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Lab Resource: Stem Cell Line

Generation of a human iPSC line, IISHDOi002-A, with a 46, XY/47, XYY mosaicism and belonging to an African mitochondrial haplogroup

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ABSTRACT

We have generated a human iPSC line, IISHDOi002-A, from commercial primary normal human dermal fibroblasts belonging to an African mitochondrial haplogroup (L3), and with a 46, XY/47, XYY mosaicism. For this purpose, reprogramming factors Oct3/4, Sox2, Klf4 and cMyc were delivered using a non-integrative methodology that involves the use of Sendai virus.

Resource table.

Unique stem cell line identifier	IISHDOi002-A
Alternative name(s) of stem cell line	FCE-FiPS4F-7
Institution	Instituto de Investigación Sanitaria Hospital 12 de Octubre, i + 12
Contact information of distributor	Dr. M. Esther Gallardo egallardo.imas12@h12o.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Sex: Male
Cell source	Normal human neonatal dermal fibroblasts (Lonza, CC2509)
Clonality	Mixed
Method of reprogramming	Sendai virus
Genetic modification	NO
Type of modification	N/A
Associated disease	N/A
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	May 2017
Cell line repository/bank	N/A
Ethical approval	Fibroblasts were obtained after signing informed consent. This study was reviewed and approved by the Institutional Ethical Committee of the "Instituto de Investigaciones Biomédicas Alberto Sols", CSIC-UAM, 406329 1.

Resource utility

Functional studies using iPSCs generated from patients with mitochondrial diseases (MD) require the use of the most appropriate controls. An isogenic control is not always possible. The availability of the iPSC line, IISHDOi002-A, will be very useful for functional studies of MD patients belonging to a L3 haplogroup.

Resource details

The establishment of a human iPSC line, IISHDOi002-A, was performed using non-integrative methodology that involves the use of Sendai viruses containing the reprogramming factors Oct3/4, Sox2, Klf4 and c-Myc (Takahashi et al., 2007). For this purpose, commercially available fibroblasts from a healthy neonatal donor were acquired in Lonza (CC2509). Subsequently, mitochondrial haplogroup determination was carried out in the IISHDOi002-A line and in the original fibroblasts by PCR amplification of mitochondrial DNA (mtDNA) regions containing the nucleotides positions that define the L3 haplogroup (m.10400 and m.3594) followed by direct sequencing of the generated amplicons (Table 1). The identification of the mtDNA nucleotide variations m.10400C and m.3594 T showed that fibroblasts and the generated iPSC line IISHDOi002-A belong to

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the African mitochondrial haplogroup L3 (Fig. 1A). IISHDOi002-A iPSC colonies displayed a typical ES-like colony morphology and growth behavior (Fig. 1B) and they stained positive for alkaline phosphatase (AP) activity (Fig. 1C). The endogenous expression of the pluripotency associated transcription factors *OCT4*, *SOX2*, *KLF4*, *NANOG*, *CRIPTO* and *REX1* was evaluated by quantitative real time polymerase chain reaction (qPCR) (Fig. 1D). Immunofluorescence analysis revealed expression of the pluripotency-associated transcription factors OCT4, SOX2 and NANOG, and surface markers SSEA3, SSEA4, TRA1-81 and TRA1-60 (Fig. 1E). We also confirmed the clearance of the vectors and the exogenous reprogramming factor genes by RT-PCR after nine culture passages (Fig. 1F). The iPSC line has been adapted to feeder-free culture conditions and a karyotype analysis after more than 20 culture passages has been carried out. This analysis displayed a 46, XY (28)/47, XYY (2) mosaicism in the iPSC line (Fig. 1G). We also confirmed the presence of abnormal cells at 4–4.5% in two different aliquots of the original fibroblasts by FISH analysis of 200 interphase cells [nuc ish (DYZ1x2, DYZ1x1) [9/200] (Fig. 1G). These data suggest that this mosaicism could be a causal finding in the fibroblasts and have a somatic origin. The level of mosaicism in the fibroblasts has been maintained around 4.5% during more than 30 passages. In any case, males with an extra copy of the Y chromosome typically have no unusual physical features although they could have fertility problems. Regarding the case reported here, the identified mosaicism, to our knowledge, is not related with any noteworthy phenotypic trait present in the carrier. We also verified by DNA fingerprinting analysis that the line IISHDOi002-A was derived from the patient's fibroblasts (Supplementary Fig. S1). In addition, the line was confirmed by PCR analysis to be mycoplasma-negative (Fig. 1H). Finally, the capacity of the IISHDOi002-A iPSC line to differentiate into the three germ layers (endoderm, mesoderm and ectoderm) was evaluated *in vitro* using an embryoid body based assay (Fig. 1I).

