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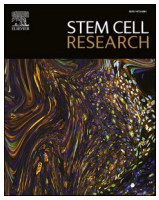

Lab Resource: Single Cell Line

Generation of induced pluripotent stem cell line (RCMGi008-A) from human skin fibroblasts of a cystic fibrosis patient with compound heterozygous F508del/CFTRdele2.3 mutations in CFTR gene

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A B S T R A C T

Skin fibroblasts obtained from a 20-year-old woman with clinically manifested and genetically proven (F508del/CFTRdele2.3) cystic fibrosis were successfully transformed into induced pluripotent stem cells (iPSCs) by using Sendai virus-based reprogramming vectors including the four Yamanaka factors, OCT3/4, SOX2, KLF4, and c-MYC. The iPSCs showed a normal karyotype, expressed pluripotency markers and exhibited the potential to differentiate into three germ layers in spontaneous differentiation assay. This iPSC line may be used for development of a personalized treatment including genome editing, disease modelling, cell differentiation and organoid formation, pharmacological investigations and drug screening.

1. Resource Table:

Unique stem cell line identifier	RCMGi008-A
Alternative name(s) of stem cell line	P9L1
Institution	Research Centre for Medical Genetics, Moscow, Russia
Contact information of distributor	MD Ekaterina Kondrateva, ekaterina.kondratyeva@gmail.com
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 20–24 Sex: female Ethnicity: Caucasian
Cell Source	skin fibroblast
Clonality	clonal
Method of reprogramming	Sendai virus (4 transgenes: 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	Cystic Fibrosis
Gene/locus	Gene: CFTR Locus: 7q31.2 Mutation:

(*continued on next column*)

(*continued*)

Unique stem cell line identifier	RCMGi008-A
	NM_000492.3(CFTR):c.[1521_1523delCTT] NM_000492.3(CFTR):c.54-5940_273 + 10250del
Date archived/stock date	December 2019
Cell line repository/bank	https://hpscreg.eu/cell-line/RCMGi008-A
Ethical approval	This study was approved by FSBSI RCMG Ethics Committee (Protocol # 1 from 28.01.2016)

2. Resource utility

Induced pluripotent stem cells line was derived from a cystic fibrosis donor who is compound heterozygous for F508del and CFTRdele2.3(21 kb) mutations in the *CFTR* gene. This line may provide a model for development of a personalized etiotropic treatment including genome editing, for disease modelling, cell differentiation and organoid formation, pharmacological investigations and drug screening.

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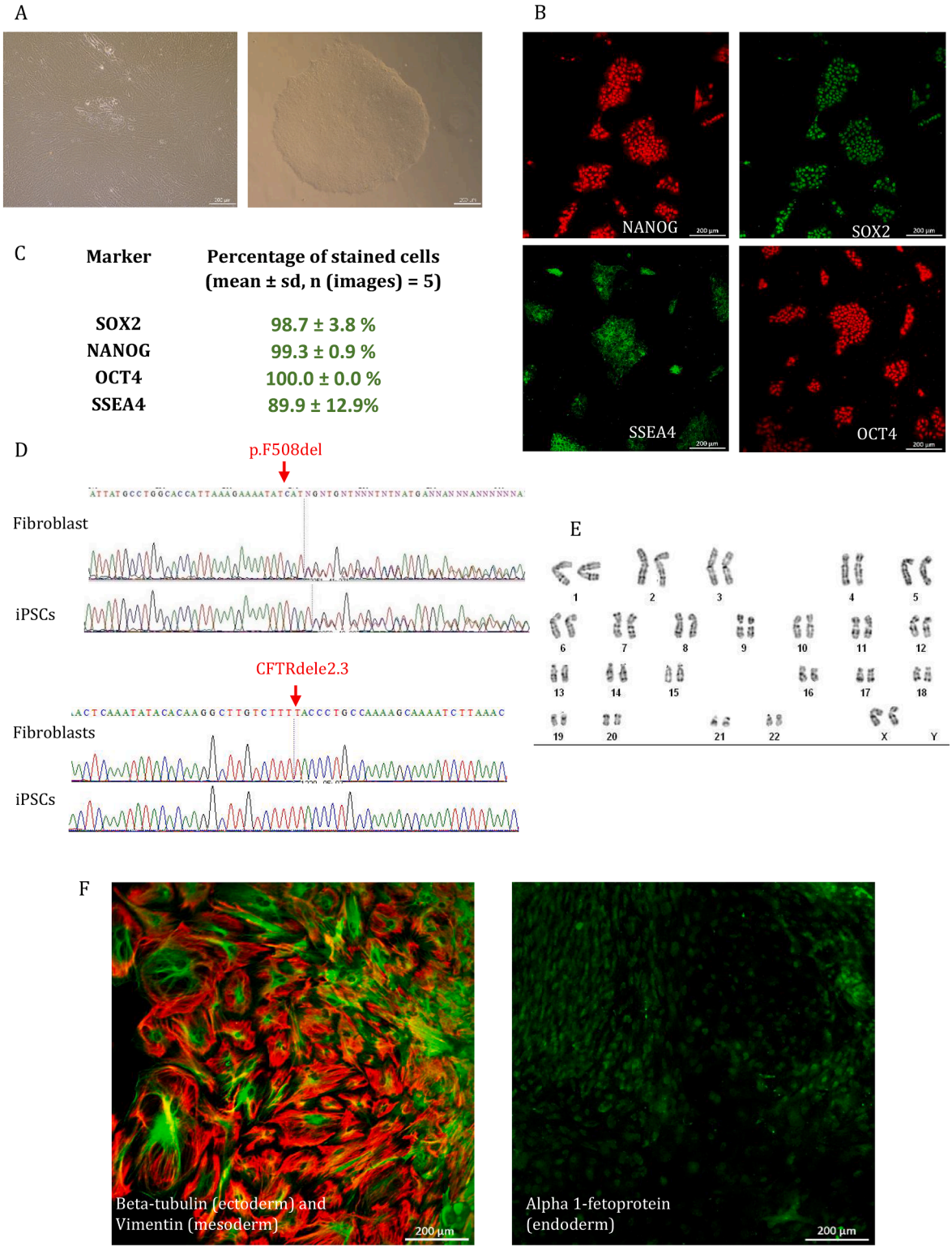

