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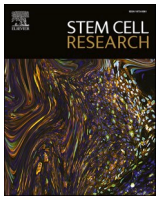

Lab Resource: Single Cell Line

Generation of induced pluripotent stem cell line (RCMGi012-A) from fibroblasts of patient with mucopolysaccharidosis type VI

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A B S T R A C T

Skin fibroblasts obtained from a 5-year-old girl with genetically proven (two heterozygous mutations in *ARSB* gene) and clinically manifested mucopolysaccharidosis type VI were successfully transformed into induced pluripotent stem cells by using Sendai virus-based reprogramming vectors including the four Yamanaka factors namely SOX2, OCT3/4, KLF4, and c-MYC. These iPSCs expressed pluripotency markers, had a normal karyotype and the potential to differentiate into three germ layers in spontaneous differentiation assay. The line may be used for cell differentiation and pharmacological investigations, and also may provide a model for development of a personalized treatment including drug screening and genome editing.

Resource table

Unique stem cell line identifier	RCMGi012-A
Alternative name(s) of stem cell line	P11L3
Institution	Research Centre for Medical Genetics, Moscow, Russia
Contact information of distributor	MD Ekaterina Kondrateva,
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 5 Sex: female Ethnicity: Caucasian
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus (OCT3/4, SOX2, KLF4, and c-MYC)
Genetic Modification	Yes
Type of Modification	Hereditary
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Mucopolysaccharidosis type VI
Gene/locus	Gene: *ARSB* Locus: 5q14.1 Mutation:

(*continued on next column*)

Resource table (*continued*)

	NM_000046.5:c.966G>A NC_000005.9: g.78241273_78248429delins77831940_77883742inv NC_000005.9:g.77831892_77883763delins [78248357_78248437inv; TAGCCTGGGAACATA]
Date archived/stock date	December 2022
Cell line repository/ bank	https://hpscreg.eu/cell-line/RCMGi012-A
Ethical approval	The study was approved by the local ethics committee of the Federal State Budgetary Institution "Research Centre for Medical Genetics" (the approval number 2015–5/3)

1. Resource utility

Induced pluripotent stem cells (iPSCs) line was derived from donor with mucopolysaccharidosis type VI who is heterozygous for two mutations in the *ARSB* gene. These iPSCs may be subsequently used for cell differentiation, organoid formation and disease modelling, development of a treatment including pharmacological investigations and genome editing.

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2. Resource details

Maroteaux–Lamy syndrome (mucopolysaccharidosis type VI, MPS-VI) is a rare genetic autosomal recessive disease from the group of lysosomal storage disorders. MPS-VI is caused by a deficiency of the lysosomal enzyme aryl-sulfatase B (ASB), encoded by the *ARSB* gene (NM_000046.5). ASB is involved in a degradation of glycosaminoglycans (GAGs) dermatan sulfate and chondroitin-4-sulfate. Accumulation of these GAGs in the cells' lysosomes and extracellular matrix leads to the progressive damage to cells and tissues with subsequent development of the multisystemic disorder. Clinical phenotypes of patients with MPS VI represent a continuum, ranging from severe early-onset forms with rapid progression of osteo-articular signs, to later-onset slowly progressing forms, which are often noted only by the presence of coarsened facial features (Harmatz and Shediac, 2017).

Here, we established an iPSC line derived from a 5-year-old girl with clinically manifested (severe growth retardation, kyphoscoliosis and hand contracture) MPS-VI. Sanger sequencing of the patient's *ARSB* gene identified heterozygous pathogenic nonsense variant c.966G>A (p.Trp322*) in the exon 5. WGS with subsequent RNA analysis determined new pathogenic variant which arose from a non-allelic homologous recombination between *ARSB* and the neighboring *LHFPL2* gene causing premature transcription termination (Bychkov et al., 2022) (Table 1).

