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Generation of the induced pluripotent stem cell line UHOMi002-A from peripheral blood mononuclear cells of a healthy male donor

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ABSTRACT

Human pluripotent stem cells (hiPSC) are highly valuable tools to model lung development and chronic bronchial diseases. We generated a hiPSC line from a highly characterized 40-year-old healthy male nonsmoking donor. Peripheral blood mononuclear cells (PBMCs) were reprogrammed using integration-free Sendai Virus. The cell line had normal karyotype, expressed pluripotency hallmarks, and differentiated into the three primary germ layers. The reported UHOMi002-A iPSC line may be used as a control to model lung development, study human chronic bronchial diseases and drug testing.

1. Resource table

Unique stem cell line identifier	UHOMi002-A
Alternative name(s) of stem cell line	Hy03
Institution	Institute for Regenerative Medicine & Biotherapy (IRMB)
Contact information of distributor	John De Vos john.devos@inserm.fr
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 40 Sex: Male
Cell Source	Peripheral Blood Mononuclear Cells (PBMCs)
Clonality	Clonal
Method of reprogramming	hOCT4, hSOX2, hC-MYC, hKLF4 (CytoTune™-iPS 2.0 Sendai Reprogramming Kit – Invitrogen, Thermo Fisher Scientific Inc.)
Genetic Modification	NO
Type of Modification	None
Associated disease	Healthy control
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
	2018-02-12

Date archived/stock date	
Cell line repository/bank	N/A
Ethical approval	The study was approved by the regional scientific ethical committee (CPP Sud Med IV) under the number ID-RCB: 2013-A00892-43/CILIPS, Promoter University Hospital of Montpellier) and informed consent was obtained from the patient.

2. Resource utility

UHOMi002-A iPSC line was generated from a healthy non-smoker donor. This cell line may serve as a control with other cell lines derived from patients with chronic lung disease, for lung disease modelling and drug screening.

3. Resource details

In this study, peripheral blood mononuclear cells (PBMCs) were obtained from a 40-year-old non-smoker healthy man. He was strictly asymptomatic and had a normal lung function.

To generate the UHOMi002-A, PBMCs were isolated by standard Ficoll gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare)