Fig 1. Characterization of RCMGi008-A cell line.

3. Resource details

Cystic fibrosis (CF) is a monogenic multisystem and one of the most common hereditary diseases: its average frequency among Caucasians is about 1 to 2000–3000 newborns (Chen et al., 2021). This pathology is caused by mutations in the *CFTR* gene, and the most frequent one is F508del (allele frequency is 85.8% among CF patients) according to CFF Patient Registry 2020 Annual Data Report (Marshall et al., 2020). CFTRdele2.3(21 kb) mutation (other name p.Ser18Argfs*16) is one of the most common mutations (allele frequency is 6.21%) in ethnic Russians population according RF CF Registry (Kashirskaya, 2018). Both of these mutations are associated with a severe disease phenotype. Despite

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)	SSEA-4: 89.9%, NANOG: 99.3%, SOX2: 98.7%, OCT-4: 100%	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46,XX Resolution: 1000	Fig. 1 panel E
Identity	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	20 STR sites analyzed, 20 matching	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sanger sequencing	Heterozygous type of mutation F508del; specific PCR product of CFTRdele2.3	Fig. 1 panel D
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Testing by MycoReport (Evrogen) Negative	Supplementary files, Fig. 1
Differentiation potential	In vitro spontaneous differentiation: embryoid bodies formation and immunocytochemisty	Positive expression of differentiation markers: Endoderm: Alpha 1-Fetoprotein Ectoderm: Tubulin beta class I Mesoderm: Vimentin	Fig. 1 panel F
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

the success of pathogenesis-based and palliative therapy, in most cases CF is incurable disease, which makes the development of new treatment consideration like gene therapy also promising. Somatic cells such as fibroblasts can be reprogrammed into iPSCs, which can be used to develop cell or gene and cell therapy. Here, we established an iPSC line derived from a 20-year-old woman with CF, clinically manifested and genetically proven (F508del/CFTRdele2.3mutations). Skin 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, respectively. iPSC clone named RCMGi008-A was purified (Fig. 1A). The expression of pluripotency markers SSEA4, OCT4, SOX2, and NANOG was detected and counted by immunocytochemistry assay (Fig. 1B and C) at passage 14. Fibroblasts and iPSCs lines carry F508del in heterozygous state (Fig. 1D). To verify the CFTRdele2.3 deletion, specific PCR products were obtained and sequenced too (Fig. 1D). IPSCs karyotype was analysed by in-house GTG-banding analysis of at least 15 metaphase spreads based on ISCN 2016. At passage 16 RCMGi008-A showed a normal 46,XX karyotype (Fig. 1E). Potential of iPSCs to differentiate into three germ layers was shown in spontaneous differentiation assay with embryoid bodies (EB) formation. EB were formed and analyzed by immunocytochemistry assay, which was positive for alpha 1-fetoprotein (endodermal marker), beta III tubulin (ectodermal marker), and vimentin (mesodermal marker) (Fig. 1F). In addition, 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. It revealed that these iPSCs at passage 16 were not infected by mycoplasma. STR analysis was performed using a kit that evaluates the genetic signatures of 20 microsatellite markers (listed in the STR report). It confirmed the origin of the cell lines from the fibroblasts of the indicated patient. Sendai virus genome and transgenes elimination was confirmed on passage 17 according to the manufacturer' protocol for CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen) using the provided primers (listed in Table 2). Stable iPSCs line was collected in Moscow Branch of the Biobank "All-Russian Collection of Biological Samples of Hereditary Diseases" (Table 1).

