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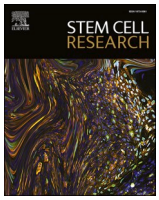

Lab Resource: Multiple Cell Lines

Generation of human induced pluripotent stem cell lines from two down syndrome patients, including a down syndrome/Alzheimer's disease case (FLENIi002-A) and a beta-amyloid-resistant case (FLENIi003-A)

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A B S T R A C T

Two human induced pluripotent stem cell (hiPSC) lines, FLENIi002-A and FLENIi003-A, were generated from peripheral blood mononuclear cells (PBMCs) using the lentiviral-hSTEMCCA-loxP vector. FLENIi002-A was derived from a 52-year-old Down syndrome patient with Alzheimer's disease and amyloid-beta brain accumulation. FLENIi003-A was derived from a cognitively unimpaired 51-year-old Down syndrome patient exhibiting no brain amyloid-beta deposition. Both lines retained the trisomy 21 genotype, and their pluripotency and differentiation potential were confirmed.

1. Resource table

Unique stem cell lines identifier	FLENIi002-A FLENIi003-A
Alternative name(s) of stem cell lines	FBDS1 FBDS2
Institution	Instituto de Neurociencias, Fleni (INEU-Fleni-CONICET)
Contact information of distributor	Leonardo Romorini (lromorini@fleni.org.ar); Ezequiel Surace (esurace@fleni.org.ar)
Type of cell lines	iPSC
Origin	Human
Additional origin info required	Age: 52 (FLENIi002-A), 51 (FLENIi003-A) Sex: Male (both patients) Ethnicity if known: Caucasian (both patients)
Cell Source	Peripheral blood mononuclear cells (PBMCs) (both lines)
Clonality	Clonal
Method of reprogramming	Lentiviral EF1a-hSTEMCCA-loxP vector expressing *OCT-4*, *SOX-2*, *c-MYC* and *KLF4*

(*continued on next column*)

(*continued*)

Unique stem cell lines identifier	FLENIi002-A FLENIi003-A
Genetic Modification	YES
Type of Genetic Modification	Trisomy 21 (both lines)
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-qPCR
Associated disease	Down syndrome-Alzheimer's disease (FLENIi002-A) Down syndrome (FLENIi003-A)
Gene/locus	N/A
Date archived/stock date	8–11/2022
Cell line repository/bank	https://hpscreg.eu/cell-line/FLENIi002-A https://hpscreg.eu/cell-line/FLENIi003-A
Ethical approval	The study was approved by the local Ethics Committee (Ethics Committee for Biomedical Research of the Fleni Institute) (code number:21–16). Written informed consent was obtained from each patient.

