



Lab resource: Stem Cell Line

# Establishment of an induced pluripotent stem cell line (ICGi025-A) from fibroblasts of a patient with 46,XY,r(8)/45,XY,-8 mosaicism



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## ABSTRACT

Ring chromosomes are structural aberrations commonly associated with disease phenotype. We consider necessary to create the iPSCs with a ring chromosome 8, which can be used for disease modeling and related research. The ICGi025-A iPSCs line was obtained by the reprogramming of the skin fibroblasts from a 1-year-old boy with 46,XY,r(8)/45,XY,-8 mosaicism, developmental delay, microcephaly, dysmorphic features, diffuse muscle hypotonia, moderate proximal muscle weakness, feeding problems, and motor alalia. The iPSCs had expression of the pluripotency-associated markers. *In vitro* differentiated cells expressed the markers of the cells of three germ layers. That data allowed us to conclude that ICGi025-A cells were pluripotent.

## Resource Table

Unique stem cell line identifier	ICGi025-A
Alternative name(s) of stem cell line	iTAF11-4
Institution	The Federal Research Center Institute of Cytology and Genetics The Siberian Branch of the Russian Academy of Sciences
Contact information of distributor	Maria Gridina <a href="mailto:gridinam@gmail.com">gridinam@gmail.com</a>
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 1 Sex: male Ethnicity: Caucasian skin fibroblast
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 Delay
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A

## Resource Table (continued)

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 NRM, "Biobank of the population of Northern Eurasia"
Ethical approval	Scientific Ethics Committee of Research Institute of Medical Genetics, Tomsk NRM: 106/2017

## 1. Resource utility

There are relatively few cases with the ring chromosome 8. The ICGi025-A line was established from the cells of 1-year-old boy with 46,XY,r(8)/45,XY,-8 mosaicism. The line is a useful tool for studying both pathogenic mechanism and the fate of the ring chromosome in human iPSCs.

## 2. Resource details

The culture of skin fibroblasts TAF11, was obtained from the skin biopsy of a 1-year-old male with severe phenotype and 46,XY,r(8)/

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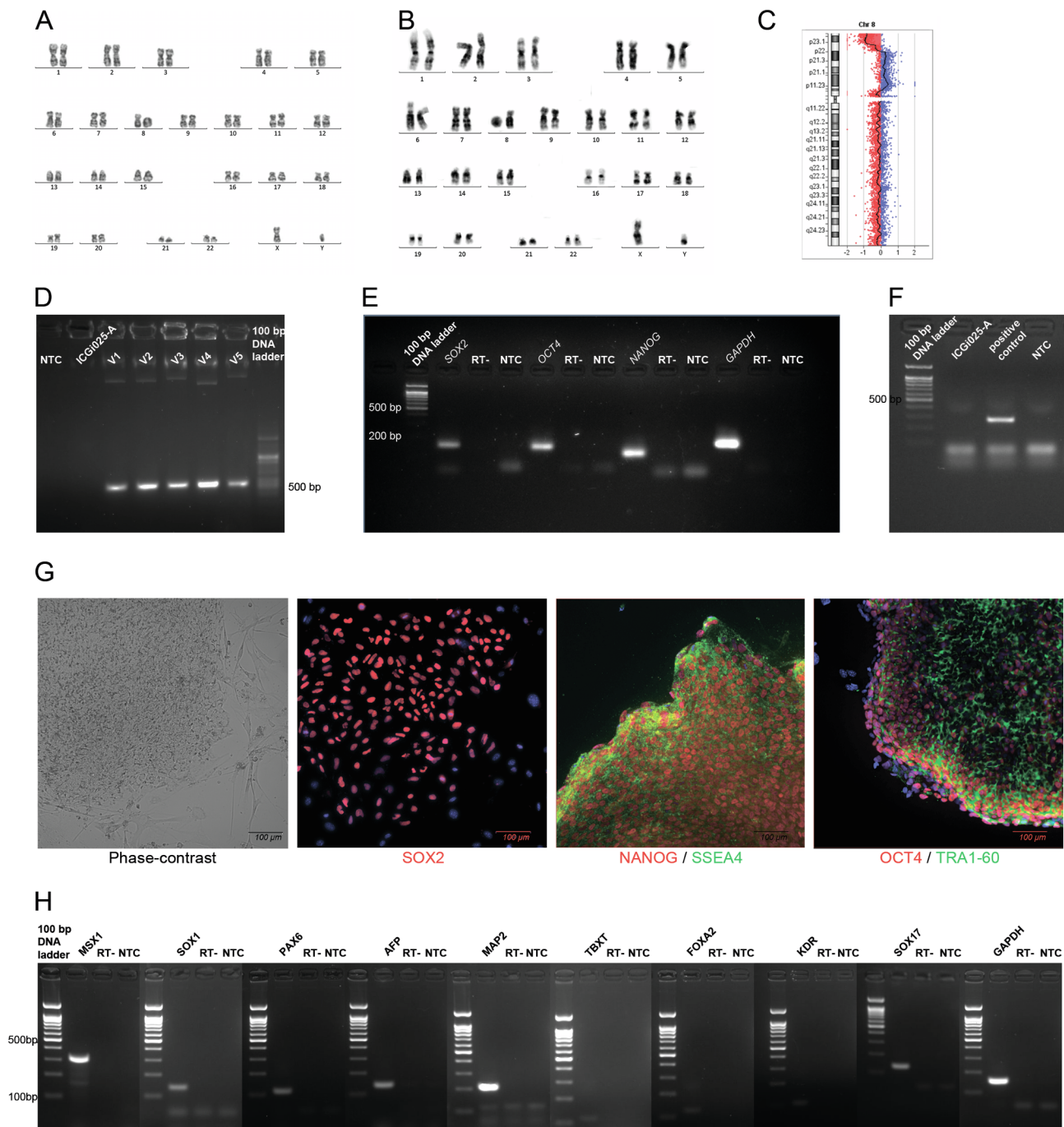


