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Lab Resource: Single Cell Line

Human induced pluripotent stem cell line (QBRIi013-A) derivation from a 6-year-old female diagnosed with Autism spectrum disorder (ASD) and intellectual disability (ID)

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A B S T R A C T

Autism spectrum disorder (ASD) is a childhood-onset neurodevelopmental disorder characterized by social interaction, behavior, and communication challenges. Here, we generated an induced pluripotent stem cell (iPSC) line, QBRIi013-A using a non-integrating Sendai virus from a 6-year-old female diagnosed with ASD and intellectual disability. The QBRIi013-A cell line was fully characterized and exhibited a pluripotency capacity and trilineage differentiation potential. Furthermore, it showed normal karyotype and genetic identity to the patient's PBMCs. Consequently, this iPSC line provides a valuable cell model in understanding the molecular mechanism underlying the complexities of ASD pathogenesis.

1. Resource Table

Unique stem cell line identifier	QBRIi013-A
Alternative name(s) of stem cell line	ASD-NDRC-1
Institution	Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar
Contact information of distributor	Sara A. Abdulla (saabdulla@hbku.edu.qa)
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 6 years Sex: Female Ethnicity: Egyptian
Cell Source	Peripheral Blood Mononuclear Cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Integration-free Sendai virus vector contain OCT4, SOX2, c-MYC and KLF4
Genetic Modification	NO
Type of Modification	NO
Associated disease	Autism spectrum disorder with Intellectual disability
Gene/locus	Gene: *N/A* Locus: N/A Mutation: N/A
Method of modification	N/A N/A
Unique stem cell line identifier	QBRIi013-A
Name of transgene or resistance	
Inducible/constitutive system	N/A
Date archived/stock date	2021–06-13
Cell line repository/bank	N/A
Ethical approval	Blood sample was obtained with full informed consent, and the protocol was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (IRB) committee of Qatar Biomedical Research Institute (QBRI-IRB:2018–024)

2. Resource utility

Our iPSC line is derived from a female child with Autism Spectrum Disorder (ASD) and intellectual disability (ID). This iPSC line provides an *in vitro* model for investigating the underlying mechanisms that contribute towards the complexities of ASD pathogenesis.