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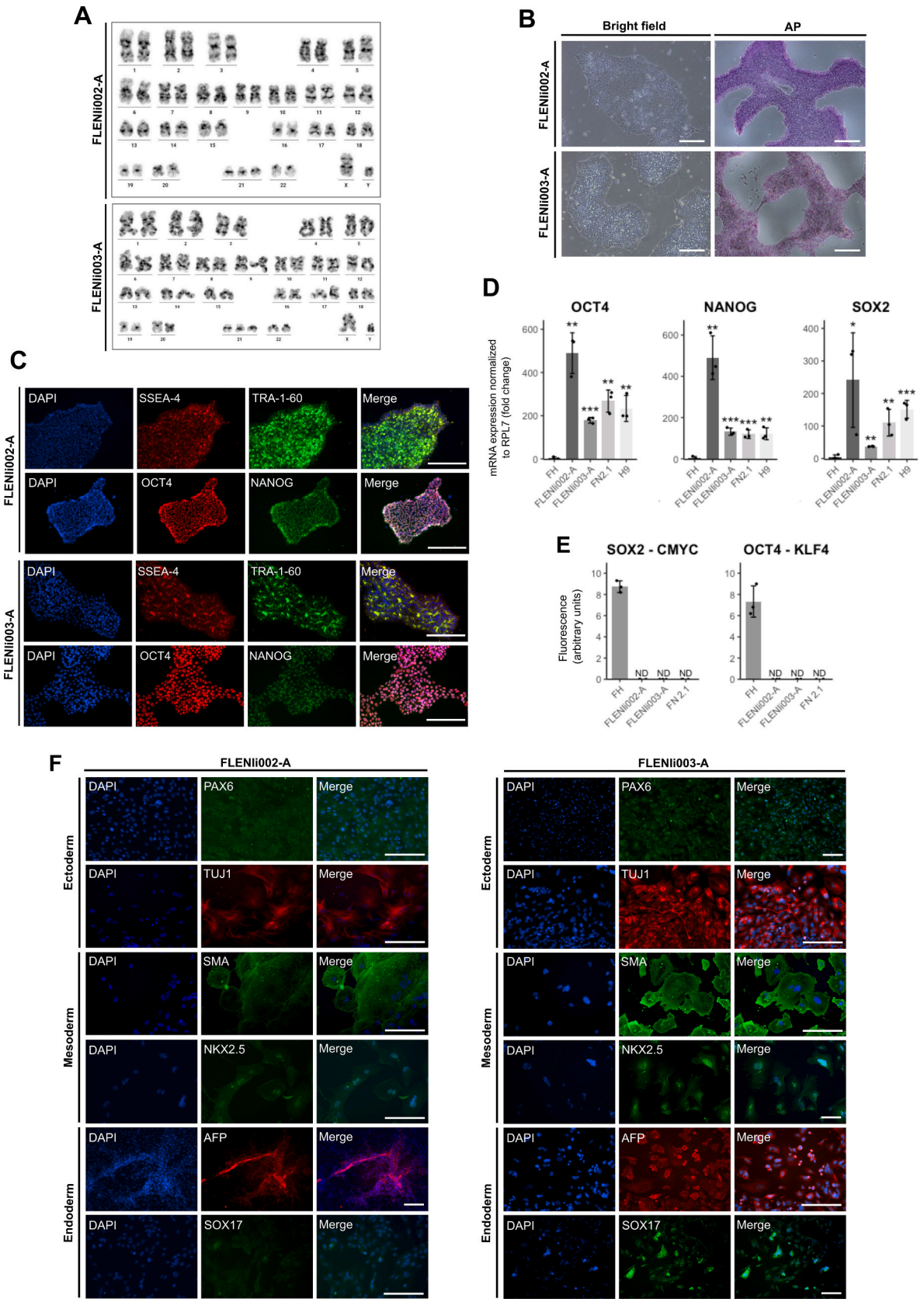

Fig. 1. Characterization of the hiPSC lines FLENIi002-A and FLENIi003-A. (A) Karyotype analysis. (B) hiPSC morphology and AP activity. (C) Immunocytochemistry for pluripotency markers SSEA-4, TRA1-60, OCT4, NANOG, and nuclear marker DAPI. (D) Expression of pluripotency markers analyzed by RT-qPCR. (E) Silencing of transgenes introduced by the STEMCCA lentiviral vector confirmed by RT-qPCR. (F) Immunocytochemistry for germ layer markers: mesoderm (SMA and NKX2.5), endoderm (AFP and SOX17), and ectoderm (PAX6 and TUJ1).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal (both lines)	Fig. 1 panel B
Phenotype	Immunocytochemistry	Positive for pluripotency markers OCT-4, NANOG, TRA-1–60, and SSEA4 (both lines)	Fig. 1 panel C
	RT-qPCR	Cells express *OCT-4*, *SOX-2* and *NANOG* (both lines)	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	47,XY,+21, both cell lines Resolution: 440	Fig. 1 panel A
mtDNA analysis (IF APPLICABLE)	N/A	N/A	N/A
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	26 sites tested, matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	N/A	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR (Negative)	Supplementary file
Differentiation potential	Embryoid body formation	Expression of α-smooth muscle actin (SMA), NK2 homeobox 5 (NKX2.5), SRY-Box Transcription Factor 17 (SOX17), α-fetoprotein (AFP), paired box protein Pax-6 (PAX6) and Class III β-tubulin (TUJ1) was used as a proof of three germ layers formation (both lines)	Fig. 1 panel F

