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Lab Resource: Multiple Cell Lines

Generation of Human Induced Pluripotent Stem Cell line from PBMCs of Healthy Donors using Integration-free Sendai virus Technology

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We have generated three well characterized human induced pluripotent stem cell (iPSC) lines derived from Peripheral Blood Mononuclear Cells (PBMC) of three healthy individuals. The PBMCs were primed and reprogrammed using a non-integrating sendai virus vector, iPSC lines showed full differentiation potential. These lines are available as YBLi001-A, YBLi002-A and YBLi003-A. The authenticity of these iPSC lines were confirmed by expression of pluripotent markers, *in vitro* directed differentiation towards three germ layers: ectoderm, mesoderm, and endoderm; karyotyping and STR analysis. These iPSC lines could be used as healthy controls for the studies involving disease-specific-iPSCs, e.g. drug toxicity and efficacy testing.

Resource table.

Unique stem cell line identifier	YBLi001-A YBLi002-A YBLi003-A			
Alternative name of stem cell line	YBL/IPSC01/Control YBL/IPSC02/Control YBL/IPSC05/Control			
Institution	Yashraj Biotechnology, Navi Mumbai, India			
Contact person and email	Dr. Shweta Bhatt, email: shweta.bhatt@yashraj.com			
Types of cell lines	iPSC			
Date archived/stock date	12 April 2021			
Origin	Human			
Additional origin info required for human ESC or iPSC	iPSC line names	Gender	Age	Ethnicity
	YBLi001-A	Male	38 years	Asian (Indian)
	YBLi002-A	Male	34 years	Asian (Indian)
	YBLi003-A	Female	37 years	Asian (Indian)
Disease status	Healthy			
Cell source	PBMC			
Clonality	Clonal			
Methods of reprogramming	Non-integrating Sendai virus			
Key marker	Pluripotent stem cell markers: SSEA4, OCT4, NANOG, TRA-1–60 and Alkaline Phosphatase			
Authentication	Identity and purity of line confirmed			
Cell line repository/bank	YBLi001-A https://hpscreg.eu/cell-line/YBLi001-A YBLi002-A https://hpscreg.eu/cell-line/YBLi002-A YBLi003-A https://hpscreg.eu/cell-line/YBLi003-A			
Ethical Approval	Institutional Ethics Committee (IEC), Yashraj Biotechnology Limited Institutional Committee for Stem Cell Research (IC-SCR), Yashraj Biotechnology Limited			

Resource utility: These human iPSC lines could serve a cell resource as healthy controls for the studies involving patient-specific iPSCs and for studies on assessing the efficacy and toxicity of drug substances.

Resource details: The pioneering work by Takahashi and Yamanaka in 2006 on the induced pluripotent stem cell (iPSC) technology has revolutionized the understanding of various diseases by diseases modeling (Takahashi et al., 2007). According to Basu et al. (2016), the Indian population is genetically diverse as a result of numerous historical and cultural occurrences. Nutritional and environmental characteristics that are unique to the Indian subcontinent further emphasize these distinctions. Age and gender matched control lines produced from healthy people are required to examine any disease in populations of Indian ethnicity. Similar ethnicity healthy control lines reduce the impact of gene-environment interactions, enabling a more thorough investigation of the pathophysiology. We have used a non-integrating

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viral vector containing human OCT3/4, KLF4, SOX2 and C-MYC transcription factors to transduce PBMCs. These three healthy iPSC lines exhibited the typical appearance of embryonic stem cells (Fig. 1A) and normal karyotypes (46, XY or 46, XX), as confirmed by G-banding karyotyping at passage 5 (Fig. 1 panel D and Supplementary Fig. 1), and exhibited positive expression of OCT4A and SSEA-4, as measured by flow cytometry, expression of alkaline phosphatase (Fig. 1A) and OCT 4A, NANOG, Sox2, TRA-1-60 and SSEA-4, as determined by immunofluorescence staining (Fig. 1B). Further, the pluripotency of these iPSC lines was confirmed by *in vitro* directed differentiation towards three germ layers; endoderm (SOX-17, FOXA2), mesoderm (GATA-4, Brachyury) and ectoderm (TUJ1, PAX6) (Fig. 1G). Short tandem repeat (STR) DNA profiling analysis showed the genotypes of these three iPSC lines 100 % matched with source donor's PBMC and also confirmed the purity of the cell lines population, indicating that there is no cross-contamination from any other cell line (Fig. 1 panel D and Supplementary Fig. 1).

