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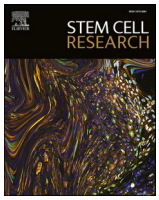

Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line (CIBIOi007-A) from a Lafora disease patient

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A B S T R A C T

An induced pluripotent stem cell (iPSC) line was generated from peripheral blood mononuclear cells (PBMCs) of a 24-year-old male patient affected by Lafora disease. The patient is homozygous for the c.721C>T, p.(Arg241*) nonsense variant in the *EPM2A* gene, which codes for the laforin protein. The generated iPSC line possesses a normal karyotype, expresses pluripotency markers, and can differentiate into derivatives of the three germ layers. It is configured as a useful tool for both disease modeling and therapy development.

1. Resource Table

Unique stem cell line identifier	CIBIOi007-A https://hpscreg.eu/cell-line/CIBIOi007-A
Alternative name(s) of stem cell line	N/A
Institution	Department of Cellular, Computational and Integrative Biology – CIBIO, University of Trento, Italy
Contact information of distributor	Graziano Lolli – graziano.lolli@unitn.it
Type of cell line	iPSC
Origin	Human
Additional origin info requiredfor human ESC or iPSC	Age: 24 Sex: Male Ethnicity if known: Caucasian
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	CytoTune™-iPS 2.0 Sendai Reprogramming Kit – Thermo Fisher Scientific (hOCT4, hSOX2, hc-Myc, hKLF4)
Genetic Modification	YES
Type of Genetic Modification	Hereditary
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR (Fig. 1 Panel C)
Associated disease	Lafora disease
Gene/locus	*EPM2A* gene, homozygous c.721C > T, p.(Arg241*) nonsense variant
Date archived/stock date	November 2021
Cell line repository/bank	N/A
Ethical approval	The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Casa Sollievo della Sofferenza Hospital (protocol no. 177CE, 7 November 2017).

2. Resource utility

The generated iPSC line can be differentiated into nervous system cells, serving as an *in vitro* model for Lafora disease (LD), which could be used to further dissect the disease's molecular mechanism and implement a screening assay for drug development purposes.

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3. Resource details

LD is the most severe form of teenage-onset progressive epilepsy, with no resolutive therapy available and only palliative treatments adopted in clinical practice (Nitschke et al., 2018; Verhalen et al., 2018). It is characterized by the accumulation in brain cells of Lafora bodies (LBs), which are composed of insoluble polyglucosans. LD is an autosomal recessive disorder driven by inactivating mutations in either the *EPM2A* or *EPM2B* genes, coding for the laforin and malin proteins, respectively. The affected individuals have unbalanced glycogen synthesis/degradation and branching/debranching activities, resulting in the accumulation of the neurotoxic LBs, mainly dictated by the missing action of the malin/laforin complex on the PTG (Protein Targeting to Glycogen) protein (Semrau et al., 2022).

The CIBIOi007-A iPSC line has been generated from PBMCs of a 24-year-old male patient affected by LD. Genetic and electroclinical characteristics of the donor patient have been previously described (d'Orsi et al., 2020). The reprogramming was performed using a Sendai viral vector encoding hOCT4, hSOX2, hc-Myc, and hKLF4. The absence of vector integration was assessed by RT-PCR (Fig. 1 Panel C). The CIBIOi007-A line exhibited normal hiPSC morphology (Fig. 1 Panel A), and the homozygous c.721C>T, p.(Arg241*) nonsense mutation in *EPM2A* gene was confirmed by Sanger sequencing (Fig. 1 Panel B). The pluripotency markers OCT4, SOX2, and NANOG were comparably expressed to a commercial WT hiPSC line at the mRNA level by RT-qPCR (Fig. 1 Panel D). The iPSC line also marked positive for OCT4, SOX2, and TRA-1–60 at the protein level by immunofluorescence assay (Fig. 1 Panel E). The differentiation potential into the three germ layers has been proven by embryoid body formation. After 21 days of differentiation, cells stained positive for ectoderm (Nestin and TUBB3), mesoderm (αSMA and TBXT), and endoderm (FOXA2 and KRT8) markers (Fig. 1 Panel F). The iPSC line resulted in negative mycoplasma contamination (Supplementary Fig. 1). The CIBIOi007-A line was authenticated by SNP array analysis, resulting in a 98.9 % concordance (supplementary data). The CNVs profile returned a duplication of about 2 kb in the chromosomic region 2q12.3q13 present in both patient PBMCs and derived hiPSCs (supplementary data). This data confirmed the cell line identity and the absence of genomic rearrangement due to the reprogramming. Table 1 summarizes the characteristics of the CIBIOi007-A iPSC line. Reagents used in this work are listed in Table 2.