Fibroblasts were transformed into iPSCs by using Sendai virus-based reprogramming vectors expressing the four Yamanaka factors, OCT3/4, SOX2, KLF4, and c-MYC, with 5:5:4 MOI ratio for KOS, c-Myc and Klf4 viruses, respectively. iPSC clone named RCMGi0012-A was purified (Fig. 1A). At passage 8 the expression of pluripotency markers SSEA4, OCT4, SOX2, and NANOG was detected and counted by immunocytochemistry assay (Fig. 1B, 1C). Sanger sequencing confirmed the presence of the c.966G>A mutation in heterozygous state and the presence of specific product of second mutation in fibroblasts and iPSC line (Fig. 1D). Potential of iPSCs to differentiate into three germ layers was proven in spontaneous differentiation assay by formation of embryoid bodies (EB) and analyzing them by immunocytochemistry staining which was positive for FOXA2 (endodermal marker), tubulin beta (ectodermal marker), and vimentin (mesodermal marker) (Fig. 1F). iPSCs karyotype was inspected by in-house Giemsa-banding of at least 15 metaphase spreads based on ISCN 2016. At passage 15 RCMGi0012-A showed a normal diploid 46,XX karyotype, without any detectable abnormalities (Fig. 1G). Short tandem repeat (STR) analysis confirmed the origin of the cell line RCMGi012-A from the fibroblasts of the indicated patient. Sendai virus genome and transgenes elimination was confirmed on passage 15 according to the manufacturer' protocol for CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen) using the provided primers (listed in Table 2). Mycoplasma testing was carried out using MycoReport kit (Evrogen) which is based on end-point PCR with amplification of species-specific regions of 16S rRNA genes and showed that this line at passage 15 were not infected by mycoplasma. Stable iPSC line was collected in Moscow Branch of the Biobank "All-Russian Collection of Biological Samples of Hereditary Diseases".

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemisty)	Positive for pluripotency markers: SSEA-4, NANOG, SOX2, OCT-4	Fig. 1 panel B
	Quantitative analysis (Immunocytochemisty counting)	100 % SSEA-4, 99.9 % NANOG, 99.9 % SOX2, 100 % OCT-4	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46,XX Resolution: 1000	Fig. 1 panel F
Identity	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	20 STR sites analyzed, 17 matching, 3 non-informative	Available with the authors
Mutation analysis (IF APPLICABLE)	Sanger sequencing	Heterozygous type of *ARSB* mutation c.966G>A Non-allelic homologous recombination between *ARSB* and *LHFPL2*	Fig. 1 panel D
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Testing by MycoReport (Evrogen) Negative	Supplementary files Fig. 2
Differentiation potential	In vitro spontaneous differentiation: embryoid bodies formation and immunocytochemisty	Positive expression of differentiation markers: Endoderm: FOXA2 Ectoderm: Beta tubulin III Mesoderm: Vimentin	Fig. 1 panel E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	Not performed
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	Not performed
	HLA tissue typing	Not performed	Not performed

3. Materials and methods

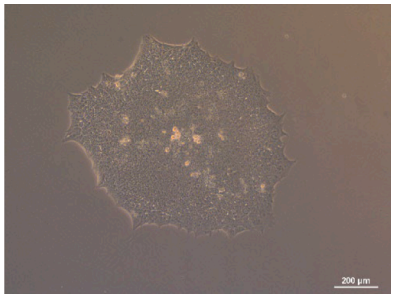
3.1. iPSCs generation and culturing

Fibroblasts culture was obtained from biopsy material by cultivation in Amniocar medium (PanEco). On second passage cells were transferred to fibroblast medium (DMEM high glucose, GlutaMAX™ and pyruvate (Life Technologies), 10% fetal bovine serum (FBS), 1% MEM non-essential amino acids solution (Gibco), 50 U/ml penicillin, 50 μg/ml streptomycin). On 1st day reprogramming of 100,000 cells/well was performed using mentioned kit, and on the 8th day the cells were reseeded into plates pre-coated with Matrigel (Corning). iPSC clones were picked from 14th day and cultured in TeSR™-E8™ medium (E8) (Stemcell Technologies) at 37°C, 5% CO_2 with passaging every 4–7 days (split ratio 1:6) using Gentle Cell Dissociation Reagent (GCDR) and with 5 μM Y27632 ROCK inhibitor (Stemcell Technologies) for 24 h.