1. Material and methods

Ethical statement: This work was approved by the Institutional Ethics committee (IEC) and Institutional Committee for Stem Cell Research (IC-SCR) of Yashraj Biotechnology, Maharashtra, India. Healthy volunteers were enrolled in IEC and IC-SCR approved studies, protocol #YBLBC17SB. Samples, whole blood, were collected by Aspira Pathlab and Diagnostics (NABL certificate No. MC-2447). Project is approved by National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT) registration ID: NAC-SCRT/134/20200209 and Drugs Controller General of India (DCGI) registration ID: ECR/305/Indt/MH/2018.

Isolation and reprogramming of PBMC, iPSC culture: Peripheral blood was collected from three healthy individuals, 38 years old Male, 34 years Male and 37 years old Female, after obtaining approval from the IEC and IC-SCR. PBMCs were isolated from blood using Ficoll-based density gradient centrifugation (Histopaque-1077, Sigma, 10771), cryopreserved in CryoStor CS10 (Stem Cell Technologies, 07930), and stored in liquid nitrogen till further use. PBMCs were thawed and seeded onto one well of a 6-well tissue culture dish in PBMC medium containing StemPro™-34 SFM Medium (Thermo Fisher, 10639–011) with cytokines; 100 ng/mL SCF, 100 ng/mL FLT-3, 20 ng/mL IL-3 and 20 ng/mL IL-6. After 2 days, fresh complete StemPro™-34 medium containing cytokines was added without disturbing the cells. One day before transduction, 0.5 mL of medium was gently removed and 1 mL of fresh complete StemPro™-34 medium containing cytokines was added without disturbing the cells. Cells were counted using hemocytometer, and volume of each virus was calculated to reach the required target MOI using the live cell count and the titer information as on the Certificate of Analysis (CoA) of CytoTune™ 2.0 reprogramming vector.

Volume of virus (μL) = MOI (CIU/cell) × number of cells/Titer of virus (CIU/mL) x 10^{-3} (mL/μL)