4. Materials and methods

4.1. Cell culturing and reprogramming

All cells were cultured in humidified incubators at 37 °C and 5 % CO_2. According to the manufacturer's instructions, PBMCs were reprogrammed using the CytoTune-iPS 2.0 Sendai Reprogramming Kit

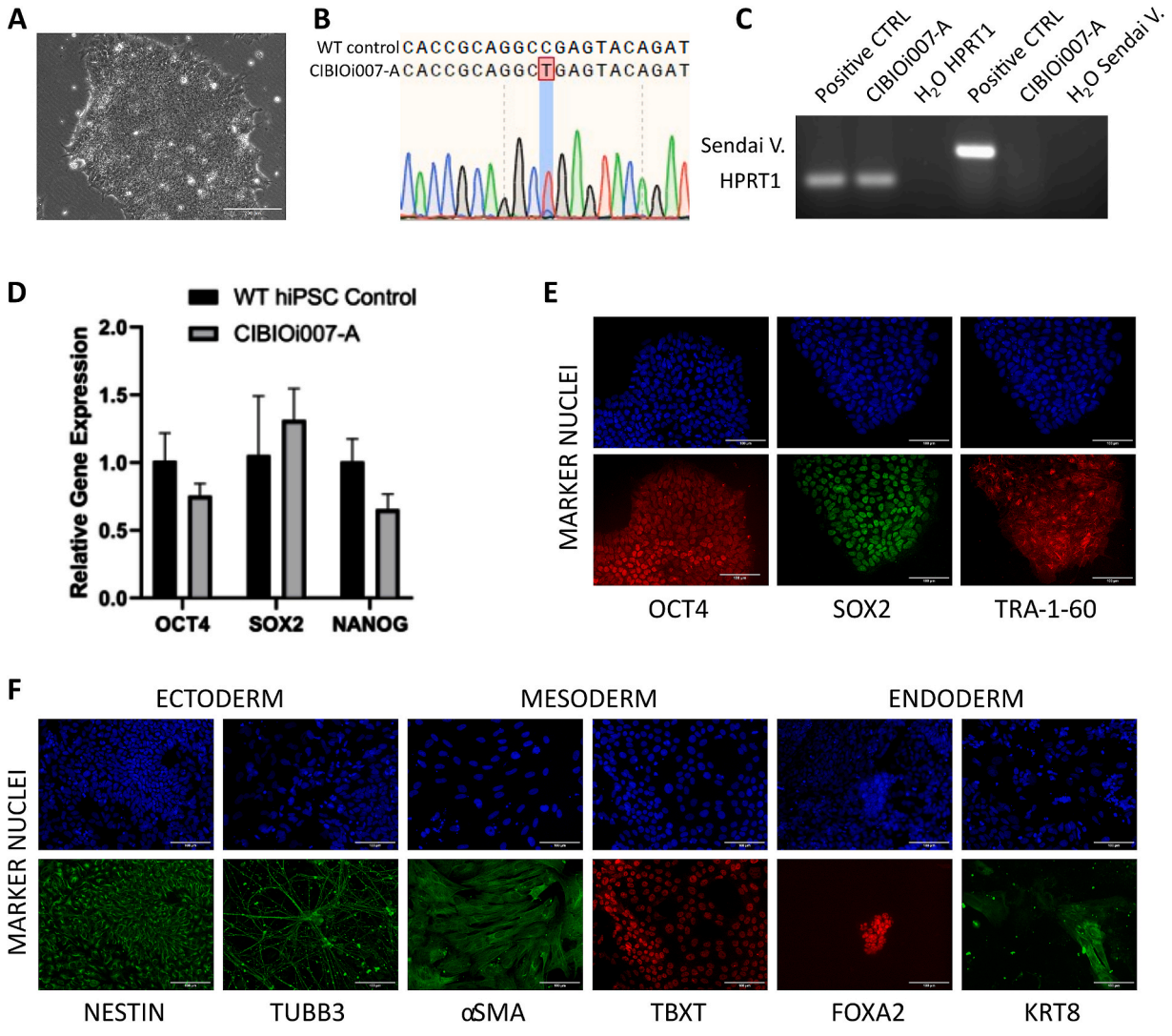

Fig. 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 Panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for pluripotency markers: OCT4, SOX2, TRA1-60	Fig. 1 Panel E
	Quantitative analysis (RT-qPCR)	Positive for pluripotency markers: OCT4, SOX2, Nanog	Fig. 1 Panel D
Genotype	SNP Array	Resolution: 500 kb	Supplementary file
mtDNA analysis (IF APPLICABLE)	*Sanger Sequencing, NGS, Long-Read Sequencing and analysis software*	*SNVs/Indels concordance analysis with the parental cell line*	N/A
Identity	SNP Array	Confirmed identity 98.9 % concordance	Supplementary fileSubmitted in the archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	homozygous c.721C > T, p.(Arg241*) nonsense variant on EPM2A gene	Fig. 1 Panel B
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	e.g. Fig. 1/supplementary
Differentiation potential	Embryoid body formation and spontaneous differentiation	Immunostaining of specific germ layer markers: Nestin and TUBB3 for Ectoderm; α-SMA and TBXT for Mesoderm;FOXA2 and KRT8 for Endoderm	Fig. 1 Panel F
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm: Nestin and TUBB3 Mesoderm: α-SMA and TBXTEndoderm: FOXA2 and KRT8	IF with specific antibodies
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

Table 2
Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse anti-OCT4	1:200	Santa Cruz, Cat# sc-5279	RRID: AB_628051
Pluripotency Markers	Rabbit anti-SOX2	1:300	Abcam, Cat# ab97959	RRID:AB_2341193
Pluripotency Markers	Mouse anti-TRA-1-60	1:200	Santa Cruz, Cat# sc-21705	RRID: AB_628385
