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Lab resource: Stem Cell Line

Generation of one iPSC line (IMEDEAi007-A) by Sendai Virus transduction of PBMCs from a Psoriasis donor

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A B S T R A C T

Psoriasis is a chronic inflammatory skin disease that speeds up the life cycle of skin cells, forming scales and red patches that are itchy and sometimes painful. It is a complex disease of autoimmune origin and genetic predisposition with more than 10 different loci associated. Here we described the production of an iPSC line generated by Sendai Virus (Klf4, Oct3/4, Sox2 and c-Myc) reprogramming of Peripheral Blood Mononuclear Cells (PBMCs) from a Psoriasis patient. The iPSC line generated has normal 46XY karyotype, is free of SeV genome and transgenes insertions, express high levels of pluripotency markers and can differentiate into all three germ layers.

1. Resource Table

Unique stem cell line identifier	IMEDEAi007-A
Alternative name(s) of stem cell line	hiPS IMEDEA-PBMCs CL1.12
Institution	IMEDEA - Instituto Mediterráneo de Estudios Avanzados
Contact information of distributor	Daniel Bachiller; d.b@csic.es
Type of cell line	iPSCs
Origin	Human
Additional origin info	Age: 35 years Sex: Male Ethnicity if known: Caucasian
Cell Source	Original cell type: Peripheral Blood Mononuclear Cell
Clonality	Clonal cell line
Method of reprogramming	CytoTune™-iPS 2.0 Sendai Reprogramming Kit
Genetic Modification	NO
Type of Modification	N/A
Associated disease	Psoriasis 1, Susceptibility to PSORS1 (OMIM entry #177900)
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	9th June 2020
Cell line repository/bank	Registered in the Human Pluripotent Stem Cell Registry (https://hpscreg.eu)
Ethical approval	Patient informed consent obtained/Comité Ético de Investigación Clínica de las Islas Baleares (Spain) Approval number IB3422/17PI

2. Resource utility

Although some treatments have shown disease remission, psoriasis is still incurable. Due to the absence of psoriasis specific biomarkers and the lack of a reliable animal model, iPSC could be used to build 3D personalized psoriatic skin constructs, which in combination with microfluidic platforms, will contribute to a better understanding of the diseases as well as to speed up drug development.

3. Resource details

Psoriasis is a complex chronic immune-mediated inflammatory skin disease that affects 2–3% of the population and which cause is still not fully understood. It is characterized by a dysregulation of immunological cell functions that trigger inflammatory reactions involving T-helper cells, dendritic cells and macrophages which, in turn, induce the hyperproliferation of keratinocytes by the secretion of different cytokines. The lack of suitable pre-clinical models mimicking the complex phenotype of the disease constitutes the main obstacle to study the pathogenesis of psoriasis and to develop new therapies. In the last decade, there has been a strong investment in the development of an *in vitro* 3D skin models (Niehues and van den Bogaard, 2018) that offer the opportunity to assess the contribution of different factors to the aetiology of the disease. In this context, the use of psoriatic fibroblast/keratinocytes in skin equivalents has demonstrate to partially preserve their psoriatic characteristics (Desmet et al., 2017), however the scarcity, limited size and heterogeneity of psoriasis skin biopsies and

