



Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line (FLENIi004-A) from an Autosomal-Dominant Alzheimer's disease PSEN1 p.M146L patient

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ABSTRACT

Human induced pluripotent stem cell (hiPSC) line FLENIi004-A was generated from peripheral blood mononuclear cells (PBMCs) using the lentiviral-hSTEMCCA-loxP vector. FLENIi004-A was derived from a 41-year-old Argentinean Caucasian male patient with Autosomal-Dominant Alzheimer's Disease, who carried the heterozygous *PSEN1* p.M146L mutation. The hiPSC line retained the *PSEN1* mutation, and its pluripotency and trilineage differentiation potential were confirmed.

Resource Table:

Unique stem cell line identifier	FLENIi004-A
Alternative name(s) of stem cell line	FBAD1
Institution	Instituto de Neurociencias, Fleni (INEU-Fleni-CONICET)
Contact information of distributor	Leonardo Romorini ()
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 41Sex: maleEthnicity if known: Caucasian
Cell Source	Erythroblast expansion and enrichment from peripheral blood mononuclear cells (PBMCs).
Clonality	Clonal
Method of reprogramming	Lentiviral EF1a-hSTEMCCA-loxP vector expressing <i>OCT-4</i> , <i>SOX-2</i> , <i>c-MYC</i> and <i>KLF4</i>
Genetic Modification	YES
Type of Genetic Modification	Congenital mutation
Evidence of the reprogramming	RT-qPCR
transgene loss (including genomic copy if applicable)	
Associated disease	Alzheimer's Disease

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Unique stem cell line identifier	FLENIi004-A
Gene/locus	<i>PSEN1</i> (rs63750306;c.436A > T; p. M146L)
Date archived/stock date	2025-07-22
Cell line repository/bank	https://hpscreg.eu/cell-line/FLENIi004-A
Ethical approval	The study was approved by local Ethics Committee (Comité de ética en investigaciones biomédicas del Instituto Fleni). Approval number: 04/14. Written informed consent was obtained from the patient.

1. Resource utility

Autosomal-Dominant Alzheimer's disease (ADAD) is a neurodegenerative disorder characterized by progressive cognitive decline. The FLENIi004-Ai hiPSC line carries the extensively studied *PSEN1* p.M146L (A > T) variant, making it a valuable model for *in vitro* studies of Alzheimer's disease pathophysiology, particularly in underrepresented Latin American populations.

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<https://doi.org/10.1016/j.scr.2026.103958>

Received 23 September 2025; Received in revised form 28 November 2025; Accepted 3 March 2026

Available online 6 March 2026

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2. Resource details

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia in the adult population. While most AD cases are sporadic, approximately 5% are familial, caused by autosomal dominant mutations in genes such as *Presenilin-1* (*PSEN1*), *PSEN2*, and *APP*. Among these, *PSEN1* variants are the most prevalent. The iPSC line FLENIi004-A was derived from erythroblasts obtained from peripheral blood mononuclear cells (PBMCs) of a clinically diagnosed 41-year-old male patient with Autosomal-Dominant Alzheimer’s Disease (ADAD) of Argentine origin, who carries the heterozygous *PSEN1* (NM_000021.4:c.436A > T) (p.M146L) mutation. Genomic coordinates correspond to Chr14:73173663 A > T in GRCh38/hg38 and Chr14:73640371 A > T in GRCh37/hg19. This pathogenic variant is one of the most frequently reported in ADAD cases worldwide

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel C
Phenotype	RT-qPCR	Cells express <i>Oct4</i> and <i>Nanog</i>	Fig. 1 panel D
	Immunocytochemistry	Positive for pluripotency markers OCT-4, NANOG, TRA1–60, and SSEA4	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46, XYResolution: 440	Fig. 1 panel B
mtDNA analysis (IF APPLICABLE)	N/A	N/A	N/A
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A	N/A
		26 sites tested, matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous mutation c.436A > T	Fig. 1 panel A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR. Negative.	Supplementary file
Differentiation potential	Embryoid body formation	Mesoderm: α-smooth muscle actin (SMA), NK2 homeobox 5 (NKX2.5) Endoderm: SRYBox Transcription Factor 17 (SOX17), Forkhead box protein A2 (FOXA2) Ectoderm: SRYBox Transcription Factor 1 (SOX1), βIII-tubulin (TUJ1).	Fig. 1 panel G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

and has been associated with early-onset disease. Table 1 and 2.

Reprogramming of patient-derived erythroblasts was carried out using the EF1a-hSTEMCCA-loxP lentiviral vector as described previously (Staerk et al., 2010), which encodes the Yamanaka transcription factors *OCT4*, *SOX2*, *KLF4*, and *c-MYC* in a single polycistronic cassette. After colony selection and expansion, the presence of the *PSEN1* p. M146L (A > T) variant was confirmed in the iPSC line by Sanger sequencing (Fig. 1A). Short tandem repeat (STR) profiling confirmed identity matching with the donor sample.

