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Lab resource: Stem Cell Line

Generation of iPSC line ICGi024-A from human skin fibroblasts of a patient with ring chromosome 18

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A B S T R A C T

Ring chromosome 18 is a rare chromosomal disorders that usually originate *de novo* and correlate with clinical manifestation: developmental delay as well as microcephaly, brain and ocular malformations, hypotonia and skeletal abnormalities. We generate iPSC clonal cell line ICGi024-A with pluripotency properties which were demonstrated *in vitro* by three germ layer differentiation capacity. ICGi024-A can be used for disease modeling and fundamental investigation of ring chromosome instability.

Resource Table

Unique stem cell line identifier	ICGi024-A
Alternative name(s) of stem cell line	iTAF12-19
Institution	The Federal Research Center Institute of Cytology and Genetics The Siberian Branch of the Russian Academy of Sciences
Contact information of distributor	Anna A. Khabarova anya.khabarova@gmail.com
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 3 Sex: male Ethnicity if known: Caucasian
Cell Source	skin fibroblast
Clonality	Clonal
Method of reprogramming	Transgene free episomal plasmid vectors (SOX2, KLF4, OCT4, L-MYC, LIN28, p53 carboxy-terminal dominant-negative fragment (mp53DD), EBNA1)
Genetic Modification	Yes
Type of Modification	Congenital *de novo* mutation
Associated disease	Developmental and speech delay, dysmorphic features, and café au lait spots
Gene/locus	N/A
Method of modification	N/A N/A
Name of transgene or resistance	
Inducible/constitutive system	N/A
Date archived/stock date	2019
Cell line repository/bank	Collective Center of ICG SB RAS "Collection of Pluripotent Human and Mammalian Cell Cultures for Biological and Biomedical Research"; Bioresource collection of the Research Institute of Medical Genetics, Tomsk NRMC, "Biobank of the population of Northern Eurasia"
Ethical approval	Scientific Ethics Committee of Research Institute of Medical Genetics, Tomsk NRM: 106/2017

(*continued on next column*)

Resource Table (*continued*)

1. Resource utility

Ring chromosome 18 is a rare chromosome abnormality. The ICGi024-A line was established from the fibroblasts of a 3-year-old boy with 46,XY,r(18). The ICGi024-A hiPSC line is a good model for studying the instability of the ring chromosome because of unlimited proliferative ability of human iPSCs (Table 1).

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel C
Phenotype	Qualitative analysis Immunocytochemistry and RT-PCR	Positive for pluripotency markers: OCT4, NANOG, SOX2, SSEA4 and TRA-1–60	Fig. 1 panel F, G, I
	Quantitative analysis. Immunocytochemistry counting	% of positive cells POU5F1 (OCT-4) 95.1% TRA 1–60 97.8% NANOG 87.8% SSEA-4 99.1%	Fig. 1 panel F and G
Genotype	Karyotype (G-banding)	46,XY,r(18)[50]/45,XY,-18[6]/47,XY,r(18),r(18)[1]	Fig. 1 panel B, H
Identity	Microsatellite PCR (mPCR) OR STR analysis	DNA Profiling not performed The STR profile of the ICGi024-Acell line totally matched with that of the parental TAF12 fibroblasts (loci analyzed:D3S1358, TH01, D12S391, D1S1656, D10S1248, D22S1045, D2S441, D7S820, D13S317, FGA, TPOX, D18S51, D16S539, D8S1179, CSF1PO, D5S818, vWA, D21S11, SE33).	
Mutation analysis (IF APPLICABLE)	Sequencing Southern Blot OR WGS	N/A N/A	
Microbiology and virology	Mycoplasma	Negative	Fig. 1 panel E
Differentiation potential	Embryoid body formation (*In vitro* spontaneous differentiation)	Differentiation potency: Endoderm: positive for expression of *AFP, SOX17, FOXA2 (HNF3B)* Mesoderm: positive for expression of *MSX1, FLK1, TBXT* Ectoderm: positive for expression of *SOX1, MAP2, PAX6*	Fig. 1 panel J
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	N/A N/A	