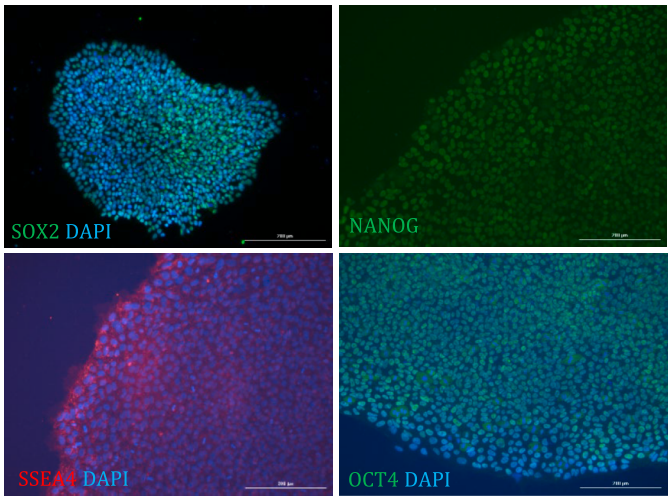
3.2. Immunocytochemistry counting

Cells were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0,25% Triton X-100 at RT for 10 min and blocked with 1% BSA in PBS at RT for 30 min. Antibodies were diluted in 1% BSA. Samples were incubated with primary and secondary antibodies at RT for 1 h, then nuclei were counterstained using DAPI (Abcam) at RT for 10 min. Fluorescent images were captures using Lionheart FX Automated Microscope (BioTek), processed and analyzed using CellProfiler 3.0.0. Cell cytoplasm and nuclei were determined, the average fluorescence intensity of staining per cell was measured and the percentage of positives cells in the green and red channels was counted.

A



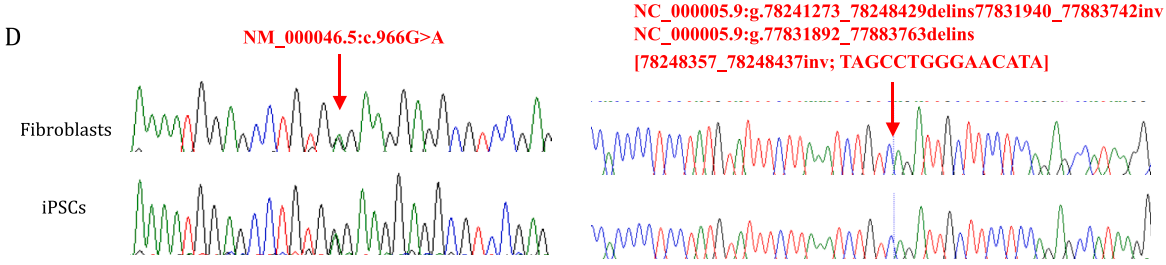
B



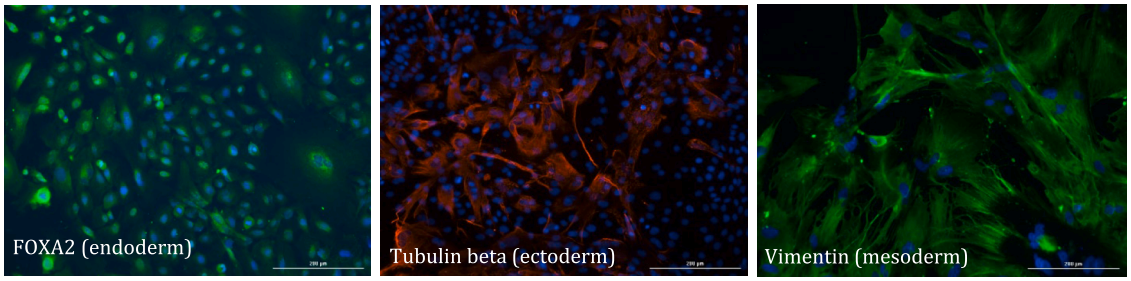
C

Marker	Percentage of stained cells (mean ± sd, n (images) = 5)
SOX2	99.9 ± 0.13 %
NANOG	99.9 ± 0.13 %
OCT4	100.0 ± 0.12 %
SSEA4	100.0 ± 0.12 %

D



E



F

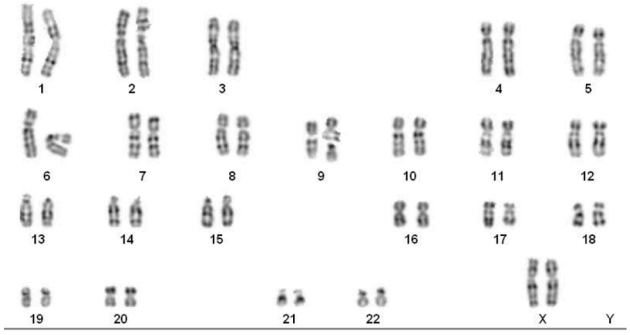


Fig. 1. ….