3. Resource details

Autism Spectrum Disorder (ASD) is a highly heritable childhood-

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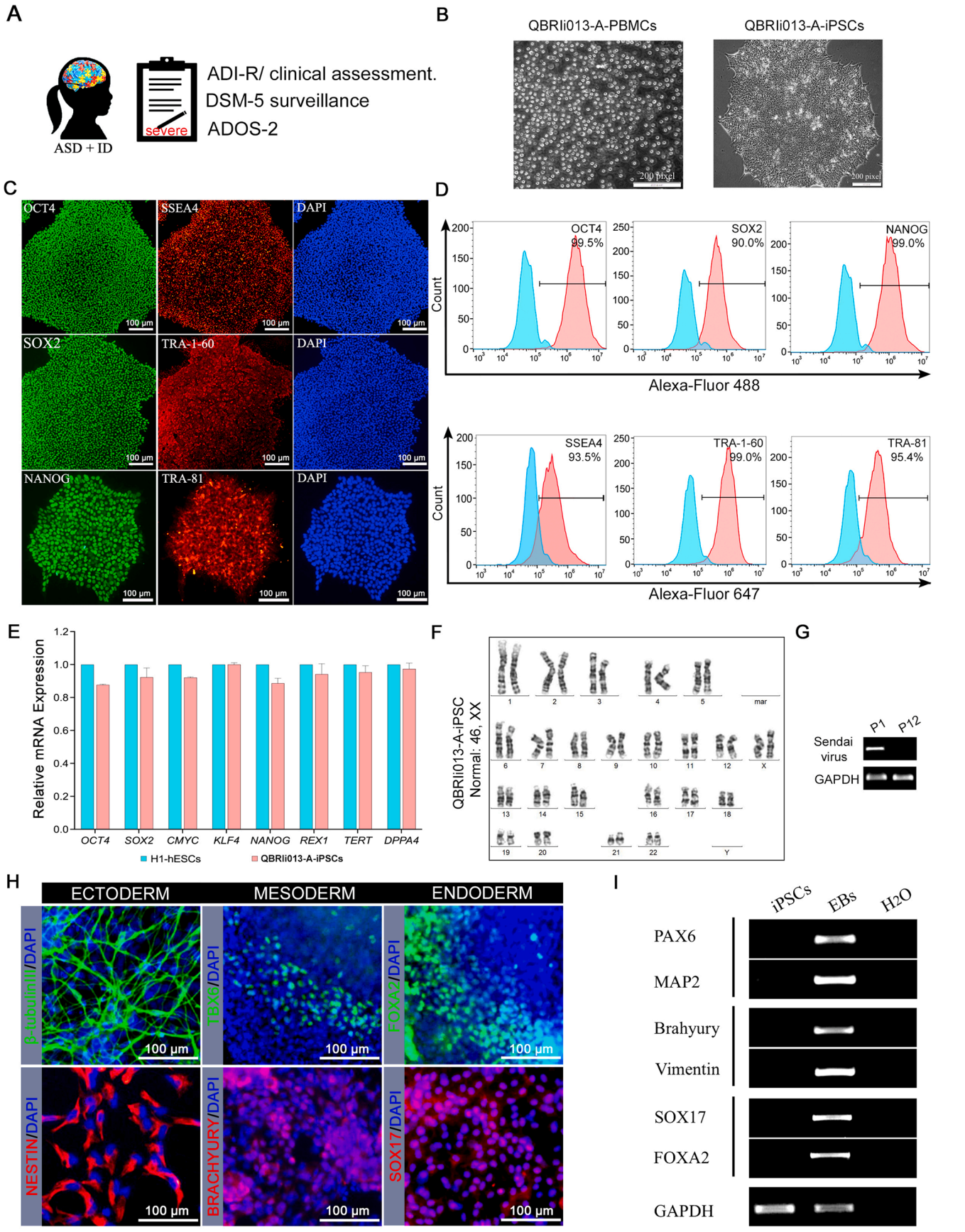

Fig. 1. Generation and characterization of iPSCs QBRIi013-A: The patient is diagnosed (A) and recruited for PBMCs isolation and reprograming of iPSC. The generated iPSC showed the typical morphological characters (B) with expression of pluripotency markers (C, D, E) with normal karyotyping (F). (H and I) clarified the trilineage differentiation capability of the generated QBRIi013-A iPS cell line after sendai virus releasing (G).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1, panel B
Phenotype	Qualitative analysis: Immunocytochemistry	Assess staining of pluripotency markers: OCT4, SOX2, NANOG, SSEA4, TRA-1–60 and TRA-81.	Fig. 1, panel C
	Quantitative analysis: Flow cytometry and qPCR	Flow cytometry: OCT3/4: 99.5%, SOX2: 90.50%, NANOG: 99.0%, SSEA4: 93.5, TRA-1–60: 99.0% and TRA-81: 95.40%. qPCR: *OCT4, SOX2, c-MYC, KLF4, NANOG, REX1, TERT1,* and *DPPA4*	Fig. 1, panel D and E
Genotype	Karyotype (G-banding) and resolution	46XX with mean resolution of 300.	Fig. 1, panel F
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	15 loci, 100% matched	Submitted in archive with journal
Mutation analysis	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR	Fig. 1/ supplementary
	Sendai virus	Negative by RT-PCR	Fig. 1, panel G
Differentiation potential	Embryoid body formation and trilineage expression.	The embryoid body formed and expressed β-tubulin, NESTIN, TBX6, Brachyury, FOXA2 and SOX17 (immunostaining) as well as the expression of *PAX6, MAP2, BRACHYURY, VIMENTIN, FOXA2,* and *SOX17* (RT-PCR).	Fig. 1, panel H and I
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