2. Resource details

Human skin fibroblasts TAF12 were derived from a 3-year-old male with developmental and speech delay and ring chromosome 18 karyotype (46,XY,r(18)(p11.1q23)[38]. arr[hg19] 18p11.32p11.21 (14316_13530125) × 1 dn, 18q23(76744252_77982126) × 1 dn) (Fig, 1A, H). Fibroblasts were reprogrammed into iPSCs through episomal vector transfection (Okita et al., 2013). Vectors did not integrate in the genome as was shown by PCR (Fig. 1D). The ICGi024-A cells had typical morphology of human iPSCs under feeder-dependent conditions in phase contrast microscopy (Fig. 1C), and expressed *OCT4* (95.1%) and *NANOG* (87.8%) pluripotency markers (red) in the nucleus and *SSEA4* (99.1%) and *TRA-1–60* (97.8%) surface markers (green), as detected by immunofluorescence staining (Fig. 1F, G) (Table 2). RT-PCR showed the presence of expression of *OCT4*, *SOX2, NANOG* and *GAPDH* (as a control) in ICGi024-A and ICAGi001-A (as a control of previously described iPS cell line), NTC – no template control with water and without cDNA, RT- - control with RNA without revertase adding (Fig. 1I). Nuclei stained by DAPI (blue). ICGi024-A cell line had 46,XY,r(18)[50]/45,XY,-18[6]/47,XY,r(18),r(18)[1] karyotype (Fig. 1B). The STR profile of the ICGi024-A cell line fully matched with that of the parental TAF12 fibroblasts (loci analyzed:D3S1358, TH01, D12S391, D1S1656, D10S1248, D22S1045, D2S441, D7S820, D13S317, FGA, TPOX, D18S51, D16S539, D8S1179, CSF1PO, D5S818, vWA, D21S11, SE33). The ICGi024-A cell line was negative for Mycoplasma contamination (Fig. 1E). The ICGi024-A cell line was able to differentiate into cells of the three germ layers following embryoid body formation that was assessed by RT-PCR for endodermal (*AFP, SOX17* and *FOXA2*), mesodermal (*MSX1, FLK1* and *TBXT*) and ectodermal (*SOX1, MAP2* and *PAX6*) genes (Fig. 1J). These results clearly demonstrate that the ICAGi001-A cells are pluripotent.

3. Materials and methods

Cell culture, immunocytochemistry and immunocytochemistry counting, *In vitro* differentiation of iPS cells, karyotyping and RT-PCR analysis was performed as it was previously described in Khabarova et al. (2019). For confirmation the absence of *Mycoplasma* contamination by PCR we usd primers from Choppa et al. (1998).

For generation of iPSCs 5×10^5 of the fibroblasts were electroporated at 1650 V, 10 ms, 3 pulses with 6 μg episomal vectors cocktail in the volume 100 μl by using Neon Transfection System. The episomal reprogramming vectors expressed GFP (addgene #41858), OCT3/4 (addgene #41813), MYC and LIN28 (addgene #41855), shRNA against p53 (addgene #41856), SOX2 and KLF4 (addgene #41814), EBNA1 (addgene # 41857). On day 3, the cells were seeded on feeder layer ($25 \times 10^3/cm^2$) in iPSC medium (DMEM-F12 with 20% KSR 1% GlutaMAX-I, 1% MEM NEAA, 1% Pen Strep, 0.1 mM 2-mercaptoethanol, and 10 ng/ml bFGF (Invitrogen)). From the day 7 to 16 the culture medium was changed daily. On day 16, colonies with iPSC morphology were picked up and expanded. iPSCs were cultured at 37 °C in an atmosphere of 5% CO2 and passed mechanically with split ratio 1:5.

STR analysis for parental TAF12 fibroblasts and the ICGi024-A cells was performed by Gordiz (http://gordiz.ru/).