Table 2
Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	SSEA4 Monoclonal Antibody (MC813-70), Mouse	1:100	Thermo Fisher Scientific, Cat # 41-4000	RRID: AB_2533506
	Anti-Oct4 Antibody, Rabbit	1:100	Abcam, Cat # ab18976	RRID:AB_444714
	Nanog (D73G4) XP Antibody, Rabbit	1:200	Cell Signaling Technology, Cat# 4903	RRID: AB_10559205
	Sox2 (D6D9) XP Antibody, Rabbit	1:400	Cell Signaling Technology, Cat # 3579	RRID: AB_2195767
Differentiation Markers	Recombinant Anti-Vimentin Antibody [EPR3776], Rabbit	1:250	Abcam, Cat # Ab92547	RRID: AB_10562134
	Recombinant Anti-FOXA2 antibody [EPR446], Rabbit	1:200	Abcam, Cat # 108,396	RRID: AB_10863255
	Anti-beta III Tubulin antibody [2G10], Mouse	1:300	Abcam, Cat # ab131205	RRID: AB_2256751
Secondary antibodies	Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:150	Abcam, Cat #: A21206	RRID: AB_2535792
	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	1:150	Cat# A-11032,	RRID: AB_2534091
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sanger sequencing	*ARSB* c.966G>A locus	693 bp	CTGGGCCTTACTTATGTCTG TCAAAGACCTTCCATGGAGG	
	Specific product of non-allelic homologous recombination between *ARSB* and *LHFPL2*	309 bp	GCACCCACTGTCCACAATCC CGATTAGCATTGGTCTGAAGGTG	
Sendai virus elimination	SeV	181 bp	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528 bp	ATGCACCGCTACGACGTGAGCGC ACCTTGACAATCCTGATGTGG	
	Klf4	410 bp	TTCCTGCATGCCAGAGGAGCCC AATGTATCGAAGGTGCTCAA	
	c-Myc	532 bp	TAACTGACTAGCAGGCTTGTCG TCCACATACAGTCCTGGATGATGATG	

3.3. Mutation verification

Genomic DNA was isolated from fibroblasts and iPSCs using Quick-DNA Miniprep Kit (Zymo Research). *ARSB* locus with c.966G>A and specific product of non-allelic homologous recombination were PCR-amplified (98°C 3′; 35x (95°C 5″, 60°C 5″, 72°C 30″)) using HF-Fuzz DNA polymerase (Dialat) in Eppendorf Mastercycler (Eppendorf). Sanger sequencing of PCR products (95°C 3′; 25x (95°C 15″, 50°C 15″, 60°C 2′)) was performed using ABI Prism 3130XL Genetic Analyzer (Applied Biosystems).

3.4. Karyotyping

iPSCs were cultured as described until reaching 70–80% confluency. 10 μg/ml colchicine solution (PanEco) was added to cells for 40 min to arrest mitosis. Then cells were treated by hypotonic solution (0.075M KCl) at 37C for 13 min and fixed by twice incubation (30 and 20 min) in cooled solution made of 3 parts methanol and 1 part glacial acetic acid. The chromosome spread was analyzed under the Axio Imager 2 microscope (ZEISS).

3.5. Spontaneous differentiation

On the 13th passage, the iPSCs were detached using GCDR and transferred in E8 with Y27632 to ultra-low attachment plate (Corning). On day 3 the half of medium was replaced and on the day 6 all the medium was replaced with EB medium consisting of Advanced DMEM/F12 (Gibco), 20% KO Serum replacement (Gibco), 1% MEM NEAA, 2 mM L-glutamine (Gibco), 50 U/ml penicillin, 50 μg/ml streptomycin. Thereafter, the medium was replaced every 2 days with an addition of 1% FBS and then stepwise increase it from 1 to 10%. In about 2 weeks EB were transferred onto gelatin-coated plates in EB medium with 10% FBS. After 7–14 days the expression of markers was analyzed using immunocytochemistry.

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.

Acknowledgements

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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.2023.103259.

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