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Lab Resource: Multiple Cell Lines

Derivation of a MUSIi012-A iPSCs from mobilized peripheral blood stem cells

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A B S T R A C T

CD34$^+$ cells were isolated from mobilized peripheral blood of a healthy donor and reprogrammed by nucleofection with episomal plasmids carrying L-MYC, LIN28, OCT4, SOX2, KLF4, EBNA-1, and shRNA against p53. The obtained MUSIi012-A cell line maintained the pluripotent phenotype, the ability to differentiate into all three germ layers, and a normal karyotype.

Resource table

Unique stem cell line identifier	MUSIi012-A
Alternative name(s) of stem cell line	SiPSC3.6 cell line
Institution	Siriraj Center of Excellence for Stem Cell Research, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
Contact information of distributor	Chuti Laowtammathron (chuti.lao@mahidol.ac.th) Surapol Issaragrisil (surapolsi@gmail.com)
Type of cell line	iPSC
Origin	Human
Additional origin info	Gender: Female Ethnicity: Thai
Cell source	Mobilized peripheral blood stem cells (CD34$^+$)
Clonality	Clonal
Method of reprogramming	Plasmids
Genetic modification	No
Type of modification	N/A
Associated disease	N/A
Gene/locus	N/A
Method of modification	No
Name of transgene or resistance	No
Inducible/constitutive system	No
Date archived/stock date	July 19, 2016
Cell line repository/bank	N/A
Ethical approval	Siriraj Institutional Review Board (SiRB) (COA no. Si248/2011), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

1. Resource utility

The MUSIi012-A line was established from mobilized peripheral blood stem cells of a healthy donor. This cell line can be used as an *in vitro* model for studying stem cell properties, and may be a good representative for studying epigenetic memory of induced pluripotent stem cells.

2. Resource details

Mobilized peripheral blood was obtained from a normal healthy volunteer by apheresis machine. Peripheral blood mononuclear cells (PBMCs) were isolated from apheresis blood using Isoprep (Matrix Technologies). Hematopoietic stem cells (CD34$^+$ cells) were purified using an EasySep™ Human CD34 Positive Selection Kit (Stem Cell

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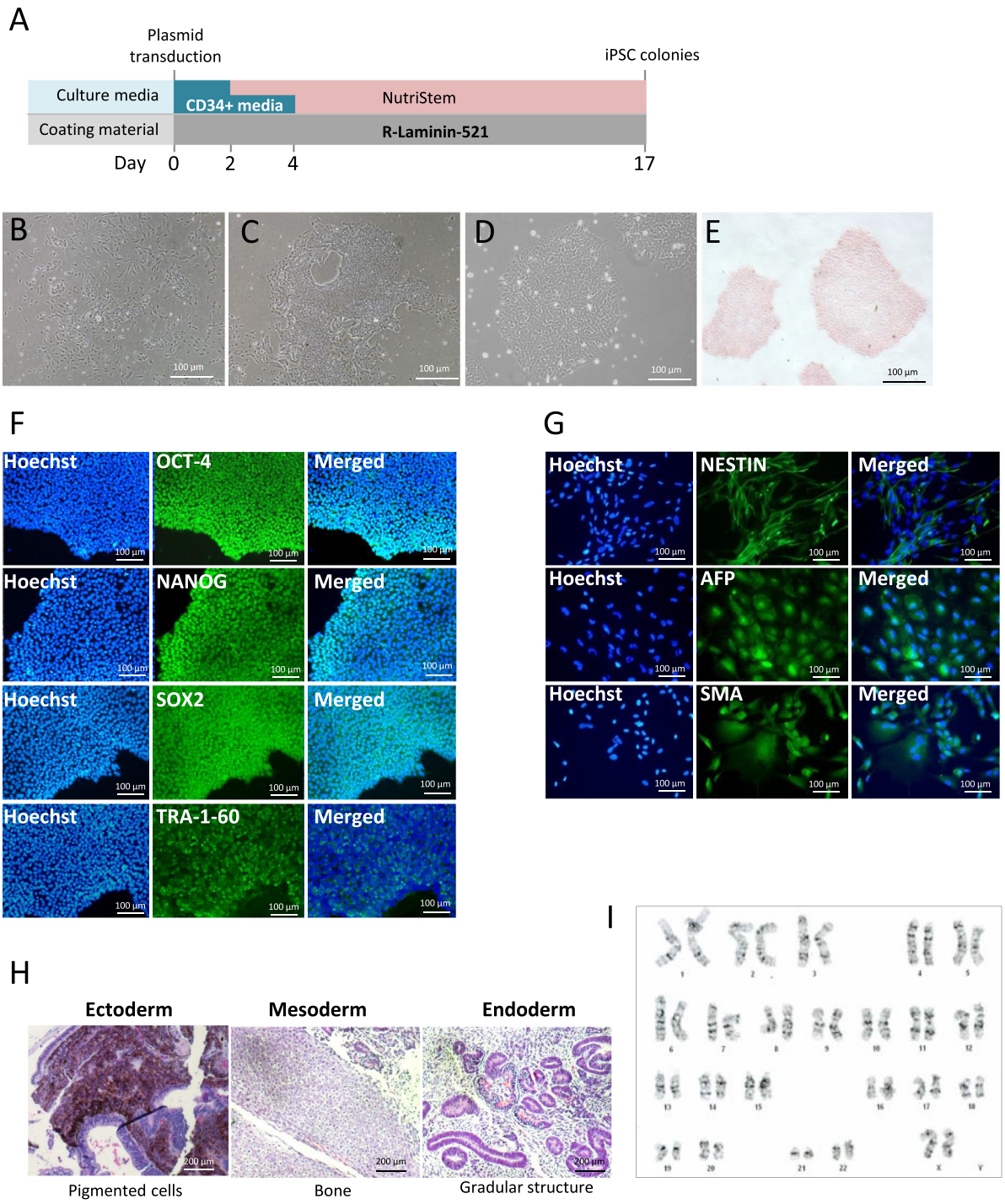