Cells were harvested and seeded in the required number of wells of a

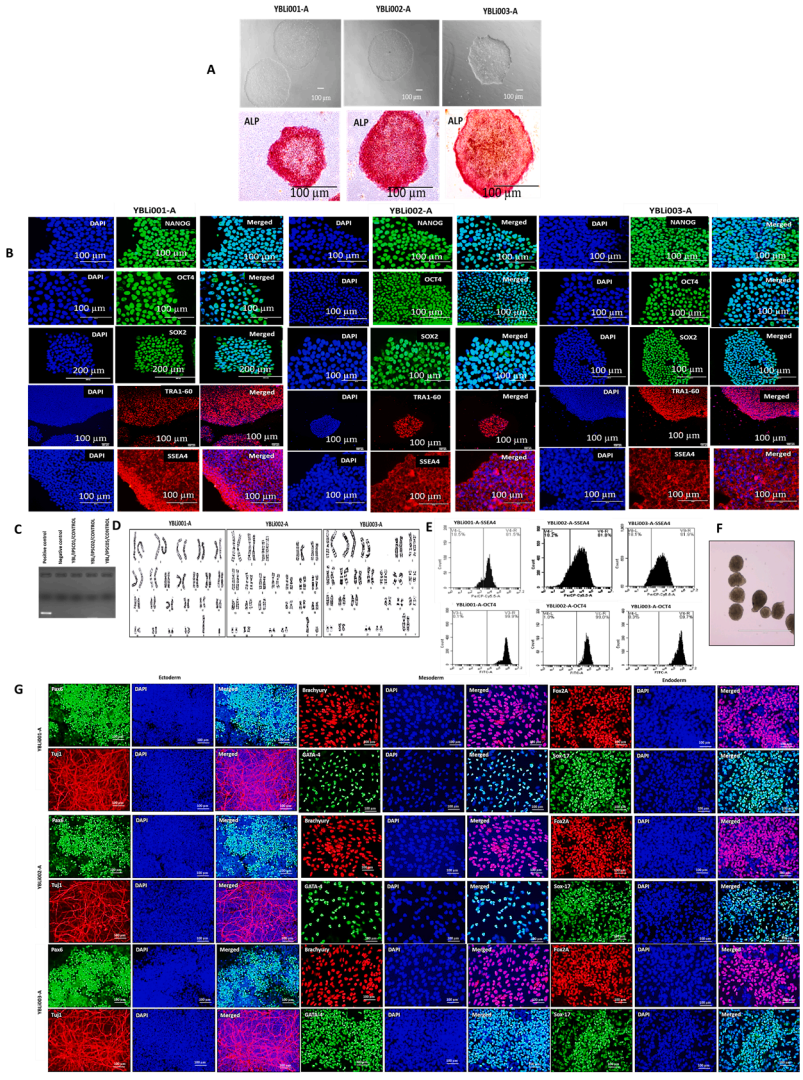

Fig. 1. A. Phase contrast of 3 iPSC lines and alkaline phosphatase staining; B. immunofluorescence staining of pluripotency markers (NANOG, OCT4, SOX2, TRA-1-60 and SSEA4) of 3 iPSC lines; C. PCR for Sendai virus clearance of 3 iPSC lines; D. Karyotying of 3 iPSC lines E. Flowcytometric Analysis of pluripotency markers (OCT4 and SSEA4) of 3 iPSC lines. F. Embroid Body (YBLi001-A), G. Expression of endoderm (SOX17, FOXA2), mesoderm (GATA-4, Brachyury) and ectoderm (TUJ1, PAX6) germ layer markers of 3 iPSC lines.

24-well plate (1.0×10^5 cells/well) in a minimum volume (~100 μl) for transduction. The calculated volumes of each of the three CytoTune™ 2.0 Sendai viruses were added to 0.4 mL of pre-warmed StemPro™-34 medium containing cytokines and 4 μg/mL of Polybrene. The solution was mixed thoroughly by pipetting the mixture gently up and down. Next step was completed within 5 min. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 overnight. Next day, virus was removed by centrifuging the cells at 400 × g for 10 min. Supernatant was aspirated and discarded. Cells were resuspended in 0.5 mL of complete StemPro™-34 Medium containing cytokines (as mentioned above) in the 24-well plate. After transduction, the cells were cultured for 2 days at 37° C in a humidified atmosphere of 5% CO_2 without any media change. After two days, 1×10^4 to 1×10^5 live cells were seeded in vitronectin (Stem Cell Technologies, 100-0763) coated plates in complete StemPro™-34 medium without the cytokines. Next 2–4 days, cells were maintained with half media change of StemPro™-34 medium without cytokines. On 4th day, cells were adapted from StemPro™-34 medium to complete mTeSR 1 media (Stem Cell Technologies, 85850) by half media change. Further for next 20 days, plated PBMCs were maintained in complete mTeSR 1 media. Cell clumps indicative of reprogrammed cells were visible on day 8 after viral transduction. On day 20, cells formed reprogrammed cell clumps that were manually picked up and plated on vitronectin plates. Colonies were expanded manually for 3–4 passages. Subsequent passaging was done by enzymatic dissociation using StemPro Accutase (Thermo Fisher, A1110501) and ReLeSR (Stem Cell Technologies, 05872). All cell culture work was done at 37° C in humidified atmosphere containing 5% CO_2 in a BSL2 facility.

Immunocytochemistry: Cells were cultured on 8 well chamber slides, fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.1% Triton-X-100 for 10 min. After blocking with 1% Bovine Serum Albumin, incubated in primary antibody overnight at 4^0 C. Next day, incubated with secondary antibody for 60 min at room temperature. Subsequently, cell nuclei were labeled with 4′, 6-Diamidin-2-phenylindo (DAPI, Invitrogen). Images were acquired with Invitrogen™ EVOS™ FL Digital Inverted Fluorescence Microscope (Thermo Fisher Scientific).

Flowcytometry: Cell suspension was fixed with BD Cytofix and permeabilized using Perm/Wash buffer (BD Biosciences, 554723) and stained with antibodies (Table 2). 10, 000 events were acquired with BD Accuri™ C6 Plus Flow Cytometer (BD Biosciences).

RNA isolation and cDNA synthesis: For gene expression analysis, total RNA was isolated using the RNA extraction kit (Qiagen, 74,004). 1 μg of total RNA was converted to cDNA using the iScript cDNA synthesis kit (Biorad, 1708890); 1 μg of the cDNA was used to run the PCR reaction using Thermo Scientific PCR Master Mix (Thermo Fisher Scientific, K0171) in thermal cycler (Biorad). PCR products were analyzed on 2% agarose gel electrophoresis.

Detection of SeV genome and transgenes: After 14 passages, hiPSC lines were tested for SeV residues. PCR was performed using primers and instructions (Table 2) as recommended by the manufacturer. The reprogramming leftovers was used as positive control RNA. Negative control RNA was obtained from the hiPSC line Gibco® Episomal hiPSC Line, (Gibco, A18945). Data included in Fig. 1 C.

Karyotyping: Karyotyping was performed at passage 7 by GTG-banding analysis performed by Medgenome Labs Private Limited, Mumbai. Cells were treated with KaryoMAX® Colcemid™ Solution (ThermoFisher Scientific, 15212–012) overnight at 37 °C and thereafter processed following standard procedures in routine diagnostics. Data included in Supplementary Fig. 1.

