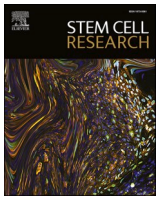
Stem Cell Research 77 (2024) 103422

Contents lists available at ScienceDirect

Stem Cell Research

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



Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line (SHUPLi002-A) from PBMCs of a healthy female donor

Beixu Li [a,b,1], Xing Ye [c,1], Junyi Lin [d], Kaijun Ma [e], Meng He [d,*], Youxin Fang [f,*]

[a] School of Policing Studies, Shanghai University of Political Science and Law, Shanghai, China
[b] Shanghai Fenglin Forensic Center, Shanghai, China
[c] Department of Forensic Medicine, School of Basic Medical Sciences, Gannan Medical University, Jiangxi, China
[d] Department of Forensic Medicine, School of Basic Medical Sciences, Fudan University, Shanghai, China
[e] Shanghai Key Laboratory of Crime Scene Evidence, Institute of Forensic Science, Shanghai Public Security Bureau, Shanghai, China
[f] Department of Neurology, Minhang Hospital, Fudan University, Shanghai, China

A B S T R A C T

Human induced pluripotent stem cells (iPSCs) have good multi-lineage differentiation potential, which is an ideal model for studying the pathogenesis of diseases and drug screening.Here, we generated an iPSC line, SHUPLi002-A, from peripheral blood mononuclear cells (PBMCs) of a healthy 28-year-old female individual using episomal vectors. SHUPLi002-A is characterized by expression of classical pluripotent stem cell markers as well as teratoma formation assays to evaluate their differentiation capacity in vivo.

Resource Table:

Unique stem cell line identifier	SHUPLi002-A
Alternative name(s) of stem cell line	iPS-26
Institution	School of Policing Studies, Shanghai University of Political Science and Law, Shanghai, China
Contact information of distributor	Beixu Li: libeixu@shupl.edu.cn
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 28 Sex: Female Ethnicity if known: Han Chinese
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Episomal vectors mediated delivery of Oct4, Sox2, Lin28, Klf4 and L-Myc
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	N/A
Gene/locus	N/A
Date archived/stock date	2021/11/17
Cell line repository/bank	https://hpscreg.eu/cell-line/SHUPLi002-A
Ethical approval	Ethics committee of basic medical school of Fudan University (2017-Y030)

1. Resource utility

The iPSC line generated from a healthy female will serve as a control for investigating disease pathogenesis and drug development. Furthermore, it's also an inexhaustible resource for allogeneic cell therapy (Yamanaka, 2020).

2. Resource details

Human peripheral blood mononuclear cells (PBMCs) isolated from a 28-year-old healthy woman, who did not exhibit any disease-related symptoms, were reprogrammed into induced pluripotent stem cells (iPSCs) by a non-integrating system containing episomal vectors expressing *Oct4, Sox2, Lin28, Klf4* and *L-Myc* previously described (Okita et al., 2011). The established iPSC line was termed as SHUPLi002-A (Table 1). The iPSC line displayed characteristic embryonic stem cell (ESC)-like morphology (Fig. 1A). Pluripotency was

* Corresponding authors.
E-mail addresses: 042101055@fudan.edu.cn (M. He), youxin_fang@163.com (Y. Fang).
[1] The first two authors contributed equally to this work.

https://doi.org/10.1016/j.scr.2024.103422
Received 22 February 2024; Received in revised form 9 April 2024; Accepted 12 April 2024
Available online 15 April 2024

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Qualitative analysis: Immunocytochemistry	Positive for pluripotency markers: NANOG, SOX2, TRA-1–60 and SSEA4	Fig. 1D
	Quantitative analysis: Flow cytometry	SOX2: 98.9 %, OCT4: 98.7 %, TRA-1-60: 94.5, SSEA4:100.0 %	Fig. 1F
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 550	Fig. 1B
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	21 loci tested, 100 % matched	submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Not performed	N/A
	Southern Blot OR WGS	Not performed	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Fig. 1E
Differentiation potential	Teratoma formation	Teratoma with three germ layers formation, endoderm (gut), mesoderm (cartilage) and ectoderm (neural tube).	Fig. 1G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