Fig. 1. Characterization of MUSIi012-A cell line.

Technologies). Collected CD34$^+$ cells were subjected to reprogramming by 4D-Nucleofector™ System (Lonza) with episomal plasmid expressing OCT4, SOX2, KLF4, LIN28, hL-MYC, and EBNA-1 against p53 (Okita et al., 2011). Cells were maintained under the culture system indicated in the schematic representation shown in Fig. 1A. Transfected cells were maintained on r-Laminine-521 (Corning), which is media that supports cell reprogramming and stem cell growth (Fig. 1B). A total of 6 colonies emerged on day 10, and they were individually subcultured on day 17 of reprogramming. Fig. 1C showed a representative colony on day 10 of reprograming. The iPSC line presented here was designated MUSIi012-A (Fig. 1D). The MUSIi012-A iPSC line expressed alkaline phosphatase activity (Fig. 1E) and pluripotent markers, including OCT4, SOX2, NANOG, and TRA-1-60, as assessed by immunofluorescence staining (Fig. 1F). Quantitative analysis by immunocytochemistry counting found 99.4% of cells to be positive for OCT4 (Table 1). Ability to differentiate into three embryonic germ layers was confirmed by embryoid body (EB) formation. Expression of ectodermal marker (NESTIN), mesodermal marker smooth muscle actin (SMA), and endodermal marker alpha-fetoprotein (AFP) was detected (Fig. 1G). Teratoma formation assay also confirmed the differentiation potential into tissue of the three embryonic germ layers including ectoderm, mesoderm and endoderm (Fig. 1H). In addition, MUSIi012-A cell line exhibited a normal karyotype (46, XX) (Fig. 1I), and was found to be free from mycoplasma as determined by MycoAlert™ PLUS

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1D
Phenotype	Qualitative analysis (immunocytochemistry)	Positive to alkaline phosphatase and OCT4, NANOG, SOX2, TRA-1-60	Fig. 1E, F
	Quantitative analysis (immunocytochemistry counting)	OCT4 - 99.4%	Fig. 1F
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 450–500	Fig. 1I
	Microsatellite PCR (mPCR) or STR analysis	Not performed	N/A
Identity		16 sites tested, 100% matched	Available from the authors
Mutation analysis	Sequencing	N/A	N/A
	Southern blot or WGS	N/A	N/A
Differentiation potential	Embryoid body formation and teratoma formation	Positive to NESTIN, AFP, and SMA, which are the representative markers for ectoderm, endoderm, and mesoderm lineage, respectively.	Fig. 1G
		For teratoma, representative cells of the 3-embryonic germ layers were found.	Fig. 1H
Donor screening	HIV-1/2, hepatitis B, hepatitis C	Not performed	Not performed
Genotype additional info	Blood group genotyping	Not performed	Not performed
	HLA tissue typing	Not performed	Not performed

Mycoplasma Detection Kit (Lonza) (Supplementary Table 1).

