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## Generation of induced pluripotent stem cell lines IRMBi003-A and IRMBi003-B from a healthy donor to model Alzheimer's disease

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### ABSTRACT

Induced Pluripotent Stem Cell (iPSC) lines derived from healthy individuals are helpful and essential tools for disease modelling. Here, we described the reprogramming of skin fibroblasts obtained from a healthy 59-year-old individual without Alzheimer's disease. The generated iPSC lines have a normal karyotype, expressed pluripotency markers, and demonstrated the ability to differentiate into the three germ layers. The iPSC lines will be used as controls to study Alzheimer's disease mechanisms.

### Resource Table.

Unique stem cell line identifier	IRMBi003-A IRMBi003-B
Alternative name(s) of stem cell line	CT1 and CT4
Institution	INM, Univ Montpellier, INSERM, Montpellier, France
Contact information of distributor	Carole CROZET <a href="mailto:carole.crozet@inserm.fr">carole.crozet@inserm.fr</a>
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 59 years' old Sex: Male
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus Cytotune 2.0 kit (ThermoFisher Scientific Inc.), hOCT4, hSOX2, hC-MYC, hKLF4
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	07/26/2023

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Cell line repository/bank	<a href="https://hpscrg.eu/cell-line/IRMBi003-A">https://hpscrg.eu/cell-line/IRMBi003-A</a> and <a href="https://hpscrg.eu/cell-line/IRMBi003-B">https://hpscrg.eu/cell-line/IRMBi003-B</a>
Ethical approval	DC-2015-2363 regional scientific ethical committee CPP Sud Med IV. Informed consent was obtained from the patient.

### 1. Resource utility

The reported cell lines may be used as healthy reference cell lines for *in vitro* studies or disease modelling for Alzheimer's disease (AD). They may also be genetically modified by gene editing to generate isogenic cell lines with mutations related to the pathology (Table 1).

### 2. Resource details

This paper details the generation of two iPSC clones (CT1 and CT4) from a healthy male without AD. They could be used as healthy control iPSC lines for AD studies to perform comparative analysis with AD iPSC cell lines (Auboyer et al., 2019a; Auboyer et al., 2019b). They may also provide cell sources for the generation of isogenic cell lines with various

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mutations of interest and known to be involved in the physiopathology of AD.

Skin fibroblasts were obtained from a 59-year-old man without diagnosed disease. Fibroblasts were reprogrammed into iPSC using integration-free Sendai viruses (Life Technologies) carrying gene sequences of the four Yamanaka factors: OCT3/4, SOX2, KLF4 and c-MYC. Several iPSC colonies with normal morphology (Fig. 1A) were picked for expansion. The disappearance of the Sendai virus was successfully established by RT-PCR analysis with Sendai virus specific primers (Fig. 1B), in selected iPSC colonies after at least 10 passages. The transgenes and viral sequences were also absent in the negative controls, non-transduced human healthy fibroblasts (hF Ct) and clones (CT1 and CT4) of interest, but present in freshly transduced (7 days) fibroblasts with the Sendai viruses (hF Ct Sendai). Expression of endogenous pluripotency factors OCT4, SOX2 and NANOG was confirmed by RT-qPCR analysis (Fig. 1C). Immunofluorescence labelling validated the expression of the embryonic stem cell specific marker SSEA4 and of the two pluripotency factors OCT4 and SOX2 (Fig. 1D). Pluripotency of the two clones was assessed following *in vitro* directed differentiation of iPSC colonies during 7 days (Fig. 1E), followed by immunofluorescence staining of ectodermal and endodermal markers, respectively  $\beta$ III-tubulin and PAX6, HNF3beta/FOXA2 and SOX17, or RT-PCR for mesodermal markers, Brachyury and  $\alpha$ Smooth muscle actin (Fig. 1E). CT1 and CT4 clones presented a normal karyotype 46,XY after 33 passages for CT1, and 35 passages for CT4. Genomic stability was also assessed by detection of recurrent genetic abnormalities using the iCS-digital™ PSC analysis provided as a service by Stem Genomics (<https://www.stemgenomics.com/>), as described previously (Assou et al., 2020) and no evidence of recurrent genomic abnormalities was detected (Fig. 1F). The short tandem repeat (STR) profile established the 100 % identity of the iPSC line to parental cells (available upon request) and clones were shown to be mycoplasma free (Supplementary data).