4. Materials and methods

4.1. iPSCs derivation and cultivation

Fibroblasts culture was obtained from biopsy material by cultivation in Amniocar medium (PanEco). On second passage the 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 ug/ml streptomycin). On day 1 reprogramming of 100.000 cells/well was performed using reprogramming kit mentioned above. On the 8th day the cells were reseeded into cell culture plates pre-coated with Vitronectin (Gibco). A day later, cells were transferred to Essential 8™ Medium (E8) (Gibco) with daily replacement thereafter. On days 16–19 we observed the appearance of colonies. On day 26, some colonies were picked manually and replated. One iPSC clone was then cultured in E8 with passaging every 4–7 days depending on confluence, with 1:6 split ratio, using Gentle Cell Dissociation and with addition of Y27632 ROCK inhibitor (Stemcell Technologies) at 5 μM for 24 h. Cells were cultivated at 37 °C, 5% CO_2.

4.2. Immunocytochemistry counting

Cells were fixed with 4% paraformaldehyde at 4 °C for 20 min, permeabilized with 0,25% Triton X-100 in PBS at RT for 10 min and blocked with 1% BSA (Gibco) in PBS at RT for 30 min. Antibodies (Table 2) were diluted in 1% BSA. Samples were incubated with primary antibodies at 4 °C overnight and then with secondary antibodies at RT for 1 h in the dark. Nuclei were counterstained using DAPI (Abcam) at RT for 10 min. Fluorescent images were captures using Lionheart FX Automated Microscope (BioTek). Images were processed and analyzed using CellProfiler 3.0.0. Cell cytoplasm and nuclei were determined and then the average fluorescence intensity of staining per cell was measured and the percentage of positives cells in the green and red channels was counted.

4.3. Karyotyping

Incubation of cells in 10 ug/ml colchicine solution (PanEco) lasted for 35–40 min to arrest mitosis followed by hypotonic treatment (0.075 M KCl) at 37C for 13 min. Fixation was done by twice incubation in 3:1 cooled solution made of 3 parts methanol and 1 part glacial acetic acid for 30 and 20 min.

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 Polyclonal Antibody, Rabbit	1:200	Thermo Fisher Scientific, Cat # PA1-097X	RRID:AB_2539868
	Anti-SOX2 Antibody [9–9–3], Mouse	1:200	Abcam, Cat # ab79351	RRID:AB_10710406
Differentiation Markers	Recombinant Anti-Vimentin Antibody [EPR3776], Rabbit	1:250	Abcam, Cat # Ab92547	RRID:AB_10562134
	Anti-alpha 1 Fetoprotein Antibody [AFP-01], Mouse	1:200	Abcam, Cat # ab3980	RRID:AB_304203
	Anti-beta Tubulin antibody [1E1-E8-H4], Mouse	1:300	Abcam, Cat # ab131205	RRID:AB_11156121
Secondary antibodies	Goat Anti-Mouse IgG H&L (Alexa Fluor® 488)	1:200	Abcam, Cat # ab150113	RRID:AB_2576208
	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647)	1:200	Abcam, Cat # 150,079	RRID:AB_2722623
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sanger sequencing	*CFTR* F508del locus	503 bp	TGCATAGCAGAGTACCTGAAACAGGA TTGATCCATTCACAGTAGCTTACCCA	
	CFTR CFTRdele2.3 locus	212 bp	GAGCTTCTGAAATTAATTGACCAC ACTCAGAACCCATCATAGGATAC	
Sendai virus elimination	SeV	181 bp	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528 bp	ATGCACCGCTACGACGTGAGCGC ACCTTGACAATCCTGATGTGG	
	Klf4	410 bp	TTCCTGCATGCCAGAGGAGCCC AATGTATCGAAGGTGCTCAA	
	c-Myc	532 bp	TAACTGACTAGCAGGCTTGTCG TCCACATACAGTCCTGGATGATGATG	

4.4. Sanger sequencing

Genomic DNA was isolated from fibroblasts and iPSCs using Quick-DNA Miniprep Kit (Zymo Research). PCR was performed using Taq DNA polymerase (Evrogen) and Eppendorf Mastercycler (Eppendorf). PCR conditions were: 95 °C for 3 min; 35 cycles of 95 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 60 sec; 72 °C for 5 min. PCR products were analyzed by Sanger sequencing using ABI Prism 3130XL Genetic Analyzer (Applied Biosystems). Cycle parameters were: 95 °C for 3 min; 25 cycles of 95 °C for 15 sec, 50 °C for 15 sec, and 60 °C for 2 min.

4.5. EB Formation

On the 10th passage, the IPSCs were detached using GCDR and transferred in E8 with Y27632 to ultra-low attachment plate (Corning Costar). On day 4, the medium was replaced with new medium consisting of 1⁄2 vol of E8 and 1⁄2 vol of ES medium (Advanced DMEM/F12 (Gibco), 20% KO Serum replacement (Gibco), 2 mM L-glutamine (Gibco), 1% MEM NEAA, 50 U/ml penicillin, 50 ug/ml streptomycin). On day 7, the medium was replaced with EB medium consisting of 1⁄2 vol of ES medium and 1⁄2 vol of fibroblasts medium without FBS. Thereafter, the medium was replaced every 2–3 days with a stepwise increase in the percentage of FBS from 1 to 10%. In about one month EB were transferred onto gelatin-coated plates in EB medium with 10% FBS. After 14 days the expression of markers was analyzed using immunocytochemistry (Table 2).

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.2022.102854.

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