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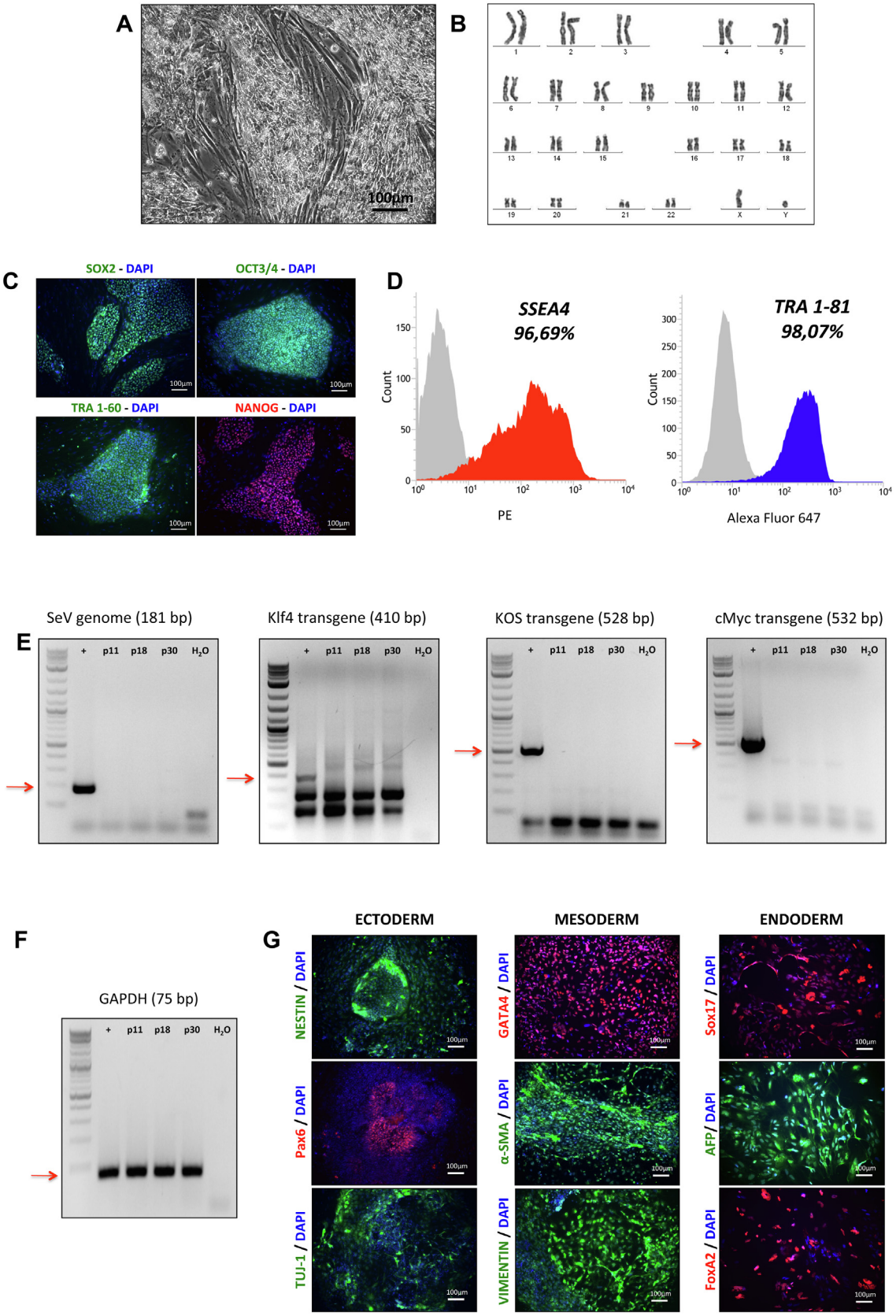

Fig.1. Characterization of a novel Psoriasis iPSC line IMEDEAi007-A. (A) Phase contrast image of the iPSC line growing on passage 19 on a feeder-coated plate (scale bar = 100 µm). **(B)** Normal male karyotype of IMEDEAi007-A after G band analysis. **(C)** The expression of SOX2, OCT3/4, TRA 1-60 and NANOG in the iPSC assessed by demonstrating absence of SeV genome and transgenes in 3 different passages of IMEDEAi007-A. **(F)** GAPDH, housekeeping control for PCR analysis. **(G)** After 10 days of directed differentiation of the iPSCs into all three germ layers: ectoderm, mesoderm and endoderm, the expression of specific markers of each one of the germ layers was tested by immunofluorescence assay.

diagnosis makes this approach difficult to be developed by different research groups. By using psoriasis-derived iPSCs lines, like IMEDEAi007-A, in the production of fibroblast and keratinocytes, it should be possible to increase the reproducibility of the results obtained at different times and places and therefore accelerate the study of this disease and the development of possible treatments.