### 3. Materials and methods

#### 3.1. iPSC generation

Fibroblasts were amplified in 10 % FBS (Lonza), DMEM, 1 % NEAA, 0.1 mM  $\beta$ -mercaptoethanol, 1 mM glutamine and 1 % penicillin/streptomycin (Gibco). Fibroblasts were reprogrammed using the Cytotune-iPSC 2.0 Sendai Reprogramming kit (Life Technologies) (KOS MOI = 5, hc-Myc MOI = 5, hklf4 MOI = 3). After 7 days, the freshly transduced cells were transferred on feeders (mytomycin-inactivated CF1 mouse

embryonic (E12.5) fibroblasts), (Charles River) on 0.1 % Gelatin (Sigma Aldrich) coated dishes in KODMEM/F-12 with 20 % KO serum, 1 % NEAA, 0.1 mM  $\beta$ -mercaptoethanol, 1 mM glutamax, 1 % penicillin/streptomycin (Gibco) and 5 ng/mL bFGF (Miltenyi Biotec). Several iPSC clones were passaged and expanded.

#### 3.2. Pluripotent stem cell cultures

iPSC were first maintained on a feeder layer and they then adapted on 0.1 % Geltrex (Gibco) coated dishes and maintained in mTeSR Plus medium (Stemcell Technologies). Colonies were mechanically passaged every 4–7 days into clumps. Cells were cultivated in a humidified incubator at 37 °C, 5 % CO<sub>2</sub>. The iPSC lines were frozen in liquid nitrogen in Cryostor and 10  $\mu$ M of Rock Inhibitor (Stemcell Technologies).

#### 3.3. PCR analysis

Total RNA was extracted from fibroblasts and iPSCs (P20) with the RNAeasy micro kit (Qiagen). cDNA were obtained using the SuperscriptIII kit (Invitrogen). Disappearance of the Sendai virus vectors and expression of mesodermal markers was assessed by PCR using specific primers (Table 2). PCR products were analysed on 2 % agarose gel and the picture was acquired using Ebox Vilber System. For the expression of the endogenous genes of pluripotency, quantitative PCR analysis was performed using the Light Cycler LC480 device (Roche Diagnostics) using SYBRGreen I Master Kit (Roche Diagnostics). The relative quantification was calculated using the 2- $\Delta\Delta$ Ct method with non-transduced fibroblasts as controls and GAPDH as housekeeping gene (Primers are listed in Table 2) using H9 human embryonic stem cell mRNA as reference and positive control.

#### 3.4. In vitro directed differentiation

iPS cells (p24) were dissociated as single cells (Gentle Dissociation Reagent, Stemcell Technologies) and differentiated into each germ layers (mesoderm, endoderm, ectoderm) in parallel with StemMACS Trilineage Differentiation Kit (Miltenyi). After 7 days, cells were fixed for immunocytochemistry.

#### 3.5. Immunofluorescence

iPSC colonies (p22) or EB were fixed on coverslips in 4 % PFA during 15 min and washed 3 times with DPBS (Gibco). Cells were permeabilized

**Table 1**  
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for OCT4, SOX2, SSEA4	Fig. 1D
	Quantitative analysis (RT-qPCR)	Expression of pluripotency markers: OCT4, SOX2, NANOG	Fig. 1C
	Karyotype and resolution	46XY, Resolution: 400	Fig. 1F
Genotype	Recurrent genomic abnormalities	No recurrent genetic abnormalities detected	
	Identity	Microsatellite PCR (mPCR) OR STR analysis	NA Available with authors
Mutation analysis (IF APPLICABLE)	Sequencing	NA	NA
	Southern Blot OR WGS	NA	NA
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary data
Differentiation potential	In vitro directed differentiation	Expression of PAX6, $\beta$ III-tubulin, SOX17 and FOXA2 by immunocytochemistry. Expression of Brachyury and $\alpha$ SMA by RT-PCR.	Fig. 1E
List of recommended germ layer markers	mRNA (RT-PCR) and protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm: PAX6, TUBB3/TUJ1 Endoderm: SOX17, FOXA2, Mesoderm: BRACHYURY, $\alpha$ SMA	Fig. 1E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	NA	NA
Genotype additional info (OPTIONAL)	Blood group genotyping	NA	NA
	HLA tissue typing	NA	NA