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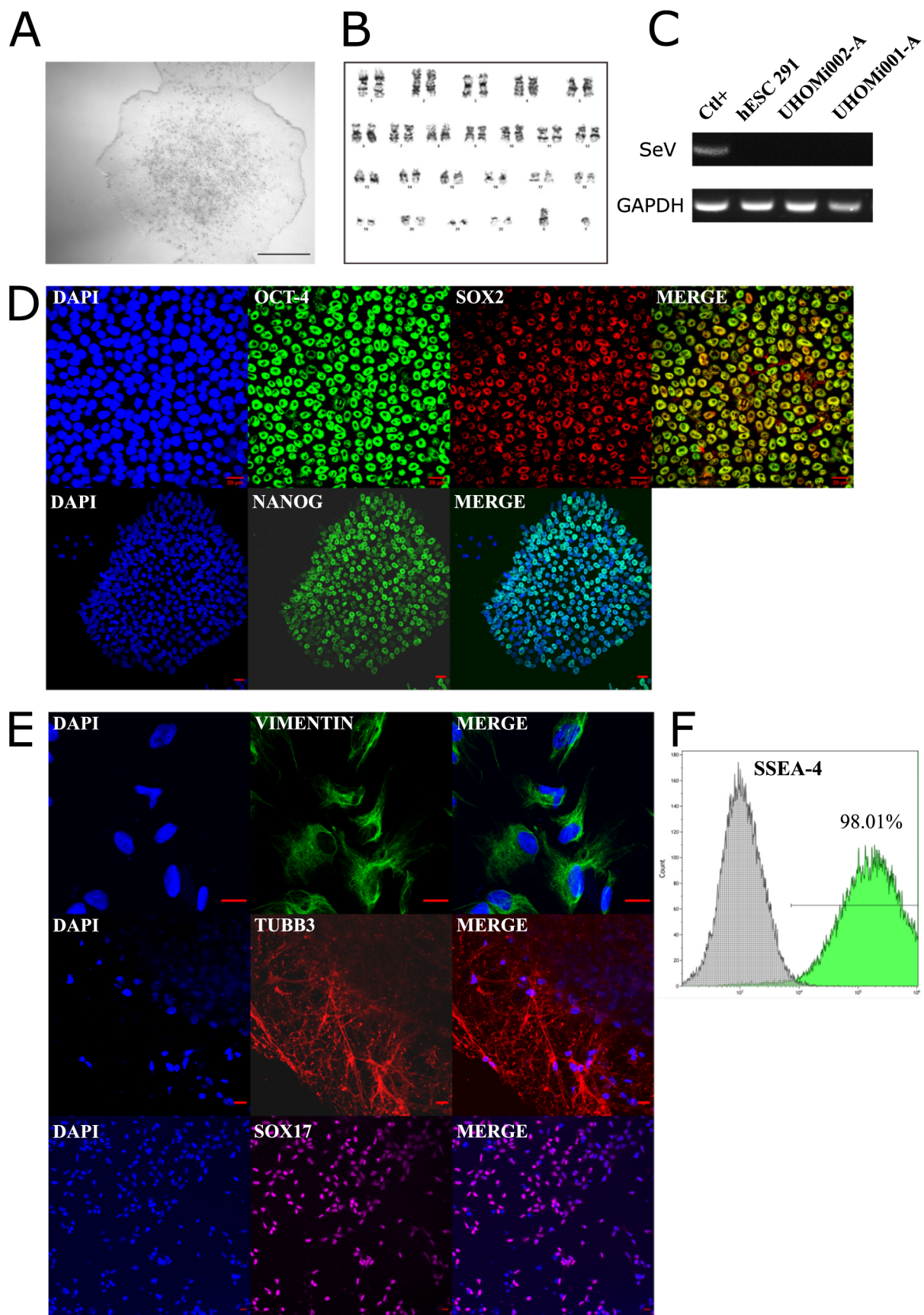


Fig. 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology Phenotype	Photography	Normal	Fig. 1 panel A
	Immunocytochemistry	Expression of pluripotency markers: OCT4, NANOG, SOX2	Fig. 1 panel D
	Flow cytometry	Positive for antigen levels & cell surface markers. SSEA-4: 98.01%	Fig. 1 panel F
Genotype	Karyotype (G-banding) and resolution	46XY, (G banding & R banding) Resolution 400–500	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR)	not performed	N. A
	STR analysis	Specific 18 loci tested, 17/18 matched between iPSC cell line and patient derived PBMCs	available with the authors
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplemental Table 1
Differentiation potential	Embryoid body formation	Embryoid bodies show the three germ layers formation: ectoderm: β III-tubulin (TUBB3); mesoderm: vimentin marker; endoderm: SRY-box transcription factor 17 (SOX17)	Fig. 1 panel E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	not shown but available with the authors
Genotype additional info (OPTIONAL)	Blood group genotyping	N.A.	N. A
	HLA tissue typing	N. A	N. A