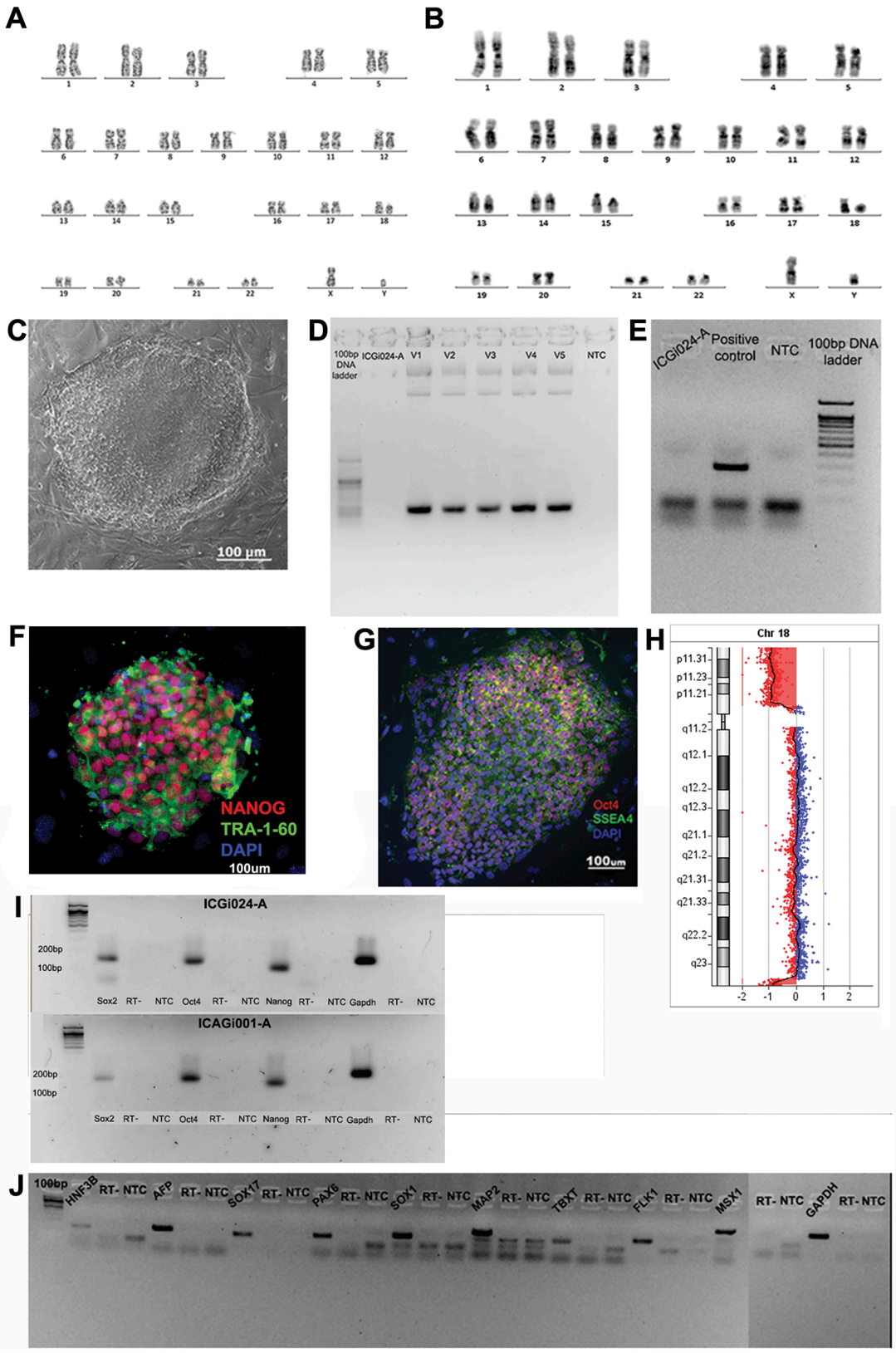

Fig. 1. Characterization of ICGi024-A line. (A) Karyotypes (human skin fibroblasts TAF12). (B) Karyotypes (ICGi024-A line). (C) Morphology of the iPSC colonies. (D) Absence of vector integration. (E) Mycoplasma contamination test. (F) Immunofluorescence staining for the pluripotency markers NANOG and TRA-1-60. (G) Immunofluorescence staining for the pluripotency markers OCT4 and SSEA4. (H) aCGH of ring chromosome 18. (I) Expression of the pluripotency markers *SOX2*, *NANOG* and *OCT4* in ICGi024-A and ICAGi-001A. (J) Expression of the endoderm (*AFP*, *SOX17* and *FOXA2*), mesoderm (*MSX1*, *FLK1* and *TBXT*) and ectoderm (*SOX1*, *MAP2* and *PAX6*) markers in the embryoid bodies and in ICGi024-A.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-NANOG	1:100	Abcam Cat# 21624, RRID: AB_446437
Pluripotency Markers	Rabbit anti-OCT4	1:200	Abcam Cat# 19857, RRID: AB_445175
Pluripotency Markers	Mouse anti- SSEA4	1:600	Abcam Cat# 16287, RRID:AB_778073
Pluripotency Markers	Mouse anti-TRA-1–60	1:600	Abcam Cat# 16288, RRID:AB_778563
Secondary antibodies	Alexa Fluor 546 Goat Anti-Rabbit IgG	1:400	Life technologies Cat# A11010, RRID:AB_143156
Secondary antibodies	Alexa Fluor 488 Goat Anti-Mouse IgG	1:400	Life technologies Cat# A32723, RRID:AB_2633275

Primers

	Target	**Forward/Reverse primer (5′-3′)**
House-Keeping Genes	*GAPDH*	GTGGACCTGACCTGCCGTCT/GGAGGAGTGGGTGTCGCTGT Expected product size: 153 bp