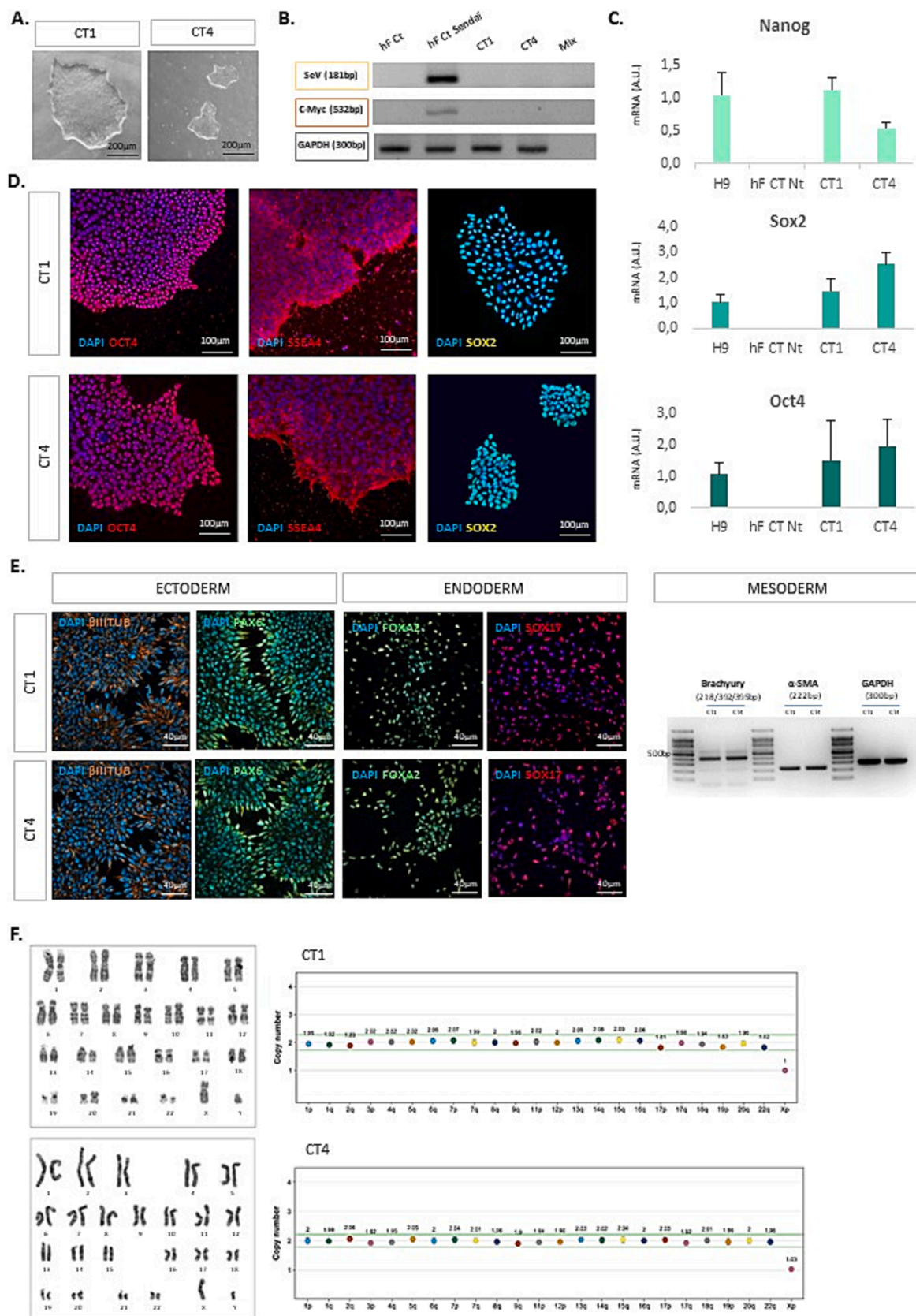


Fig. 1. .