FLENIi004-A iPSCs exhibited a normal karyotype (46, XY) (Fig. 1B),

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	mouse anti-Nanog IgG	1/200	Santa Cruz Biotechnology sc-293121	AB_2665475
Pluripotency Markers	mouse anti-Oct-4 IgG	1/200	Santa Cruz Biotechnology sc-5279	AB_628051
Pluripotency Markers	mouse anti-Sox2 IgG	1/200	Cell signaling #4900	AB_10560516
Pluripotency Markers	mouse anti-TRA-1–60 IgM	1/200	Santa Cruz Biotechnology sc-21705	AB_628385
Pluripotency Markers	mouse anti-SSEA4 IgG	1/200	Santa Cruz Biotechnology sc-21704	AB_628289
Differentiation Markers	rabbit anti-FOXA2	1/200	Invitrogen 710,730	AB_2576440
Differentiation Markers	rabbit anti-SOX17 IgG	1/200	Invitrogen PA5-72815	AB_2718669
Differentiation Markers	rabbit anti-SMA IgG	1/200	Abcam ab124964	AB_11129103
Differentiation Markers	rabbit anti-NKX2.5 IgG	1/200	Invitrogen PA5-49431	AB_2634885
Differentiation Markers	rabbit anti-SOX1 IgG	1/200	Abcam ab87775	AB_2616563
Differentiation Markers	mouse anti-TUJ1 IgG	1/200	Abcam ab78078	AB_2256751
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 594	1/400	Thermo Fisher Scientific A-11005	AB_2534073
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 488	1/400	Thermo Fisher Scientific A-11008	AB_143165
Primers				
	Target	Size of band	Forward/Reverse primer (5’-3’)	
Exogenous factors (qPCR)	STEMCCA plasmid OCT-4/KLF4	561 bp	CAACGAGAGGATTTTGAGGC/ATCGTTGAACCTCTCGGTCTCTCT	
Exogenous factors (qPCR)	STEMCCA plasmid SOX-2/c-MYC	550 bp	TTGGCTCCATGGGTTTCGGTG/AAGGGTGTGACCGCAACGTAGG	
Pluripotency Markers (qPCR)	POU5F1 (OCT-4)	105 bp	GCAGGCCCGAAAGAGAAAGCGA/TGGCTGATCTGCTGCAGTGTGG	
Pluripotency Markers (qPCR)	NANOG	120 bp	TCCTTCTCTCCCTCTCCCAT/TAGGCTCCAACCTACTCCACCTC	
House-Keeping Genes (qPCR)	RPL7	138 bp	AATGGCGAGGATGGCAAG/TGACGAAGCGAAGAAGC	
Sequencing	PSEN1 exon 5	260 bp	GTGGTAATGTGGTTGGTGAT/CCCAACCATAAGAAGAACAG	