Differentiation Markers	Mouse anti-Nestin	1:500	R&D Systems, Cat# MAB1259	RRID: AB_2251304
Differentiation Markers	Mouse anti-TUBB3	1:300	Proteintech, Cat# 66375-1-lg	RRID: AB_2814998
Differentiation Markers	Mouse anti- α-SMA	1:100	Genetex, Cat# GTX100034	RRID: AB_1240408
Differentiation Markers	Rabbit anti-TBXT	1:100	R&D Systems, Cat# MAB20851-100	N/A
Differentiation Markers	Rabbit anti-FOXA2	1:100	Novusbio, Cat# NBP2-57623	N/A
Differentiation Markers	Mouse anti-KRT8	1:100	R&D Systems, Cat# MAB3165	RRID: AB_2234521
Secondary antibodies	Donkey Anti-Mouse IgG Alexa Fluor 546	1:1000	Thermo Fisher Scientific, Cat# A-10036	RRID: AB_2534012
Secondary antibodies	Donkey Anti-Rabbit IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific, Cat# A-21206	RRID: AB_2535792
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sendai virus vectors (PCR)	SeV	181 bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
Pluripotency Markers (qPCR)	NANOG	78 bp	CCTGTGATTTGTGGGCCTG/GACAGTCTCCGTGTGAGGCAT	
Pluripotency Markers (qPCR)	SOX2	78 bp	GTATCAGGAGTTGTCAAGGCAGAG/CTAGTCTTAAAGAGGCAGCAAAC	
Pluripotency Markers (qPCR)	OCT4	71 bp	GGAAGGAATTGGGAACACAAAGG/AACTTCACCTTCCCTCCAACCA	
House-Keeping Genes (qPCR)	GAPDH	87 bp	TGCACCACCAACTGCTTAGC/GGCATGGACTGTGGTCATGAG	
House-Keeping Genes (qPCR)	β-ACTIN	183 bp	TGTACGCCAACACAGTGCTG/GCTGGAAGGTGGACAGCGA	
House-Keeping Genes (qPCR)	HPRT1	94 bp	TGACACTGGCAAAACAATGC/GGTCCTTTTCACCAGCAAGC	
Genotyping (PCR)	EPM2A	432 bp	GGATTAGAGAGAGCCTCTGGCC/CCTCTTCGTCAATGTAGACAGCC	
Targeted mutation analysis/sequencing	EPM2A	N/A	TGGAATGCTCTTTCCACTTTGCT	

(Invitrogen). Colonies with hiPSC morphology were picked after 20 days post-transduction and cultured in TeSR-E8 medium (STEMCELL Technologies) on Geltrex (Gibco). hiPSCs were dissociated with 0.5 mM EDTA (Invitrogen) in PBS 1X (Gibco) every 3–4 days and further cultured.