Fig. 1.

45,XY,-8 mosaicism. The phenotype include developmental delay, microcephaly, dysmorphic features, diffuse muscle hypotonia, moderate proximal muscle weakness, feeding problems, and motor alalia. The karyotype of the patient's fibroblasts was a 46,XY,r(8)(p23;q24.3) (Okita et al., 2013)/45,XY,-8 (Choppa et al., 1998). arr[hg19] 8p23.3-p23.1 (191530\_8079920) × 1 dn, 8p11.22-p23.1(12467484\_39587538) × 3 dn, 9p21.1(28604283\_28758185) × 1 mat. nuc ish (D8Z2 × 1)[258/499] (Fig. 1A). The 8p23.3-p23.1 deletion, 8p11.22-p23.1 duplication, and 9p21.1 deletion were detected using aCGH (Fig. 1C). We suppose that duplication at 8p23.1-8p11.22 just proximal to the terminal 8p23.3-p23.1 deletion breakpoint led to ring chromosome 8 formation. The 9p21.1 microdeletion involved only the LINGO2 gene. The clinical significance of this deletion remains unclear. The deletions and

duplication were confirmed by real-time PCR (data not shown). FISH with chromosome 8 centromere-specific alpha-satellite DNA probe was performed to verify the mosaic karyotype. According to SNP analysis, r(8) originated from maternal chromosome 8 (data not shown).

iPSCs were obtained from the skin fibroblasts via episomal vector transfection (Okita et al., 2013). Used vectors did not integrate in the genome ICGi025-A cells as was shown by PCR at the 10th passage (Fig. 1D). The ICGi025-A cells had typical morphology of human iPSCs maintaining on mitomycin-treated mouse embryonic fibroblasts (Fig. 1G). Here we described in detail only ICGi025-A, however 11 iPSC lines were generated from the donors fibroblasts.

The karyotype of ICGi025-A cells was 46,XY,r(8)(p23;q24.3)[88]/45,XY,-8[11]. arr[hg19] 8p23.3-p23.1(191530\_8079920) × 1 dn, 8p11.22-p23.1(12467484\_39587538) × 3 dn, 9p21.1(28604283\_

