixed with 4% PFA before staining for all three germ layers (Table 2).

Immunocytochemistry staining

The expression of specific pluripotency and germ layer markers were analyzed using immunocytochemistry staining. The antibodies and applied dilutions are listed in Table 2. Cells were imaged using a LSM510 confocal with META detector (Carl Zeiss) and figures generated with Zen black edition microscope software (Carl Zeiss).

Alkaline phosphatase staining

Alkaline phosphatase staining was performed by alkaline phosphatase live stain (AP) (Thermo Fisher Scientific) according to manufacturer's instructions. Pictures were taken with EVOS-FL (Thermo Fisher Scientific).

Flow cytometry

iPSC single cell suspensions were made using TrypLE Select, and stained for pluripotency markers. Marker expression was measured on an LSR-II (BD Bioscience) and analyzed using Flowjo software (Flowjo, Ashland, USA).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2017.10.001.

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