Materials and methods

iPSCs generation

Healthy human neonatal fibroblasts (Lonza, CC2509) were reprogrammed using the CytoTune-iPS 2.0 Sendai reprogramming kit following the instructions of the manufacturer. IISHDOi002-A was expanded as described in Galera et al., 2016.

AP analysis

The iPSC line was seeded on a feeder layer plate. After one week, direct AP activity was determined using the AP blue membrane substrate solution kit (Sigma, AB0300).

Mitochondrial haplogroup

DNAs from fibroblasts and IISHDOi002-A were haplogrouped by PCR and direct sequencing, using the primers described in Table 2. The mtDNA nucleotides, m.10400C and m.3594 T, defining the African haplogroup L3, have been verified.

qPCR analysis

Total mRNA was isolated using TRIZOL and cDNA using the Quantitect RT cDNA synthesis kit. The expression of the endogenous pluripotency associated genes (*OCT4*, *SOX2*, *KLF4*, *NANOG*, *CRIPTO* and *REX1*) was quantified by qPCR. Primers are listed in Table 2 (Aasen et al., 2008). All the expression values were normalized to the *GAPDH* gene. Plots are representative of at least three independent experiments.

Karyotype and FISH

Cells were treated with 10 μg/mL of Colcemid (Gibco) for 90 min at 37 °C, trypsinized, treated with KCl 0.075 M, and fixed with Carnoy's fixative for karyotype analysis and fixed three times in methanol/acetic acid (3:1) for FISH. Cells were then dropped on a microscope glass slide and dried. Metaphase cells were G banded using Wright staining. At least 30 metaphases were karyotyped. For FISH, CEP probe for chromosomes Y (DYZ1) and slides were denatured at 72 °C for 2 min and incubated at 37 °C (16 h) for hybridization. Slides were washed in SSC with 0.1% Tween 20. Coverslips were mounted on the slides with DAPI. 200 interphase cells were analyzed with a Nikon fluorescent microscope. Digital images were acquired with a monochrome CCD camera linked to Metasystem software.

Immunofluorescence analysis

Cells were grown on 0.1% gelatin-coated 35 mm culture plates, fixed with 4% paraformaldehyde 30 min at RT and permeabilized using TBS+

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1, panel B
Phenotype	Qualitative analysis: immunocytochemistry	Positive for the pluripotency markers: SSEA3, SSEA4, TRA-1-81, TRA-1-60, OCT4, NANOG, SOX2	Fig. 1, panel E
	Qualitative analysis: alkaline phosphatase activity	Positive	Fig. 1, panel C
	Quantitative analysis: gene expression (qPCR)	Positive for the pluripotency markers *OCT4*, *KLF4*, *SOX2*, *CRIPTO*, *NANOG*, *REX1*	Fig. 1, panel D
Genotype	Karyotype (G-banding) and resolution	46, XY (28)/47, XYY (2) Resolution 450–500	Fig. 1, panel G
Identity	Microsatellite PCR (mPCR)	N/A	
	STR analysis	8 loci, all matched (D2S1338, D7S820, D8S1179, D13S317, D19S433, D21S11, VWA, amelogenin)	Supplementary Fig. S1
Mutation analysis (if applicable)	Sequencing	N/A	
	Southern Blot OR WGS	N/A	
Haplogroup analysis	Sequencing	Confirmation of the L3 haplogroup: m.10400C and m.3594 T	Fig. 1, panel A
Microbiology and virology	Mycoplasma	Negative	Fig. 1, panel H
	Sendai virus silencing	Virus silenced	Fig. 1, panel F
Differentiation potential	Embryoid body formation and directed differentiation	Positive for: smooth muscle actin (SMA), β-tubulin (Tuj1) and alpha-fetoprotein (AFP)	Fig. 1, panel I
Donor screening (optional)	HIV 1+2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (optional)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