4.2. Embryoid body formation and differentiation

hiPSCs were dissociated in clumps and grown in suspension in TeSR-E8 medium with 10 μM Y-27632 (Aobious). On day 2, the medium was shifted to 1:1 Essential 6 and TeSR-E8 and changed to Essential 6 medium (Gibco) on day 4. Embryoid bodies (EBs) were plated on Geltrex-coated 48-well plates on day 6, and the E6 medium was replaced every other day, allowing for cell differentiation. On day 21, cells were fixed for marker staining.

4.3. Genotyping

Genomic DNA was extracted from hiPSCs with the Wizard SV Genomic DNA Purification System (Promega), following the manufacturer's protocol. PCR was performed with Phusion Green High-Fidelity DNA Polymerase (Thermo Fisher Scientific), and products were

purified with the Quick PCR Purification and Gel Extraction Kit (FineTest). The sequence was determined through Sanger DNA sequencing (Metabion).

4.4. RNA isolation, polymerase chain reaction (PCR), and quantitative PCR (qPCR)

RNA isolation was performed with the TRiFast II reagent (Euroclone) from hiPSCs at passage 10, and RNA was *retro*-transcribed with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific), following the respective manufacturer's protocols. Starting from cDNA, the expression of pluripotency markers was assessed by qRT-PCR, using ExcelTaq 2X Fast Q-PCR Master Mix (SMOBIO Technology). The same cDNA was used to verify the absence of the reprogramming vector with Phusion Green High-Fidelity DNA Polymerase. All the PCR protocols were performed according to the manufacturer's instructions.

4.5. Immunocytochemistry

Cells were fixed in 4 % PFA for 15 min at RT, and permeabilization was obtained with 0.5 % Triton X-100 (Sigma) in PBS 1X for 15 min at RT. Cells were blocked with 5 % FBS (Gibco) and 0.3 % Triton X-100 in PBS 1X for 1 h at RT. Then, primary antibodies were diluted with 2 % FBS, 0.2 % Triton X-100 in PBS 1X and incubated overnight at 4 °C. Cells were rinsed three times with PBS 1X for 5 min and incubated with secondary antibodies for 2 h at RT. Finally, nuclei were stained with 1:10000 Hoechst in PBS 1X for 10 min at RT.

4.6. Mycoplasma test

Cells were tested for mycoplasma at passage 10 with the PlasmoTest Mycoplasma contamination detection kit (InvivoGen), following the manufacturer's protocol.

4.7. SNP-array analysis

High-resolution SNP-array analysis was conducted using the CytoScan HD array (Thermo Fisher Scientific) as previously described (Palumbo et al., 2020). Data analysis was performed using Chromosome Analysis Suite Software version 4.3 (Thermo Fisher Scientific), following a standardized pipeline (Di Muro et al., 2024). CNVs $\geq$500 Kb in length and overlapping $\geq$100 consecutive probes were retained to minimize the detection of false-positive calls.

CRediT authorship contribution statement

Gabriele Trentini: Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Giulia Cazzanelli:** Investigation, Formal analysis. **Marina Cardano:** Validation, Methodology, Investigation. **Orazio Palumbo:** Validation, Software, Methodology, Investigation, Data curation. **Mario Benvenuto:** Validation, Methodology, Data curation. **Pietro Palumbo:** Validation, Methodology, Data curation. **Francesca Agriesti:** Methodology. **Claudia Piccoli:** Methodology. **Luciano Conti:** Resources. **DEFEAT-LD Study Group:** Resources. **Massimo Carella:** Visualization, Supervision, Project administration, Funding acquisition, Conceptualization. **Graziano Lolli:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Giuseppe d'Orsi:** Writing – review & editing, Visualization, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Graziano Lolli reports financial support was provided by European Union. Giuseppe d'Orsi reports financial support was provided by European Union. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2025.103792.

Data availability

Data will be made available on request.

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