onset neurodevelopmental disorder characterized by social reciprocity deficits and stereotyped or repetitive behaviors accompanied by restricted interests and impaired communication capabilities. The worldwide prevalence of ASD is increasing and is estimated to be around 1.5%. However, the severity and the clinical manifestations of ASD vary between individuals. Consequently, this adds layers of difficulties towards drug development and understanding the fundamental molecular mechanisms that contribute towards the outcome of ASD (Reilly et al., 2017). Stem cell biology represents an innovative and powerful avenue to uncover the sophisticated mechanisms of ASD by studying neurodevelopment *in vitro* through patient-specific induced pluripotent stem cells (iPSCs). In our study, an iPS cell line was derived from peripheral blood mononuclear cells (PBMCs) of a 6-year-old female diagnosed with ASD. The child was recruited from a large longitudinal population-based study (Salloum-Asfar et al., 2021). According to the Autism Diagnostic Interview-Revised (ADI-R) (Rutter et al., 2003), she was initially clinically identified by expert clinicians, at 2.5 years age, at the Child Development Center (CDC) - Rumeilah Hospital in Qatar. Later, the child was reassessed two times, at the age of 4 and the age of 6; time of sample collection used in this study. Alongside ASD, the child was further diagnosed with intellectual disability (ID) and a sleeping disorder. Based on the Diagnostic and Statistical Manual of Mental Disorders Surveillance (DSM-5) criteria (A. American Psychiatric Association, A.P. Association, 2013) as well as the Autism Diagnostic Observation Schedule, Second Edition-2 (ADOS-2, module 1) (Lord et al., 1999), the child was classified as a severe ASD case (Fig. 1A). The non-integrating Sendai virus vectors with Yamanaka factors (OCT4, SOX2, KLF4, and c-MYC) were used to induce reprogramming of the isolated PBMCs into pluripotent like cells. The selected iPSC colonies were picked up and expanded for extensive characterization (Table 1). Morphologically, our (QBRIi013-A) cell line displayed typical characteristics of normal embryonic stem cells (Fig. 1B). Moreover, the generated iPSCs expressed the pluripotency markers; OCT4, SOX2, NANOG, SSEA4, TRA-1-60, and TRA-81 as indicated through immunofluorescence stainings (Fig. 1C). Quantifying these specific protein markers through flow cytometry further showed high expression percentages (Fig. 1D). Additionally, the mRNA expression levels of the self-renewal markers (*OCT4, SOX2, c-MYC, KLF4, NANOG, REX1, TERT1,* and *DPPA4*) were quantified equally and comparable to hESCs (Fig. 1E). Furthermore, G-banding karyotyping analysis revealed that the (QBRIi013-A) iPSC line has a normal diploid (46, XX) karyotype (Fig. 1F). Reprogramming of the viral vector was silenced as examined by RT-PCR at passage 12 (Fig. 1G). The functional characterization assessment of the generated iPS cell line, in this case, demonstrated its ability for embryoid body (EBs) formation with the capability of differentiation into multiple cell types of the three germ layers. These spontaneously differentiated EBs expressed the ectodermal (Beta tubulin III, NESTIN, PAX6, and MAP2), mesodermal (TBX6, BRACHYURY, and VIMENTIN) and endodermal (FOXA2, and SOX17) markers (Fig. 1H and I). (QBRIi013-A) line was confirmed as a mycoplasma free cell line (Supplementary Fig. 1), and the STR analysis authenticated its identity with the patient PBMCs (Supplementary Table).

4. Materials and methods

4.1. Cell culture and reprogramming

The subject's peripheral blood was collected in tubes with an anticoagulant and used to isolate the PBMCs using Ficoll-Paque (Sigma-Aldrich). PBMCs were cultured in StemPro-34 medium with cytokines for 3–5 days then reprogrammed using a CytoTune-iPS 2.0 Sendai reprogramming kit (Thermo Fisher Scientific, A16518). The reprogramed iPSC colonies were cultured in mTESR-1 medium (Stem Cell Technologies) on Matrigel (Corning)-coated plates. The established colonies were routinely passaged non-enzymatically using the ReLeSR™ (STEMCELL technologies) and then replated on previously Matrigel coated plates (200 μL for 24 well plate 400 μL for 12 well plate and 800 μL for 6 well plate). The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2.