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry		Dilution	Company Cat # and RRID
	Antibody		
Pluripotency Markers	Rabbit polyclonal anti SOX2 Rabbit Polyclonal anti OCT4 Rabbit polyclonal anti NANOG PerCP Mouse monoclonal SSEA4 Mouse IgG3 PerCP Isotype Control (Clone 133316) antibody	1:50	Santa Cruz Technology Cat# sc-20088 RRID AB_2255358 Santa Cruz
		1:400	Technology Cat# RRID AB_2167703 Abcam Cat# ab80892 RRID
		1:250	AB_2150114 Bd pharmlingen Cat# 561565 RRID AB_10894210 R&D
		1:250	Cat#C007C RRID AB_1207939
		1:250	
Differentiation Markers	Polyclonal goat anti SOX17 Polyclonal goat anti Vimentin Mouse anti β III-tubulin	1:150	R&D Systemsaf1924 Santa Cruz Biotechnology Cat# sc-7557, RRID:
		1: 50	AB_793998 Covance Research Products Inc. Cat# MMS-435P, RRID:
		1:1000	AB_2313773
Secondary antibodies	donkey anti mouse Alexa fluor 555 donkey anti rabbit Alexa fluor 488 donkey anti goat Alexa fluor 647	1:2000	Thermo Fisher Scientific Cat# A-31570, RRID: AB_2536180 Thermo
		1:2000	Fisher Scientific Cat# A-21206, RRID: AB_2535792 Thermo Fisher
		1:2000	Scientific Cat# A-21206, RRID: AB_2535862
Primers			
	Target	Forward/Reverse primer (5'-3')	
Virus clearance (qPCR)	SeV product size: 181 bp	GGATCACTAGGTGATATCGAGC ACCAGACAAGAG TTTAAGAGATATGTATC	
House-Keeping Genes	GAPDH (PCR) product size: 300 bp	CTG GCG TCT TCA CCA CCA TGG CAT CAC GCC ACA GTT TCC CGG	

(Visser et al., 1988) and carefully washed with PBS plus fetal bovine serum (FBS) 10%. PBMCs were cultured in StemSpan supplemented with Erythroid Expansion Medium (SSEM, Stemcell Technologies), according to the manufacturer's instructions. Erythroid differentiation leads to erythroid progenitor cells expansion, as confirmed by flow cytometry with anti-CD36 and anti-CD71 antibodies between day 7 to day 10. The four reprogramming factors OCT3/4, SOX2, KLF4, and C-MYC were transiently expressed in the erythroid progenitor cells using the integration-free Sendai virus gene-delivery method. UHOMi002-A had a normal morphology (Fig. 1, panel A, bright field microscopy; scale bar: 50 μ m) and had structurally and numerically normal diploid karyotype (46, XY), (Fig. 1, panel B). The generated iPSC line was tested for the absence of exogenous reprogramming vector which was analysed by PCR using Sendai virus vector (SeV) specific primers. After about 10 passages, the elimination of the reprogramming vector SeV was confirmed in the iPSC line using PCR, which was selected for further analysis (Fig. 1C, third panel, UHOMi002-A). The viral sequence was also absent in the negative non transduced human embryonic stem cells hESC 291 and in the previously published hiPSC cell line UHOMi001-A (Ahmed et al., 2018) (Fig. 1C, panel 2 and 4 respectively) but present in positive control.

Expression of pluripotency markers was evidenced by immunocytochemistry staining, using antibodies against human OCT3/4, SOX2 and NANOG (Fig. 1, D scale bar 20 μ m) and by flow cytometry using the cell surface markers SSEA4 (Fig. 1, panel F). The ability of

UHOMi002-A to differentiate spontaneously into the three germ layers in vitro was confirmed by embryoid body (EB) formation. EB formation was performed by transferring about 20 iPSC clumps to low-adhesion dishes (Falcon) in E8 medium. After 24 h, EB were suspended in differentiation medium composed of RPMI-1640 medium (Gibco) supplemented with 10% FBS (Gibco) and 1% Penicillin-streptomycin. The medium was changed every two days for 30 days. After this period, the EB were plated on Geltrex-coated dishes and cultured for five days. Cells were then fixed in 4% paraformaldehyde for immunofluorescence analysis. They expressed ectodermal (β -III-tubulin), mesodermal (vimentin) and endodermal proteins (SOX17) (Fig. 1, panel E).