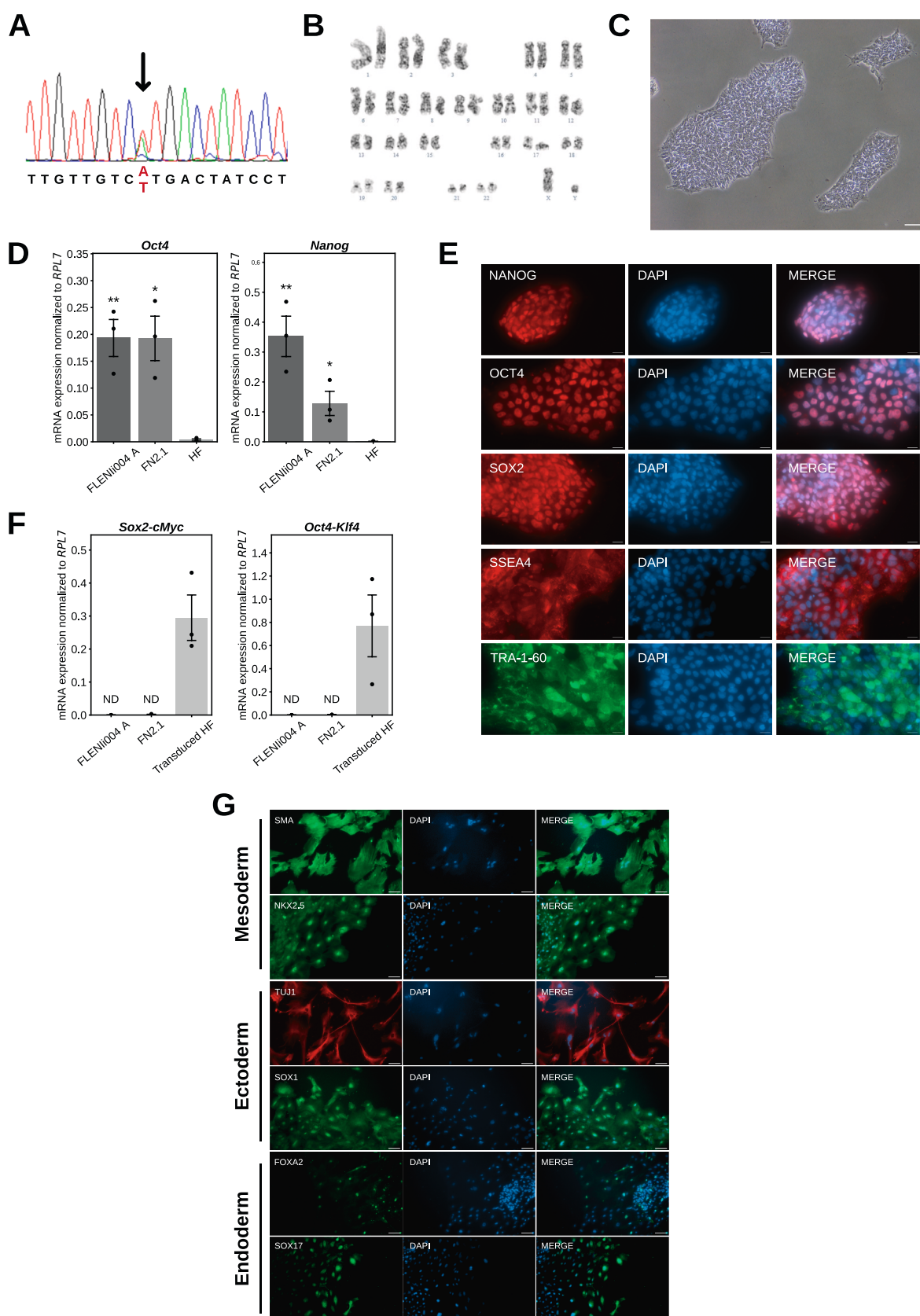


Fig. 1. Characterization of the hiPSC line FLENI004-A. (A) Sanger sequencing showing the *PSEN1* p.M146L (A > T) variant in the iPSC line. (B) Karyotype analysis. (C) hiPSC morphology. (D) Expression of pluripotency markers analyzed by RT-qPCR. (E) Immunocytochemistry for pluripotency markers NANOG, OCT4, SOX2, SSEA-4, TRA1-60, and nuclear marker DAPI. (F) Silencing of transgenes introduced by the STEMCCA lentiviral vector confirmed by RT-qPCR. (G) Immunocytochemistry for germ layer markers: mesoderm (SMA and NKX2.5), ectoderm (TUJ1 and SOX1), and endoderm (FOXA2 and SOX17).

and displayed typical morphology of pluripotent stem cells, characterized by flat colonies with a high nucleus-to-cytoplasm ratio and defined borders (Fig. 1C, scale bar: 100 μ m). Pluripotency was validated by both RT-qPCR and immunofluorescence for key endogenous markers, including nuclear OCT4, SOX2, NANOG, and surface markers TRA-1-60 and SSEA-4 (Fig. C and D, scale bar: 20 μ m). Human fibroblasts (HF) and an established hiPSC line (FN2.1) (Questa et al., 2016) were used as negative and positive controls, respectively. Importantly, the silencing of exogenous transgenes introduced by the STEMCCA vector was confirmed by RT-qPCR using primers specific for the viral polycistronic sequences. No residual expression of *OCT4-KLF4*, or *SOX2-c-MYC* from the vector was detected in FLENi004-A cell line, indicating complete reprogramming and independence from the exogenous reprogramming cassette (Fig. 1F, ND: no detected). As positive controls, HF at day 6 post-transduction and hiPSC line FN2.1 were included.

Finally, functional pluripotency was further confirmed via *in vitro* undirected differentiation using the embryoid body (EB) formation assay. Cells differentiated into the three embryonic germ layers, as demonstrated by the expression of β -III tubulin (TUBJ1) and SRY-Box Transcription Factor 1 (SOX1) for ectoderm, Smooth Muscle Actin (SMA) and NK2 homeobox 5 (NKX2.5) for mesoderm, and Forkhead box protein A2 (FOXA2) and SRY-Box Transcription Factor 17 (SOX17) for endoderm, assessed by immunostaining (Fig. 1G, scale bar: 100 μ m).