4.2. Immunocytochemistry and flow cytometry

Cells were fixed at passage 10 with 4% PFA (paraformaldehyde) (Santa Cruz Biotechnology, USA) for 20 min. The fixed cells were washed by PBS then permeabilized with 0.5 % Triton X-100 (Sigma-Aldrich, USA) in PBS (PBST) and subsequently blocked with 6% bovine serum albumin (BSA) in PBST. The primary antibodies were added to the cells overnight at 4 °C and the secondary antibodies for 1 h at room temperature. The nuclei were counterstained using DAPI staining. Acquisition of our images were through Olympus IX 53, an inverted fluorescence microscope. Flow cytometry quantifications were achieved via the BD Accuri C6 flow cytometer used to analyze stained cells and FlowJo for the corresponding data analysis. The list of antibodies used can be found in Table 2.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	Rabbit anti-OCT4	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Rabbit anti-SOX2	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Rabbit anti-NANOG	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Mouse anti SSEA4	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Mouse anti TRA-1–60	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Mouse anti TRA-81	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Differentiation Markers	Mouse Anti- Tubulin β 3 (TUBB3)	1:500	BioLegend Cat# 801201, RRID:AB_2313773
Differentiation Markers	Mouse anti-Nestin, clone 10C2 antibody	1:500	Millipore Cat# MAB5326, RRID:AB_2251134
Differentiation Markers	TBX6	1:500	R and D Systems Cat# AF4744, RRID: AB_2200834
Differentiation Markers	Mouse anti- Brachyury	1:500	Abcam Cat# 140,661
Differentiation Markers	FOXA2	1:500	Santa Cruz Biotechnology Cat# sc-6554, RRID: AB_2262810
Differentiation Markers	Mouse anti SOX17	1:2000	OriGene Cat# TA500096, RRID:AB_2255344
Secondary antibodies	Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-21206, RRID: AB_2535792
Secondary antibodies	Donkey anti-Goat IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-11055, RRID: AB_2534102
Secondary antibodies	Donkey anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	1:500	Thermo Fisher Scientific Cat# A-31570, RRID: AB_2536180
Secondary antibodies	Donkey anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-21202, RRID: AB_141607
Secondary antibodies	Donkey anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	1:500	Thermo Fisher Scientific Cat# A-31571, RRID: AB_162542
Primers			
	Target	**Forward/Reverse primer (5′-3′)**	
Pluripotency Markers (qPCR)	OCT4 (144 bp)	GACAGGGGGAGGGGAGGAGCTAGG/ CTTCCCTCCAACCAGTTGCCCCAAAC	
Pluripotency Markers (qPCR)	SOX2 (151 bp)	GGGAAATGGGAGGGGTGCAAAAGAGG / TTGCGTGAGTGTGGATGGGATTGGTG	
Pluripotency Markers (qPCR)	c-MYC (136 bp)	TTCTGTGGAAAAGAGGCAGG/ TGCGTAGTTGTGCTGATGTG	
Pluripotency Markers (qPCR)	KLF4 (92 bp)	CAGTCCCGGGGATTTGTAGC/ GAAGAAGGTGGGGTGAGCAT	
Pluripotency Markers (qPCR)	NANOG (192 bp)	CATGA GTGTGGATCCAGCTTG/ CCTGAATAAGCAGATCCATGG	
Pluripotency Markers (qPCR)	REX1(145)	TCACAGTCCAGCAGGTGTTTG/ TCTTGTCTTTGCCCGTTTCT	
Pluripotency Markers (qPCR)	TERT (107 bp)	TTGAAATCGCGCAAACCTGC/ GCCTCATCCTTTGTCCTGGT	
Pluripotency Markers (qPCR)	DPPA4 (120 bp)	ACTGAACGGCCATATCCGAG/ TCCCTCGACTTCTCTGTGGA	
Ectodermal differentiation (RT-PCR)	PAX6 (98 bp)	GCGGAAGCTGCAAAGAAATAG/ GGGCAAACACATCTGGATAATG	
Ectodermal differentiation (RT-PCR)	MAP2 (212 bp)	CAGGTGGCGGACGTGTGAAAATTGAGAGTG/ CACGCTGGATCTGCCTGGGGACTGTG	
Mesodermal differentiation (RT-PCR)	BRACHYURY (274 bp)	GCCCTCTCCCTCCCCTCCACGCACAG/ CGGCGCCGTTGCTCACAGACCACAGG	
Mesodermal differentiation (RT-PCR)	VIMENTIN (280 bp)	GAACGCCAGATGCGTGAAATG/ CCAGAGGGAGTGAATCCAGATTA	
Endodermal differentiation (RT-PCR)	SOX17 (773 bp)	TCCTGGAGGAGCTAAGGAAA/ GCCACTTCCCAAGGTGTAAA	
Endodermal differentiation (RT-PCR)	FOXA2 (478 bp)	CTGTGTAGACTCCTGCTTCTTC/ CCCTCCCTCCTTCTTGAAATAAT	
House-Keeping Genes (RT-PCR)	GAPDH (156 bp)	ACGACCACTTTGTCAAGCTCATTTC/ GCAGTGAGGGTCTCTCTCTTCCTCT	
Sendi virus (RT-PCR)	SENDAI VIRUS (181 bp)	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
Mycoplasma primer (RT-PCR)	Mycoplasma (280 bp)	GGGAGCAAACAGGATTAGATACCCT/ TGCACCATCTGTCACTCTGTTAACCTC	