Microsatellite analysis showed matching profiles of short tandem repeats (STR) amplified from a genomic DNA UHOMi002-A iPSC cells and patient-derived PBMCs (matching of 17/18 loci: TAF9, D21S11, D21S1270, D13S634, D18S391, D13S742, AMXY, D21S1411N, D13S305, D21S213N, D18S1112N, D13S628, DY23, D18S535, D21S224, D21S370N, SRY and D18S386; available with authors). The UHOMi002-A cell line was also confirmed to be free of mycoplasma. Of note, ratio < 0.9 means that the sample was negative for mycoplasma

4. Materials and methods

4.1. Reprogramming of PBMCs to iPSCs

Erythroid progenitor cells were transduced by Sendai-virus using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific), following manufacturer's instruction. The calculated volumes of each of the three CytoTune™ 2.0 Sendai-virus (SeV) were added to the (KOS MOI = 5, hc-Myc MOI = 5, hKlf4 MOI = 3). The colonies with an embryonic stem cells (ES)-like appearance were manually isolated based on morphology.

4.2. Cell culture of iPSC

UHOMi002-A line was cultured on fresh Matrigel-coated plates (BD Biosciences) or on Geltrex matrix (Thermo Fisher Scientific) in E8 medium (Thermo Fisher Scientific), according to the manufacturer's instruction. The cells were cultivated at 37 °C in normoxic conditions and 5% CO₂ and passaged manually. They were adapted by passaging them using EDTA dissociation (Versene Solution, Thermo Fisher Scientific) every week into clumps, using 1:10 split ratio.

4.3. Karyotyping

Karyotyping was performed at mechanic passage 20 on R banded metaphases using standard procedures. At least 15 metaphases were examined per sample with an approximate resolution of 400 bands using a conventional microscope and IKAROS software (Metasystems). Karyotyping was performed using a service provider (Department of Medical Genetics, Nîmes Hospital).

4.4. Immunofluorescence labelling

Cells were fixed in 4% PFA for 15 min at room temperature (RT) and washed 3 times in phosphate-buffered saline (PBS, Gibco). The cells were permeabilized with 0.5% Triton X-100 (Sigma Aldrich) for 15 min, blocked with 10% normal donkey serum (Sigma Aldrich) for 1 h at RT. Cells were incubated with the primary antibodies in PBS BSA 1% 0.1% Triton X-100 overnight at 4 °C (listed in Table 2). Secondary antibodies were used at the dilution of 1:2000 during one hour at RT. Cell nuclei were stained with DAPI (Sigma Aldrich). Images were captured with Zeiss Axioimager Z1 Apotome and Confocal Microscopy Zeiss LSM700.

4.5. Flow cytometry analysis

Cells were harvested using cell dissociation reagent (Versene Solution) and washed twice in PBS. Cells were then incubated 15 min at room temperature with a Live Cell/Dead Cell discriminator staining (Violet Zombie, Biolegend). Cells were incubated with a conjugated primary or the control isotype antibody for 30 min at 4 °C. After

staining, cells were washed twice and analysed by flow cytometry using Beckman Coulter Gallios and Kaluza software.

4.6. Testing for Sendai virus clearance

Total RNA was isolated from iPS cells within the first week after transfection with Sendai virus and iPSCs after passage 15. RNA extraction and RT were performed as previously described (qRT-PCR section). PCR was performed using Q5 High-Fidelity DNA Polymerase with the primers listed in Table 1. The PCR mix was composed of cDNA (50 ng), the forward and reverse primers 1.5 µL at 10 µM for each, dNTPs (10 nM), Q5 buffer and Q5 DNA Pol5 (New England BioLabs). DNA was melted for 3 min at 95 °C, then 35 cycles (95 °C during 15 s, 55 °C for 15 s and 72 °C for 30 s), and final elongation was performed at 72 °C for 2 min using C100 Thermal Cycler (Bio-Rad). Products were analysed on 2.5% agarose gel, ran at 130 V electric field. Finally, the picture was acquired using ChemiDoc™MP Imaging System (Bio-Rad).

4.7. Short tandem repeat analysis (STR)

DNA extraction was performed as described below. STR analysis of generated iPSCs and parental PBMCs was performed to detect 18 loci. The method has been previously described (Ahmed et al., 2018).

4.8. Mycoplasma testing

Cell culture supernatants were tested using bioluminescence MycoAlert® Detection Kit (Lonza) according to manufacturer's instructions.

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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