1.0.1. Resource utility

The FLENIi002-A line was derived from a Down syndrome (DS) patient with Alzheimer's disease (AD) and amyloid-beta deposits, while the FLENIi003-A line was generated from a Down syndrome patient resistant to amyloid-beta (Aβ) deposition and without signs of Alzheimer's disease. These hiPSC lines provide valuable resources for investigating the factors that confer protection against amyloid-beta pathology in DS.

2. Resource details

DS results from trisomy 21 and is linked to various phenotypic features, including an increased risk for AD due to the triplication of the *APP* gene (located in 21q21). Individuals with DS over 40 years of age typically have Aβ deposits in their brains. However, some individuals show resistance to these deposits despite advanced age (Lao et al., 2017; Tudorascu et al., 2019; Zammit et al., 2020; Zammit et al., 2021). The FLENIi002-A cell line was derived from a 51-year-old patient with DS who exhibited both AD and Aβ plaques. In contrast, the FLENIi003-A line was obtained from a 52-year-old patient with DS who demonstrated resistance to Aβ pathology and showed no clinical signs of AD.

PBMCs were obtained from both patients, and reprogramming into hiPSCs was performed using the EF1a-hSTEMCCA-loxP lentiviral vector expressing *OCT-4*, *SOX-2*, *c-MYC*, and *KLF4* pluripotency genes, following a feeder-free protocol (Somers et al., 2010; Chen et al., 2011). Both hiPSC lines retained the trisomy 21 genotype (Fig. 1A). Morphologically, both lines displayed the typical characteristics of hiPSCs, including compact multicellular colonies with a high nucleus-to-cytoplasm ratio and clear colony borders. High levels of Alkaline Phosphatase (AP) activity were observed (Fig. 1B). Immunofluorescence staining further validated the robust expression of pluripotency markers OCT-4, NANOG, SSEA-4 and TRA-1–60 (Fig. 1C). RT-qPCR confirmed expression of stemness markers *SOX-2*, *POU5F1* (*OCT-4*), and *NANOG*, with a primary culture of human fibroblasts (HF) serving as a negative control (Fig. 1D). The established hiPSC line FN2.1 (Questa et al., 2016) and H9 human embryonic stem cells were used as positive controls. Silencing of the transgenes inserted by the STEMCCA lentiviral vector was confirmed by RT-qPCR using specific primers for exogenous gene expression (Fig. 1E), with hiPSCs (FN2.1) and transduced fibroblasts from day 6 of reprogramming (HF) as negative and positive controls, respectively. *In vitro* differentiation assays confirmed the pluripotent potential of both lines. Undirected differentiation through the embryoid body method demonstrated their ability to generate the three germ layers, as evidenced by immunofluorescence staining for Smooth Muscle Actin (SMA, mesoderm), NK2 homeobox 5 (NKX2.5, mesoderm), Alpha-Fetoprotein (AFP, endoderm), SRY-Box Transcription Factor 17 (SOX17, endoderm), paired box protein Pax-6 (PAX6, ectoderm) and Class III β-tubulin (TUJ1, ectoderm) (Fig. 1F). (See Tables 1 and 2).