Short tandem repeat (STR) genotyping: STR typing was performed at passage 7 by Medgenome Labs Private Limited, Bangalore. Data included in Supplementary Fig. 1.

Trileaneage differentiation: iPSC lines at passage 7 were differentiated towards three germ layers: ectoderm, mesoderm, and endoderm using STEMdiff™ Trilineage Differentiation Kit as per manufacturer's instructions.

Table 1
Characterization and Validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line Normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Expression of pluripotency markers: OCT4, SOX2, NANOG, TRA-1-60 and SSEA4	Fig. 1 panel B
	Flowcytometery	Expression of pluripotency markers: OCT4 and SSEA4	Fig. 1 panel E
	PCR	Clearance of vector against sendai virus	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XY and 46XX, no abnormalities observed, Resolution 500	Fig. 1 panel D and Supplementary Fig. 1
Identity	STR analysis	16 loci from the genomic DNA of iPSC lines were matched with loci from genomic DNA of PBMCs	Supplementary Fig. 1
Microbiology	Mycoplasma, Endotoxin and Bioburden testing	Mycoplasma by luminescence-Negative Endotoxin by quantitative LAL test-Negative Bioburden by direct inoculation-Negative	Supplementary Table 1
Differentiation potential	Embryoid body formation and Trilineage differentiation	Embryoid body formed In vitro spontaneous differentiation, yielded expression of endoderm (SOX-17, FOXA2), mesoderm (GATA-4, Brachyury) and ectoderm (TUJ1, PAX6)	Fig. 1 panel F & G
Donor screening	HIV	Negative	Data available with authors

Embryoid body (EB) formation: iPSC colonies at passage 7 were enzymatically detached, transferred to a low attachment plate in mTeSR 1 medium. Suspension EBs were seeded onto Matrigel coated 4-well chambers after 7 days, grown for another 8 days and fixed for immunochemistry.

Endotoxin, Mycoplasma and Bioburden testing: Spent media from optimally confluent iPSC cultures were collected after 48 h and tested using MycoAlert™ mycoplasma detection kit, (Lonza, LT07-318) as per the manufacturer's instructions, performed routinely. Bioburden testing performed routinely by plating spent media on Nutrient Agar Plate (Himedia, MP001)- for bacterial count and on Sabouraud Dextrose Agar Plate (Himedia, MPH063)- for fungal count. Endotoxin test was performed routinely using Pierce LAL Chromogenic Endotoxin Quantitation Kit (Thermo fisher, 88282) as per the manufacturer's instruction.

Table 2
Reagent details.

Vector Clearance	Target SeV	Forward Reverse	Primer sequence GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC	
Antibodies used for immunostaining				
	Antibody	Dilution	Company and cat#	RRID
Primary Antibodies	OCT-4A (C30A3) Rabbit mAb	1:200	Cell Signaling Technology 9656	AB_1658242
	SOX2 (D6D9) Rabbit mAb	1:200	Cell Signaling Technology 9656	AB_1658242
	NANOG (D73G4) XP® Rabbit mAb	1:200	Cell Signaling Technology 9656	AB_1658242
	SSEA4 (MC813) Mouse mAb	1:200	Cell Signaling Technology 9656	AB_1658242
	TRA-1–60(S) (TRA-1–60(S)) Mouse mAb	1:200	Cell Signaling Technology 9656	AB_1658242
	Mouse Anti-TUJ1	1:250	Cell Signaling Technology 5568 T	AB_11127203
	Rabbit Anti SOX17	1:250	Abcam ab224637	AB_2801385
	Rabbit Anti GATA4	1:250	Sigma 11PA073899	AB_477501
	Rabbit Anti-PAX6	1:200	Abcam ab5790	AB_305110
	Anti- Brachyury	1:250	Abcam ab20680	AB_727024
	Mouse Anti-FOXA2	1:250	Abcam Ab108422	AB_11157157
Secondary antibodies	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)	1:1000	Abcam ab150081	AB_2734747
	Goat Anti-Mouse IgG H&L (Alexa Fluor® 488)	1:1000	Abcam ab150120	AB_2631447

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103062.

References

Basu, A., Sarkar-Roy, N., Majumder, P.P., 2016. Genomic reconstruction of the history of extant populations of India reveals five distinct ancestral components and a complex structure. Proc. Natl. Acad. Sci. U.S.A. 113, 1594–1599. https://doi.org/10.1073/pnas.1513197113.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131 (5), 861–872. https://doi.org/10.1016/j.cell.2007.11.019. PMID: 18035408.