assessed by positive immunostaining of two intracellular and two extracellular pluripotent markers: NANOG, SOX2, TRA-1-60 and SSEA4 (Fig. 1D). Flow cytometry analysis was also performed to determine the exact positive percentage of the pluripotent markers: SOX2: 98.9 %, OCT4: 98.7 %, TRA-1-60: 94.5 and SSEA4:100.0 % (Fig. 1F). SHUPLi002-A iPSC line has a normal diploid 46, XX karyotype (Fig. 1B), The absence of reprogramming vectors was confirmed by PCR at passage 13 (Fig. 1C). Teratoma formation assay was performed to assess the differentiation potency of SHUPLi002-A. As shown in Fig. 1G, SHUPLi002-A could generate teratomas containing all three germ layers in vivo, including ectoderm (neural tube), mesoderm (cartilage) and endoderm (gut). Short tandem repeat (STR) analysis confirmed that the SHUPLi002-A iPSCs profile matched perfectly with that of the parental PBMCs (information available with the authors). Furthermore, SHUPLi002-A was free of mycoplasma contamination (Fig. 1E).

3. Materials and methods

3.1. PBMC reprogamming and hiPSC generation

PBMCs were isolated from the whole blood using Lymphoprep™ (StemCell Technologies) and cultured in StemSpan™ SFEM II (StemCell Technologies), supplemented with StemSpan™ Erythroid Expansion Supplement (StemCell Technologies). Eight days later, 1×10^6 PBMCs were collected and electroporated with pCXLE-hOCT3/4, pCXLE-hSK, and pCXLE-hUL (Addgene) (Okita et al., 2011) by 4D-Nucleofector™ (Lonza) using P3-primary Cell 4D-Nucleofector X Kit (Lonza) and program EO-100. Transfected cells were then seeded in 6-well plate previously coated with Matrigel (BD Biosciences) in StemSpan™ SFEM II medium for 4 days. Then the medium was changed with 2 mL mTeSR Plus medium (StemCell Technologies) supplemented with 0.25 mM sodium butyrate per well and refreshed every other day. Sodium butyrate was withdrawn on 12 days after electroporation. Colonies were manually picked after about 20 days after electroporation and expanded in mTeSR Plus medium on plates coated with Matrige in a humidified incubator (37 ℃, 5 % CO2). The medium was changed daily and cells were passaged with TrypeLE™ Express (ThermoFisher Scientific) at about 1.0×10^4 cells/cm^2 every 4–5 days with 10 μM Y2632 (Sigma).

3.2. Immunofluorescence (IF) staining

When the iPSCs (P10) cultured on coverslips reached 50 % confluence, cells were fixed in 4 % Paraformaldehyde (PFA, Sigma) for 20 min, permeabilized with 0.2 % Triton X-100 (Sigma) for 1 h, and blocked with 10 % donkey serum (ThermoFisher Scientific) for 1 h at room temperature. Then, the cells were incubated with primary antibodies at 4 ℃ overnight and stained with secondary antibodies for 1 h at room temperature. Nuclei were stained with DAPI (ThermoFisher Scientific) and images were captured by fluorescence microscope (Leica, DMi8). Both primary and secondary antibodies were diluted in 5 % donkey serum, and antibodies used are listed in Table 2.

3.3. Flow cytometry

Cells (P10) at 80 % confluence were digested with TrypeLE™ Express and washed with PBS, cells were then blocked with Human TruStain Fc Receptor Blocking Solution (BioLegend), and stained with the fluorochrome-conjugated antibodies for TRA-1–60 and SSEA4 listed in Table2. Intracellular staining (SOX2 and OCT4) was performed with the Transcription Factor Buffer Set (BD Pharmingen). Live/dead staining was performed with Fixable Viability Dye 780 (BD Pharmingen). Stained cells were analyzed on Cytoflex S (Beckman) and data analysis was performed with FlowJo software (Tree Star, Ashland, OR).

3.4. Karyotyping

Cells (P8) at 60 % confluence were treated with 0.1 μg/mL Colchicine (Sigma) for 2 h and then digested with TrypeLE™ Express. Digested cells were fixed and examined by standard G-banding analysis. The karyotyping analysis was performed at Shanghai Zhenhe Biotechnology Co, LTD, China, and a total of 20 metaphases were analyzed.

3.5. Teratoma formation assay

A total of 5×10^6 dissociated iPSCs (P10) were collected and resuspended in 200 μl 50 % Matrigel with PBS. 100 μl cells per site were then injected into subcutaneous tissue of NOD/SCID mice to form teratoma. After 6–8 weeks, teratomas were collected and processed for hematoxylin-eosin (HE) staining.