**Table 2**  
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse anti-OCT3-4 (C-10)	1:500	Santa Cruz Biotechnology Cat# sc-5279	RRID: AB_628051
	Rabbit anti-SOX2	1:100	Bethyl Cat#301-739A	RRID: AB_1211354
	Mouse anti-SSEA4 (MC-813-70)	1:500	Thermo Fisher Scientific Cat# MA1-021	RRID: AB_2536687
Differentiation Markers	Mouse anti-Beta-Tubulin III, Clone TUJ1	1:200	STEMCELL Technologies Cat#60052	RRID:AB_2,313,773
	Rabbit anti-PAX6	1:20	Thermo Fisher Scientific Cat# PA5-25970	RRID: AB_2543470
	Rabbit anti-HNF3beta/FOXA2	1:75	Millipore Cat# 07-633	RRID: AB_390153
	Mouse anti-SOX17	1:75	R&D Systems Cat# MAB1924	RRID: AB_2195646
Secondary antibodies	Donkey Anti-Mouse IgG, Alexa Fluor 555-conjugated	1:1000	Molecular Probes Cat# A21424	RRID: AB_141780
	Goat anti-Rabbit IgG, Alexa Fluor 488-conjugated	1:1000	Molecular Probes Cat# A-11008	RRID: AB_143165
	Donkey anti-Rabbit IgG, Alexa Fluor 488-conjugated	1:1000	Molecular Probes Cat# A21206	RRID: AB_2535792
	Donkey anti-Goat IgG, Alexa Fluor 555-conjugated	1:1000	Thermo Fisher Scientific Cat# A21432	RRID: AB_2535853
	Donkey anti-Mouse IgG, Alexa Fluor 488-conjugated Primers	1:1000	Molecular Probes Cat# A21202	RRID: AB_141607
	Target	Size of band	Forward/Reverse primer (5'-3')	
Transgenes from SeV vector (PCR)	SeV	181 bp	Forward: GGA TCA CTA GGT GAT ATC GAG C Reverse: ACC AGA CAA GAG TTT AAG AGA TAT GTA TC	
	c-Myc	532 bp	Forward: TAA CTG ACT AGC AGG CTT GTC G Reverse: TCC ACA TAC AGT CCT GGA TGA TGA TG	
	GAPDH	300 bp	Forward: CTG GCG TCT TCA CCA CCA TGG Reverse: CAT CAC GCC ACA GTT TCC CGG	
Mesodermal markers (PCR)	BRACHYURY/TBXT	218/392/395 bp	Forward: ACC CAG TTC ATA GCG GTG AC Reverse: CAA TTG TCA TGG GAT TGC AG	
	αSMA	222 bp	Forward: TTC AAT GTC CCA GCC ATG TA Reverse: GAA GGA ATA GCC ACG CTC AG	
Pluripotency Markers (qPCR)	OCT4	150 bp	Forward: TGT ACT CCT CGG TCC CTT TC Reverse: TCC AGG TTT TCT TTC CCT AGC	
	SOX2	144 bp	Forward: ATG CAC CGC TAC GAC GTG A Reverse: CTT TTG CAC CCC TCC CAT TTC	
	NANOG	354 bp	Forward: TGC CTC ACA CGG AGA CTG TC	

**Table 2 (continued)**

Antibodies used for immunocytochemistry/flow-cytometry			
Antibody	Dilution	Company Cat #	RRID
House-Keeping Genes (qPCR)	GAPDH	NA	Reverse: TGC TAT TCT TCG GCC AGT TG Forward: CGC TCT CTG CTC CTC CTG TT Reverse: CCA TGG TGT CTG AGC GAT GT

with 0.5 % Triton X-100 (Sigma Aldrich), 3x10min for nuclear staining or 3 min in 0.3 % Triton X-100 for the other immunostainings. Non-specific binding sites were blocked with 0.2 % Bovine Serum Albumin (MP BIOMEDICALS) in DPBS (1 h, RT). Primary and secondary antibodies were diluted (Table 2) in 0.2 % BSA-DPBS solution, and applied overnight at 4 °C and 1 h, RT respectively. Nuclei were labelled with DAPI (Life technologies) in DPBS. Cells were washed twice in DPBS and water. Coverslips were mounted with Fluorsave (Millipore). Samples were imaged with AxioObserver Zeiss.

### 3.6. Karyotype and recurrent genomic instability

Genomic stability tests and Karyotypes were performed by StemGenomics at passage 33 (CT1) and passage 35 (CT4), (<https://www.stemgenomics.com/>).

### 3.7. Short tandem repeat analysis

Microsatellite analyses were performed on DNA extracted from parental fibroblasts and iPSCs, using the following short tandem repeats: D1S439, D9S1784, D14S986, D19S913, D5S818, D13S317, D7S820, D16S539, vWA, Th01, TPOX, CSF1PO) + amelogenin.

### 3.8. Mycoplasma testing

Absence of mycoplasma contamination was assessed on cell culture supernatants (p25) using the MycoAlert® Mycoplasma Detection assay (Lonza).

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Crozet reports financial support was provided by Fondation pour la Recherche Médicale. CLUA-PROVOST reports financial support was provided by Alzheimer Foundation.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2023.103250>.

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