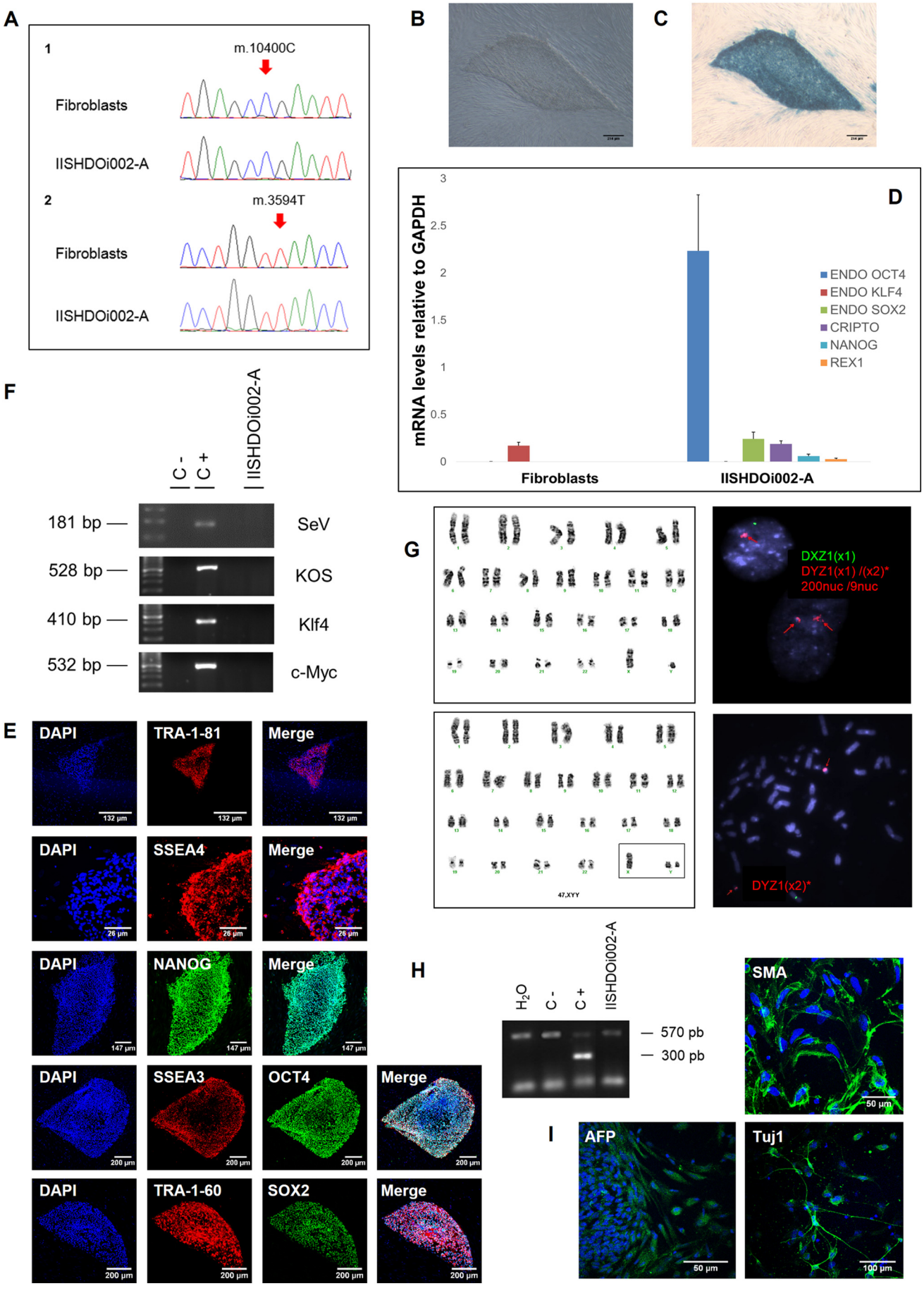

Fig. 1. Molecular and functional characterization of the IISHDOi002-A iPSC line.

(0.1% Triton X-100 in Tris-buffered saline, TBS) for 45 min. Then the cells were incubated in TBS++ (3% donkey serum, 0.3% Triton X-100 in TBS) for 2 h at RT. Primary antibodies were applied overnight at 4 °C. Secondary antibodies, 2 h at RT. Nuclei were stained with DAPI (Sigma, 28718-90-3). All the antibodies are listed in Table 2.

In vitro differentiation assay

The *in vitro* pluripotency capacity of the line IISHDOi002-A was tested by spontaneous embryoid body differentiation. The protocol we have used has been described in detail by Galera et al. (2016).

DNA fingerprinting

The markers D13S317, D7S820, VWA, D8S1179, D21S11, D19S433, D2S1338 and amelogenin for sex determination were amplified by PCR and analyzed by ABI PRISM 3100 Genetic analyzer and Peak Scanner v3.5 (Thermofisher) (Table 2).

Mycoplasma detection

Mycoplasma detection was performed by PCR (primers specified in Table 2) using 1 mL of the cell culture supernatant (3 days culture at 90% confluence). The 300 bp band represents that the sample is positive for mycoplasma. The band at 570 bp is an internal control to discard the inhibition of the polymerase.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.02.009.