3.6. Residual presence of episomal plasmids analysis

Genomic DNA was extracted from the iPSCs (P13) using TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (TaKaRa). Then the loss of residual EBNA1 and WPRE sequences from the episomal vectors was confirmed by PCR. PCR amplification was performed using 2 × Taq PCR Mastermix (TIANGEN) on PTC-100 Thermal Cycler (Bio-Rad) under the following conditions: 95 °C, 5 min; 35 cycles of [95 °C, 15 s; 60 °C, 15 s; 72 °C, 25 s]; 72 °C, 3 min. Primers used are listed in Table 2.

3.7. Mycoplasma detection

The absence of mycoplasma at passage 8 was tested using PCR amplification method. PCR amplification was performed as above but under the following conditions: 95 °C, 5 min; 35 cycles of [95 °C, 30 s; 55 °C, 30 s; 72 °C, 45 s]; 72 °C, 3 min, Primers used are listed in Table 2.

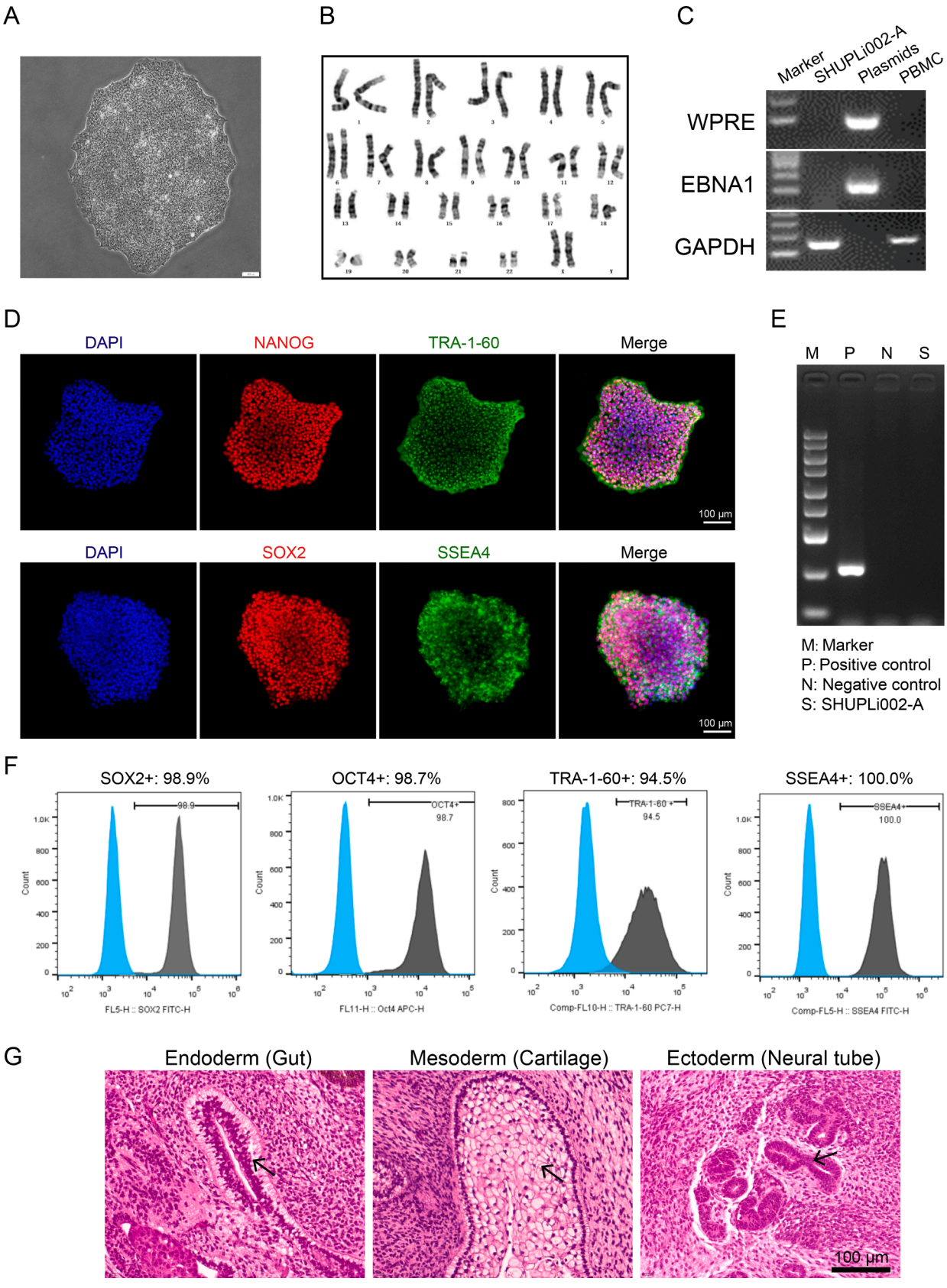


Fig. 1. Characterization of iPSCs line SHUPLi002-A

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Rabbit anti-NANOG	1:100	Abcam Cat# ab80892	AB_2150114
	Goat anti-SOX2	1:500	R and D Systems Cat# AF2018	AB_355110
	Mouse anti-SSEA-4	1:100	Santa Cruz Cat# sc-21704	AB_628289
	Mouse anti-TRA-1-60	1:2000	Millipore Cat# MAB4360	AB_2119183
Secondary antibodies	Alexa Fluor 594 donkey anti-rabbit IgG	1:1000	ThermoFisher Scientific Cat# A-21207	AB_141637
	Alexa Fluor 594 donkey anti-goat IgG	1:1000	Thermo Fisher Scientific Cat# A-11058	AB_2534105
	Alexa Fluor 488 donkey anti-mouse IgG	1:1000	Thermo Fisher Scientific Cat# A-21202	AB_141607
	Goat anti-Mouse IgM (Heavy chain) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-21042	AB_141357
Pluripotency Markers for flow-cytometry	Alexa Fluor® 647 Mouse anti-Oct3/4	1:10	BD Biosciences Cat# 560,329	AB_1645318
	Alexa Fluor® 488 anti-SOX2	1:1000	Biolegend Cat#656110	AB_2563956
	FITC anti-human SSEA-4	1:150	Biolegend Cat#330410	AB_1089205
	PE/Cyanine7 anti-human TRA-1-60	1:50	Biolegend Cat#330620	AB_2728285