Pluripotency Marker	*OCT4*	CTGGGTTGATCCTCGGACCT/CACAGAACTCATACGGCGGG Expected product size: 128 bp
Pluripotency Marker	*NANOG*	AAAGAATCTTCACCTATGCC/GAAGGAAGAGGAGAGACAGT Expected product size: 110 bp
Pluripotency Marker	*SOX2*	AAGGATAAGTACACGCTGCCC/GCTTCAGCTCCGTCTCCAT Expected product size: 128 bp
Plasmid primer	*pEP4-SF1-oriP*	TTCCACGAGGGTAGTGAACC/TCGGGGGTGTTAGAGACAAC Expected product size: 544 bp
Differentiation Markers	*AFP*	AAATGCGTTTCTCGTTGCTT/GCCACAGGCCAATAGTTTGT Expected product size: 136 bp
Differentiation Markers	*SOX17*	CTCTGCCTCCTCCACGAA/CAGAATCCAGACCTGCACAA Expected product size: 102 bp
Differentiation Markers	*FOXA2(HNF3B)*	GGAGCGGTGAAGATGGAA/TACGTGTTCATGCCGTTCAT Expected product size: 122 bp
Differentiation Markers	*MSX1*	CGAGAGGACCCCGTGGATGCAGAG/GGCGGCCATCTTCAG Expected product size: 307 bp
Differentiation Markers	*FLK1*	TGATCGGAAATGACACTGGA/CACGACTCCATGTTGGTCAC Expected product size: 131 bp
Differentiation Markers	*TBXT*	AATTGGTCCAGCCTTGGAAT/CGTTGCTCACAGACCACA Expected product size: 112 bp
Differentiation Markers	*MAP2*	CAGGTGGCGGACGTGTGAAAATTGAGAGTG/CACGCTGGATCTGCCTGGGGACTGTG Expected product size: 212 bp
Differentiation Markers	*SOX1*	CACAACTCGGAGATCAGCAA/GGTACTTGTAATCCGGGTGC Expected product size: 133 bp
Differentiation Markers	*PAX6*	GTCCATCTTTGCTTGGGAAA/TAGCCAGGTTGCGAAGAACT Expected product size: 110 bp

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.

Acknowledgments

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