Table 1

Classification	Test	Result	Data
Morphology	Photography	ICGi025-A cells had typical morphology of human iPSCs	Fig. 1 panel G
Phenotype	Immunocytochemistry	POU5F1 (OCT-4) expressed 97.4% TRA 1–60 expressed 98.6% NANOG expressed 89% SSEA-4 expressed 99.3% SOX2 expressed 96.9%	Fig. 1 panel G
Genotype	Flow cytometry Karyotype (G-banding) and resolution	N/A 46,XY,r(8)(p23;q24.3)[88]/45,XY,-8[11]. arr[hg19] 8p23.3-p23.1(191530_8079920) × 1 dn, 8p11.22-p23.1(12467484_39587538) × 3 dn, 9p21.1(28604283_28758185) × 1 mat	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR) STR analysis	N/A The STR profile of the ICGi025-Acell line totally matched with that of the parental TAF11 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	N/A	
Microbiology and virology	Southern Blot OR WGS Mycoplasma	N/A Negative	Fig. 1 panel F
Differentiation potential	e.g. Embryoid body formation OR Teratoma formation OR Scorecard OR Directed differentiation	Differentiation potency: Endoderm: <i>In vitro</i> spontaneous differentiation AFP, SOX17, FOXA2 Mesoderm: <i>In vitro</i> spontaneous differentiation MSX1, KDR, TBXT Ectoderm: <i>In vitro</i> spontaneous differentiation SOX1, MAP2, PAX6	Fig. 1 panel H
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	

28758185) × 1 mat (Fig. 1B).

The ICGi025-A cells expressed of the pluripotency-associated markers (Fig. 1G and Table 1), including *OCT4* (97.4%), *NANOG* (89%), and *SOX2* (96.9%), as was showed by RT-PCR (Fig. 1E) and immunofluorescence staining which also revealed the most cells positive for the SSEA4 and TRA-1–60 (Fig. 1G). The ICGi025-A cells were differentiated *in vitro* in embryoid bodies. RT-PCR showed the expression of endodermal (*FOXA2*, *AFP*, *SOX17*), ectodermal (*SOX1*, *MAP2*, *PAX6*) and mesodermal (*TBXT*, *KDR*, *MSX1*) genes (Fig. 1H) in the obtained embryoid body cells. Taken together that indicated the ICGi025-A cells were pluripotent.

The STR profile of the ICGi025-Acell line totally matched with that of the parental TAF11 fibroblasts (loci analyzed:D3S1358, TH01, D12S391, D1S1656, D10S1248, D22S1045, D2S441, D7S820, D13S317, FGA, TPOX, D18S51, D16S539, D8S1179, CSF1PO, D5S818, vWA, D21S11, SE33).

The presence of mycoplasma was checked and the cells were not contaminated (Fig. 1F).

Taken together, we successfully generated ICGi025-A iPSCs line.

### 3. Materials and methods

#### 3.1. Generation of iPSCs

The human skin fibroblasts were maintained in DMEM/F12 supplemented with 10% fetal bovine serum, 1% Pen Strep, 1% MEM Non-essential Amino Acid solution, 2 mM L-glutamine (all from Invitrogen) at 37 °C in 5% CO<sub>2</sub>.

500 × 10<sup>3</sup> of the fibroblasts were electroporated at 1650 V, 10 ms, 3 pulses with the 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 × 10<sup>3</sup>/cm<sup>2</sup>) of mitomycin C-treated CD-1 mouse embryonic fibroblast 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. Cells were cultured at 37 °C in an atmosphere of 5% CO<sub>2</sub>. iPSCs were passed mechanically with ratio of split 1:5.

#### 3.2. Vector's integration detection

The phenol-chloroform extraction method was used for genome DNA isolation. The integration of episomal vectors in the genome iPSCs was checked using PCR. PCR was set up with 50 ng DNA as template using primers shown in Table 2.

#### 3.3. Immunocytochemistry

The iPSCs were fixed in 3% formaldehyde for 20 min. The cells were permeabilized with 0.1% Triton X-100 in PBS for 3 min and blocked with 3% BSA in PBS for 25 min. The cells were incubated overnight with primary antibodies at 4 °C, washed twice with 0.02% Tween-20 in PBS. Secondary antibodies were incubated for 1 h at room temperature (Table 2). All antibodies were diluted in PBS with 1.5% BSA. Immunofluorescence and immunofluorescence counting (100 cells counted) were examined with a fluorescence microscope AxioObserver Z1 (Zeiss) using ZEN software in collective Microscopic Center of ICG SB RAS, Novosibirsk and Fiji soft (ImageJ).