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Episomal Plasmids (PCR)	WPRE	173 bp	TTTCCGGGACTTTCGCTTTC/ CAGAATCCAGGTGGCAACAC	
Episomal Plasmids (PCR)	EBNA1	244	GGTTTTGAAGGATGCGATTAAG/ TTTAATACGATTGAGGGCGTCT	
House-Keeping Genes (PCR)	GAPDH	153 bp	GTGGACCTGACCTGCCGTCT/ GGAGGAGTGGGTGTCGCTGT	
Mycoplasma detection (PCR)	Mycoplasma	450 bp	ACACCATGGGAGCTGGTAAT/ CTTCATCGACTTTCAGACCCAAGGCAT	

3.8. Short tandem repeat (STR) profiling

The SHUPLi002-A iPSCs and the parental PBMCs were analyzed for 21 loci (D5S818, D13S317, D7S820, D16S539, VWA, TH01, AMEL, TPOX, CSF1PO, D12S391, FGA, D2S1338, D21S11, D18S51, D8S1179, D3S1358, D6S1043, PENTAE, D19S433, PENTAD and D1S1656) by Shanghai Biowing Applied Biotechnology Co, LTD, China.

CRediT authorship contribution statement

Beixu Li: Writing – original draft, Investigation, Methodology, Data curation. **Xing Ye:** Investigation, Formal analysis, Data curation. **Junyi Lin:** Resources, Project administration. **Kaijun Ma:** Resources, Conceptualization. **Meng He:** Writing – review & editing, Project administration, Funding acquisition, Data curation. **Youxin Fang:** Writing – review & editing, Resources, Project administration, Data curation, Conceptualization.

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

No data was used for the research described in the article.

Acknowledgements

This study is supported by Shanghai Key Laboratory of Crime Scene Evidence, Institute of Forensic Science, Shanghai Public Security Bureau (2020XCWZK01) and Minhang District Health Commission Project (2021MHZ088).

References

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K., Shibata, T., Kunisada, T., Takahashi, M., Takahashi, J., Saji, H., Yamanaka, S., 2011. A more efficient method to generate integration-free human iPS cells. Nat. Methods 8 (5), 409–412.
Yamanaka, S., 2020. Pluripotent Stem Cell-Based Cell Therapy-Promise and Challenges. Cell Stem. Cell 27 (4), 523–531.