4.3. RNA extraction, RT-PCR, and real-time qPCR

Direct-zol RNA Extraction Kit (Zymo Research) used for total RNA extraction following the manufacturer's instructions. cDNAs were synthesized using superscript IV, First-Strand Synthesis System (Thermo FisherScientific). PCR products were amplified using the PCR-Master mix (Thermo Fisher Scientific). Real-time PCR was performed using GoTaq qPCR MasterMix (Promega). The used primers and the product sizes are included in table 2.

4.4. Karyotyping

100 ng/mL of KaryoMax colcemid (Thermo Fisher Scientific, USA) was added to the cells for 2 h at passage 12. The cells were exposed to a hypotonic solution (0.75 M KCL, Thermo Fisher Scientific, USA) for 20 min at 37 °C and later fixed and stored in methanol/acetic acid (3:1) solution. The cells were shipped for analysis to Universitätsklinikum, Institut für Humangenetik, Postfach, 07,740 Jena, Germany, with a mean resolution of 300 bands per haploid microscope set. According to the ISCN 2016, the karyotype formula is given reflecting the analysis of

20 metaphases.

4.5. In vitro trilineage spontaneous differentiation

The iPSCs, at passage 13, were dissociated using tryplE (Thermo Fisher Scientific, USA), and plated on ultra-low attachment plates (1:1) for spontaneous formation of embryoid bodies (EBs) by culture in DMEM/F12 media supplemented with 20% Knockout Serum Replacement (KSR), 1 mM L-glutamine, 1% NEAA, 0.1 mM 2-mercaptoethanol, and 1% (v/v) penicillin–streptomycin. After 4 days of suspension culture, the EBs were plated for an additional 14 days on Matrigel-coated plates and then examined to express different germ layer markers using the specific antibodies listed in Table 2.

4.6. Short tandem repeat (STR) profiling

The AmpFISTR Identifiler Plus PCR Amplification Kit (Applied biosynthesis, Life Technologies) was used to detect 15 different Loci for STR analysis. DNA of the generated iPS cell line was extracted under highly sterile condition using the DNA purification kit (NORGEN, BIOTEK).

4.7. Mycoplasma detection test

Mycoplasma was routinely checked in the media through RT-PCR, using specific primers for mycoplasma (Table 2), as shown at passage 15.

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.2021.102500.

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