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Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse anti-TRA-1-81	1:150	Millipore Cat# MAB4381, RRID: AB_177638
	Mouse anti-TRA-1-60	1:150	Millipore Cat# MAB4360, RRID: AB_11211864
	Rabbit anti-SOX2	1:100	Thermo Fisher Scientific Cat# PA1–16968, RRID: AB_2195781
	Mouse anti-SSEA4	1:10	Millipore Cat# MAB4304, RRID: AB_177629
	Rat anti-SSEA3	1:20	Abcam Cat# ab16286, RRID: AB_882700
	Goat anti-NANOG	1:25	R and D Systems Cat# sc-5279, RRID: AB_628051
	Mouse anti-OCT4	1:100	Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051
Differentiation markers	Mouse anti-β tubulin isotype III	1:300	Sigma-Aldrich Cat# T8660, RRID: AB_528427
	Mouse anti-AFP	1:300	Sigma-Aldrich Cat# WH0000174M1, RRID: AB_1839587
	Mouse anti-SMA	1:400	Sigma-Aldrich Cat# A2547, RRID: AB_476701
Secondary antibodies	Cy™2-conjugated AffiniPure Donkey Anti-Goat IgG (H+L)	1:50	Jackson ImmunoResearch Labs Cat# 705-225-147, RRID: AB_2307341
	Cy™2-conjugated AffiniPure Goat Anti-Mouse IgG, Fcγ Subclass 2b specific	1:50	Jackson ImmunoResearch Labs Cat# 115-225-207, RRID: AB_2338749
	Cy™2-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L)	1:50	Jackson ImmunoResearch Labs Cat# 111-225-144, RRID: AB_2338021
	Cy™3-conjugated AffiniPure Goat Anti-Rat IgM, μ chain specific	1:250	Jackson ImmunoResearch Labs Cat# 112-165-075, RRID: AB_2338249
	Cy™3-conjugated AffiniPure Goat Anti-Mouse IgG, Fcγ Subclass 3 specific	1:250	Jackson ImmunoResearch Labs Cat# 115-165-209, RRID: AB_2338698
	Cy™3-conjugated AffiniPure Donkey Anti-Mouse IgM, μ chain specific	1:250	Jackson ImmunoResearch Labs Cat# 715-165-020, RRID: AB_2340811
	Goat anti-mouse IgG (H+L), Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-11029, RRID: AB_2534088

Primers

	Target	Forward/reverse primer (5′-3′)
Pluripotency markers (qPCR)	*Endo-KLF4*	AGCCTAAATGATGGTGCTTGGT/TTGAAAACTTTGGCTTCCTTGTT
	Endo-OCT4	GGGTTTTTGGGATTAAGTTCTTCA/GCCCCCACCCTTTGTGTT
	Endo-SOX2	CAAAAATGGCCATGCAGGTT/AGTTGGGATCGAACAAAAGCTATT
	REX1	CCTGCAGGCGGAAATAGAAC/GCACACATAGCCATCACATAAGG
	CRIPTO	CGGAACTGTGAGCACGATGT/GGGCAGCCAGGTGTCATG
	NANOG	ACAACTGGCCGAAGAATAGCA/GGTTCCCAGTCGGGTTCAC
House-Keeping Genes (qPCR)	*GAPDH*	GCACCGTCAAGGCTGAGAAC/AGGGATCTCGCTCCTGGAA
Haplogroup analysis	mtDNA region: from m.9977 to m.10858	TCTCCATCTATTGATGAGGGTCT/CAACCACCCACAGCCTAATT
	mtDNA region: from m.3150 to m.3980	TACTTCACAAAGCGCCTTCC/CCCTTCGCCCTATTCTTCAT
Virus silencing	SeV	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG
	Klf4	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA
	c-Myc	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG
STR analysis	D2S1338	[6-FAM] CCAGTGGATTTGGAAACAGA/ACCTAGCATGGTACCTGCAG
	D7S820	[6-FAM] TGTCATAGTTTAGAACGAACTAACG/CTGAGGTATCAAAAACTCAGAGG
	D8S1179	[6-FAM] TTTTTGTATTTCATGTGTACATTCG/CGTAGCTATAATTAGTTCATTTTCA
	D13S317	[6-FAM] ACAGAAGTCTGGGATGTGGA/GCCCAAAAAGACAGACAGAA
	D19S433	[6-FAM] CCTGGGCAACAGAATAAGAT/TAGGTTTTTAAGGAACAGGTGG
	D21S11	[6-FAM] GTGAGTCAATTCCCCAAG/GTTGTATTAGTCAATGTTCTCC
	VWA	[6-FAM] CCCTAGTGGATGATAAGAATAATC/GGACAGATGATAAATACATAGGATGGATGG
	Amelogenin	[6-FAM] CCCTGGGCTCTGTAAAGAATAGTG/ATCAGAGCTTAAACTGGGAAGCTG
Mycoplasma detection	GPO-3/MGSO	GGGAGCAAACAGGATTAGATACCCT/TGCACCATCTGTCACTCTGTTAACCTC

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Author disclosure statement

There are no competing financial interests in this study.

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