3. Materials and methods

3.1. Cell culture

$CD34^+$ cells were isolated from the mobilized peripheral blood of a healthy donor using an EasySep™ Human CD34 Positive Selection Kit (Stem Cell Technologies). Isolated cells were transfected with episomal plasmids expressing OCT4, SOX2, KLF4, LIN28, hL-MYC, EBNA-1, and shRNA against p53 (Okita et al., 2011). Cells were maintained on r-Laminin-521-coated plates (Corning) in NutriStem Medium (Corning) at 37 °C with 5% CO_2 and 5% O_2. Six iPSC clones were isolated after reprogramming. The iPSC line presented in this work was designated as MUSIi012-A. Cells were subcultured every 5–7 days using Versene Solution (Thermo Fisher Scientific).

3.2. Immunofluorescence staining

Cells were fixed in 4% paraformaldehyde for 30 min before being permeabilized with 0.1% Triton X-100 in PBS for 40 min at room temperature (RT). Cells were then incubated in blocking solution (PBS + 10% FBS) for 1 h before the first antibodies were added, followed by incubation overnight at 4 °C. Cells were washed several times with PBS to remove excess antibody. Secondary antibodies were prepared in PBS and added into the cells, followed by incubation in the dark for 1 h at RT (Table 2). Cell nuclei were counterstained with Hoechst 33,342 for 5 min before visualization with an Eclipse Ti-S microscope (Nikon).

3.3. Karyotyping

The cells that survived were cultured for 10 additional passages prior to standard G-banding karyotyping analysis.

3.4. Embryoid body formation and in vitro differentiation

Cells were treated with 10 μM of Y-27,632 for 1 h prior to disaggregation into single cells, and the concentration was adjusted to 4.5×10^5 cells/ml. The cell suspension was then added into an AggreWell™ plate (STEMCELL Technologies). Cells were gently pipetted up and down several times to ensure even distribution of cells throughout the well, and then they were centrifuged at 100 x g for 3 min to capture the cells in the microwells. The plate was incubated at 37 °C with 5% CO_2 and 5% O_2 for 24 h for embryoid body (EB) formation. EBs were collected the following day and cultured in suspension in Knockout DMEM containing 10% KnockOut™ Serum Replacement (Gibco), 1% L-GlutaMax (Gibco), 1% nonessential amino acids (Gibco), and 0.1% β-mercaptoethanol (Gibco) for 5 days, followed by transfer to 0.1% gelatin-coated plates and culture for 7–10 more days (Laowtammathron et al., 2018).

3.5. Teratoma formation

All animal experiments were performed after receiving approval from the Siriraj Animal Care and Use Committee (SiACUC) (COA no. SiACUO009/2561). Briefly, iPSCs were treated with 10 μM of Y-27,632 for 1 h prior to harvesting. Cells were resuspended at 1×10^6 cells/200 μl of cold 30% (v/v) Matrigel in KO-DMEM basal medium, and then implanted intramuscularly into 6 to 8-week-old nude mice. Teratomas were removed 6–8 weeks after transplantation and fixed in 10% formalin overnight before being embedded in paraffin wax. Samples were sectioned and examined by hematoxylin and eosin (H&E) staining.

Table 2
Reagent details.

Antibodies used for immunocytochemistry/flo	Antibody	Dilution	Company cat # and RRID
Pluripotency markers	Mouse anti-TRA-1-60	1:100	Millipore cat # MAB4360, AB_2,119,183
	Rabbit anti-SOX2	1:100	Millipore cat # AB5603, AB_2,286,686
	Mouse anti-TARI-81	1:100	Millipore cat # MAB4381, AB_177,638
	Rabbit anti-NANOG	1:100	Thermo Fisher Scientific cat # PA1-097, AB_2,539,867
Differentiation markers	Mouse anti-AFP	1:20	Millipore Cat# ST1673-100UG, *AB_10,693,988*
	Rabbit anti-SMAI	1:50	Abcam Cat# ab5694, AB_2,223,021
	Mouse anti-NESTIN	1:200	Millipore Cat# MAB5326, AB_2,251,134
Secondary antibodies	Alexa Fluor 488 Goat Anti-Rabbit lgG	1:500	Thermo Fisher Scientific, #A11008, AB_143,165
	Alexa Fluor 488 Goat Anti-Mouse lgG	1:500	Thermo Fisher Scientific, #A-11,001, AB_2,534,069

3.6. STR analysis

STR analysis for parental cells (PBMCs) and MUSIi012.A were performed at the Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University.

3.7. Mycoplasma test

Mycoplasma contamination was determined using a MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza).

Author declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome

Declaration of Competing Interest

All authors declare no personal or professional conflicts of interest relating to any aspect of this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101597.

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