3. Materials and methods

3.1. Reprogramming and cell culture

PBMCs were isolated from blood using a Ficoll density gradient procedure (HISTOPAQUE® SIGMA, #10771). A total of 2×10^6 cells were cultured in 2 mL of erythrocyte expansion media containing StemPro™-34-SFM (Gibco™, #10639011), 50 μg/mL ascorbic acid, 50 ng/mL SCF, 10 ng/mL IL-3, 2 U/mL EPO, 40 ng/mL IGF-1, and 1 μM dexamethasone in 1 well of a 12-well dish at 37 °C, 5 % CO_2. Media was replaced on days 3 and 6. On day 9, cells were transduced with the EF1a-hSTEMCCA-loxP lentiviral reprogramming vector at a MOI of 7. Cells were plated on Geltrex-coated dishes (1 %, Gibco™, #A1413202) and further cultured according to the manufacturer's instructions for TeSR™-E7™ (STEMCELL Technologies™, #05914). At day 28 post-transduction, hiPSC colonies were mechanically isolated and expanded on Geltrex-coated dishes in mTeSR™ Plus medium (STEMCELL Technologies™, #17187501). Cell cultures were passaged every 3 to 4 days using Versene (Gibco™, #15040066) at a 1:8 ratio, with 10 μM Y-27632 ROCK inhibitor (Cell Signaling Technology, #13624) added for up to 24 h.

3.2. Karyotyping

G-banded chromosome analysis of 20 metaphases from hiPSCs (passages 18 and 10) was performed according to standard protocols..

3.3. Alkaline phosphatase assay

hiPSCs were subjected to alkaline phosphatase staining following the manufacturer's instructions (Sigma, #86R) (passages 17 and 8).

3.4. RNA isolation and RT-qPCR

RNA was extracted using TRIzol (ThermoFisher Scientific,

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	mouse anti-OCT-4 IgG	1:200	Santa Cruz Biotechnology sc-5279	AB_628051
Pluripotency Markers	rabbit anti-NANOG IgG	1:200	Cell Signaling Technology 4903	AB_10559205
Pluripotency Markers	mouse anti-SSEA4 IgG	1:200	Santa Cruz Biotechnology sc-21704	AB_628289
Pluripotency Markers	mouse anti-TRA-1–60 IgM	1:200	Santa Cruz Biotechnology sc-21705	AB_628385
Differentiation Markers	mouse anti-AFP IgG	1:200	Santa Cruz Biotechnology sc-166325	AB_2305278
Differentiation Markers	rabbit anti-SMA IgG	1:200	Abcam ab124964	AB_11129103
Differentiation Markers	rabbit anti-SOX17 IgG	1:100	Invitrogen PA5-72815	AB_2718669
Differentiation Markers	rabbit anti-NKX2.5 IgG	1:100	Invitrogen PA5-49431	AB_2634885
Differentiation Markers	rabbit anti-PAX6 IgG	1:100	Abcam ab195045	AB_2750924
Differentiation Markers	rabbit anti-TUJ1 IgG	1:400	Covance Antibody Products MMS-435P	AB_2313773
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 594	1:400	Thermo Fisher Scientific A-11005	AB_2534073
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 488	1:400	Thermo Fisher Scientific A-11001	AB_2534069
Secondary antibodies	Goat anti-Mouse IgM Alexa Fluor 488	1:400	Thermo Fisher Scientific A-21042	AB_2535711
Secondary antibodies	Goat anti-Rabbit IgG Alexa Fluor 594	1:400	Thermo Fisher Scientific A-11012	AB_2534079
Secondary antibodies	Goat anti-Rabbit IgG Alexa Fluor 488	1:400	Thermo Fisher Scientific A-11008	AB_143165
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Exogenous factors (RTPCR)	STEMCCA plasmid OCT-4/KLF4	561 bp	CAACGAGAGGATTTTGAGGC/ATCGTTGAACTCCTCGGTCTCTCT	
Exogenous factors (RTPCR)	STEMCCA plasmid SOX-2/c-MYC	550 bp	TTGGCTCCATGGGTTCGGTG/AAGGGTGTGACCGCAACGTAGG	
Pluripotency Markers (qPCR)	POU5F1 (OCT-4)	105 bp	GCAGGCCCGAAAGAGAAAGCGA/TGGCTGATCTGCTGCAGTGTGG	
Pluripotency Markers (qPCR)	SOX-2	110 bp	AGCATGGAGAAAACCCGGTACGC/ CGTGAGTGTGGATGGGATTGGTGT	
Pluripotency Markers (qPCR)	NANOG	120 bp	TCCTTCCTCTCCCCTCCTCCCAT/TAGGCTCCAACCATACTCCACCCTC	
House-Keeping Genes (qPCR)	RPL7	138 bp	AATGGCGAGGATGGCAAG/TGACGAAGGCGAAGAAGC	