#### 3.4. In vitro differentiation

iPSCs were cultured in iPSC medium without bFGF on the agarose-covered plates. Cell aggregates were growing during 16 days, the media was change every second day.

#### 3.5. RT-PCR

Total RNA was isolated by TRI Reagent (Sigma) according to manufacturer's recommendations. The RNA was treated with DNase I (Thermo Fisher Scientific). cDNA was obtained by RevertAid RT kit (Thermo Fisher Scientific) and RT-PCR was performed with HP-Taq DNA polymerase. *GAPDH* was used as an endogenous control gene. The

Table 2

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-SOX2	1:400	RSE National center for Biotechnology, Astana, Cat# NCB 1601
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_2,633,275
<b>Primers</b>			
	Target	Forward/Reverse primer (5'-3')	
House-Keeping Genes	<i>GAPDH</i>	GTGGACCTGACCTGCCGTCT/GGAGGAGTGGGTGTCGCTGT Expected product size: 153 bp	
Pluripotency Marker	<i>OCT4</i>	CTGGGTTGATCCTCGGACCT/CACAGAACTCATACGGCGGG Expected product size: 128 bp	
Pluripotency Marker	<i>NANOG</i>	AAAGAATCTTCACCTATGCC/GAAGGAAGAGGAGAGACAGT Expected product size: 110 bp	
Pluripotency Marker	<i>SOX2</i>	AAGGATAAGTACACGCTGCC/GCTTCAGCTCCGTCTCCAT Expected product size: 128 bp	
Plasmid primer	pEP4-SF1-oriP	TTCCACGAGGGTAGTGAACC/TCGGGGGTGTTAGAGACAAC Expected product size: 544 bp	
Differentiation Markers	<i>AFP</i>	AAATGCGTTTCTCGTTGCTT/GCCACAGGCCAATAGTTTGT Expected product size: 136 bp	
Differentiation Markers	<i>SOX1</i>	CACAACTCGGAGATCAGCAA/GGTACTTGTAATCCGGGTGC Expected product size: 133 bp	
Differentiation Markers	<i>MAP2</i>	CAGGTGGCGGACGTGTGAAAATTGAGAGTG/CACGCTGGATCTGCCTGGGGACTGTG Expected product size: 212 bp	
Differentiation Markers	<i>SOX17</i>	CTCTGCCTCCTCCACGAA/CAGAATCCAGACCTGCACAA Expected product size: 102 bp	
Differentiation Markers	<i>MSX1</i>	CGAGAGGACCCCGTGGATGCAGAG/GGCGGCCATCTTCAGCTTCTCCAG Expected product size: 307 bp	
Differentiation Markers	<i>KDR (FLK1)</i>	TGATCGGAAATGACACTGGA/CACGACTCCATGTTGGTCCAC Expected product size: 131 bp	
Differentiation Markers	<i>TBXT (BRACHYURY)</i>	AATTGGTCCAGCCTTGAAT/CGTTGCTCACAGACCACA Expected product size: 112 bp	
Differentiation Marker	<i>PAX6</i>	GTCCATCTTTGCTTGGGAAA/TAGCCAGGTTGCGAAGAAGT Expected product size: 110 bp	
Differentiation Marker	<i>FOXA2 (HNF-3B)</i>	GGAGCGGTGAAGATGGAA/TACGTGTTTCATGCCGTTTCAT Expected product size: 122 bp	

list of primers is shown in Table 2. “RT-” is the control without reverse transcriptase.

### 3.6. Karyotyping

Preparation of metaphase chromosomes from iPSCs was performed on passages 10–12 as previously described (Prokhorovich et al., 2007). 99 metaphase spreads were analysed using a Carl Zeiss Axioplan 2 imaging microscope, digital images were analysed using ISIS 3 (In Situ Imaging System, MetaSystems GmbH) software. Multicolor banding (MCB) was carried out using XCyte 2 mBAND probe (MetaSystems GmbH).

### 3.7. Mycoplasma contamination detection

PCR was set up with 50 ng DNA as template using primers from Choppa et al. (1998).

STR analysis for parent TAF11 fibroblasts and the ICGi025-A cells was performed by Gordiz (<http://gordiz.ru/>).

### 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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