3. Materials and methods

3.1. Reprogramming and cell culture

PBMCs were isolated from whole blood using a density gradient centrifugation protocol with HISTOPAQUE-10771 (Sigma). 2×10^6 cells were cultured in erythroid expansion medium composed of StemPro-34 (Gibco) supplemented with 50 μ g/mL ascorbic acid, 50 ng/mL SCF, 10 ng/mL IL-3, 2 U/mL EPO, 40 ng/mL IGF-1, and 1 μ M dexamethasone at 37 °C, 5% CO₂. On day 12, erythroblast-enriched cultures were transduced with the EF1 α -hSTEMCCA-loxP lentiviral vector (MOI: 5) with 4 μ g/mL polybrene. After transduction, cells were plated onto irradiated mouse embryonic fibroblast (iMEF) and cultured in MME media (DMEM, 10% FBS, 2 mM non-essential aminoacids, 0.1 mM 2-mercaptoethanol and 10 ng/mL bFGF). Upon the appearance of small colonies, medium was changed to hES medium [DMEM/F12, 20% KSR (Gibco), 2 mM non-essential aminoacids, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol and 8 ng/mL bFGF]. Around day 28, colonies with iPSC-like morphology were picked and transferred to Geltrex-coated dishes (Gibco) using mTeSR™ Plus medium (STEMCELL Technologies). Passages (1:3) were performed every 3–4 days with Versene and ROCK inhibitor.

3.2. Genotyping and STR analysis

Genomic DNA was isolated using the Wizard Genomic DNA Purification kit (Promega). *PSEN1* p.M146L mutation was screened by PCR amplification and sequenced by Sanger. STR analysis of 26 locations was performed at the Laboratorio de Huellas Digitales Genéticas (Buenos Aires, Argentina).

3.3. Karyotyping

G-band chromosome analysis of 50 metaphases from hiPSCs (passage 10) was performed by Kromos Cytogenetic Laboratory (Buenos Aires, Argentina). Standard procedures were used to assess chromosomal integrity and ploidy at 440-band resolution.

3.4. RNA isolation and RT-qPCR

Total RNA was extracted from cells (passages 5–11) using TRIzol. cDNA was synthesized from 1 μ g of total RNA with random primers and

M–MLV reverse transcriptase (Promega), following the manufacturer's instructions. RT-qPCR was conducted on a StepOnePlus Real-Time PCR System (Applied Biosystems) using FastStart Universal SYBR Green Master Mix (Roche). *RPL7* was used as a housekeeping control. Transgene expression was evaluated with primers spanning two of the transgenes within the STEMCCA polycistronic cassette, allowing specific detection of exogenous transcripts. Data were analysed using LinRegPCR (Ramakers et al., 2003).

Immunofluorescence staining.

Cells (passages 11–17) were fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X-100 for 45 min at room temperature (RT). Blocking and antibody incubations were performed in PBS containing 1% Normal Goat Serum. Primary antibodies were incubated overnight at 4 °C. Fluorescent secondary antibodies were applied for 2 h at RT. Nuclei were counterstained with DAPI. Imaging was performed using Zeiss Axio Observer 7.

3.5. *In vitro* differentiation

hiPSC colonies maintained on iMEF were used to initiate EB formation. Colonies were detached using 1 mg/mL collagenase IV and transferred to non-adherent 60 mm Petri dishes. EBs were cultured in suspension for 4 days in DMEM supplemented with 20% FBS, 2 mM non-essential aminoacids, 2 mM L-glutamine and 0.1 mM 2-mercaptoethanol. On day 4, EBs were plated onto 0.1% gelatin-coated 24-well plates and cultured in the same medium for an additional 10 days to promote undirected differentiation.

3.6. Statistical analysis

Results are expressed as mean $\pm$ SEM. Student's *t*-test was used; * *p* < 0.05, ** *p* < 0.01.

CRedit authorship contribution statement

Mercedes Florencia Vautier: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis. **Patricio Chrem Mendez:** Writing – review & editing, Resources, Investigation, Conceptualization. **Guillermo Jerez Ferreyra:** Methodology, Investigation. **Victoria Massazza:** Investigation. **Manuela Apécetche:** Investigation. **María Soledad Rodríguez Varela:** Supervision, Investigation. **Giulia Clas:** Methodology, Investigation. **Micaela Nievas:** Investigation. **Ricardo Allegri:** Writing – review & editing, Supervision, Funding acquisition. **Gustavo Emilio Sevlever:** Writing – review & editing, Funding acquisition. **María Elida Scassa:** Writing – review & editing, Supervision, Funding acquisition. **Ezequiel Ignacio Surace:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Mariela Claudia Marazita:** Writing – review & editing, Supervision, Methodology, 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.

Acknowledgments

This work was supported by the Argentinian National Scientific and Technical Research Council (CONICET) (grant number PIP11220200100270CO) and Fundación Fleni.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2026.103958>.

Data availability

Data will be made available on request.

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