#15596026), and cDNA was synthesized from total RNA using Oligo (dT) primers and M−MLV Reverse Transcriptase (Promega, #M170B) following the manufacturer's instructions. qPCR amplification (95 °C for 15 s, 60 °C for 1 min, 40 cycles) and analysis were performed using the StepOnePlus Real-Time PCR System (PE Applied Biosystems). The FastStart Universal SYBR Green Master Mix (ROX) (Roche, #04913850001) was used for all reactions. Transgene expression was assessed using primers spanning two of the four transgenes on the STEMCCA lentiviral vector sequence, allowing amplification of only cDNA generated from the exogenous mRNA expression of these genes. Cells were analyzed at passages between 10 and 18. Values were normalized to levels of the housekeeping gene *RPL7*.

3.5. In vitro differentiation

hiPSCs (passages 12 and 20) were detached using Dispase (Gibco™, #17105041) and transferred to non-adherent Petri dishes in DMEM/F12 with 1x GlutaMAX (Gibco, #35050061), 20 % KnockOut™ Serum Replacement (Gibco™, #10828), 1x non-essential amino acids (Sigma, #M7145), 0.1 μM 2-mercaptoethanol, 100 U/ml penicillin, and 50 μg/ml streptomycin to induce the formation of embryoid bodies (EBs). On day 4, EBs were plated onto 0.1 % gelatin-coated 24-well plates and cultured for an additional 17 days in DMEM/F12 supplemented with 20 % FBS (Gibco, #10270106), 1x GlutaMAX, 100 U/ml penicillin, and 50 μg/ml streptomycin.

3.6. Immunofluorescence staining

Cells were fixed with 4 % paraformaldehyde for 30 min and permeabilized with 0.1 % Triton X-100 for 30 min. Primary antibody incubation was performed overnight at 4 °C in PBS with 1 % BSA. Fluorescent dye-conjugated secondary antibodies were incubated in PBS with 1 % BSA for 1 h at room temperature. Cells were counterstained with DAPI and examined under a EVOS FLoid (Thermo Fisher Scientific) inverted microscope. Pluripotent markers were analyzed at passages 17 and 9.

3.7. STR analysis

Genomic DNA was isolated using the Wizard Genomic DNA Purification kit (Promega). STR analysis of 26 *loci* was performed at the Laboratorio de Huellas Digitales Genéticas (Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina) (passages 18 and 9).

3.8. Statistical analysis

Results are expressed as mean ± SEM (n = 3). Comparisons were analyzed by Two-tailed Student's *t*-test (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$).

CRediT authorship contribution statement

Giulia S. Clas: Validation, Methodology, Formal analysis. **Mariela Marazita:** Validation, Methodology, Formal analysis. **Lucía Pertierra:** Resources, Investigation. **Silvia Vazquez:** Investigation. **Luciana Isaja:** Methodology. **Soledad Rodríguez-Varela:** Methodology. **Sofía Mucci:** Methodology. **Belén Helou:** Investigation. **Fernanda Tapajóz:** Investigation. **Nahuel Magrath:** Investigation. **Yanina Bérgamo:** Conceptualization, Investigation, Resources. **Ricardo Allegri:** Investigation. **Gustavo E. Sevlever:** Investigation. **Maria E. Scassa:** Methodology, Investigation. **Ezequiel I. Surace:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Leonardo Romorini:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2025.103728.

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