In order to avoid possible complications arising from inherent differences in the biology of psoriasis-derived fibroblasts, the reprogramming process was carried out with a pro-erythroblast enriched PBMCs population from a 35 years old donor (diagnosed by histopathological analysis of a skin biopsy specimen). Cells were reprogrammed using the CytoTune 2.0 Sendai Reprogramming Kit carrying the 4 Yamanaka factors (Klf4, Oct3/4, Sox2 and c-Myc). The IMEDEAi007-A cell line generated showed a growth rate, behaviour and morphology (Fig. 1A) typical of human Embryonic Stem Cells (hESC), as well as a normal male genotype (46XY) (Fig. 1B). In addition, the iPSC line demonstrated to be free of the SeV genome and transgenes integrations (Fig. 1E and F) at three different cell passages (11, 18 and 30). Regarding the stemness of the line, immunocytochemical analysis showed the expression of the pluripotency-associated markers: SOX2, OCT3/4, TRA 1-60 and NANOG (Fig. 1C), and a specific and high TRA 1-81 and SSEA-4 expression was confirmed by quantitative flow cytometry (Fig. 1D). Finally, in order to probe the differentiation potential of the iPSC line, directed differentiation into all three germ layers was carried out (Fig. 1G). The expression of specific markers for ectoderm, mesoderm and endoderm in the differentiated cells was analyzed by immunocytochemistry. Short tandem repeat (STR) analysis (Table 1, data not shown) indicated a 100% match with parental PBMCs. The full characterization of the line was completed determining the absence of mycoplasma in iPSC samples (Supplementary Fig. S1).

To summarize, the iPSC generated from a Psoriasis donor has a great potential for the study of the disease, as well as a screening tool in drug development.

4. Materials and methods

4.1. iPSCs derivation and expansion

PBMCs were isolated by ficoll from a Psoriasis donor peripheral blood sample. In order to select and expand a cell population without rearrangements, PBMCs were cultured in a pro-erythroblast serum-free specific media (1:1 IMDM/Ham's F12 supplemented with: 1% ITS-X, 1% Chemically Defined Lipid Concentrate, 1% Glutamax, 0.025 g/L L-Ascorbic Acid, 5 g/L BSA, 100 ng/ml SCF, 10 ng/ml IL3, 2U/ml EPO, 40 ng/ml IGF-1, 1 μM Dexamethasone and 100 μg/ml Holo-transferrin). After 7 days of culture, 3×10^5 cells were transduced with CytoTune 2.0 Sendai Virus Reprogramming Kit (Invitrogen), using a MOI 5:5:3 (KOS:hc-Myc:hKlf4) and following the manufacturer recommendations. After 3 days, 3×10^4 transduced cells/well were seed on a p6 well MEF-CD1-coated plate. Pro-erythroblast culture medium was maintained for 4 more days, and then it was gradually changed to hiPS medium until colonies were ready to be manually isolated 15 days after reprogramming.

iPS colonies were grown on MEF CD1 until passage 3 when cells where change to human Hafi-W3R feeders. Rock Inhibitor Y-27632 was used at 10 μM during the passages to increase cell survival. Cells were cultured at 37 °C and 5% CO_2 in a humidified incubator.

4.2. Karyotype analysis

iPSCs were passed to Matrigel-coated plates and sent to *Biobanco del Sistema Sanitario Público de Andalucía* (Granada, Spain) for the karyotyping by G-banded metaphases analysis.

4.3. SeV genome and transgenes analysis

Genomic RNA was isolated (RNeasy Mini Kit, Qiagen) from iPSCs on passage 11, 18 and 30 and from a pool of iPSCs 17 days after reprogramming as a positive control. After reverse transcription of RNA (High Capacity cDNA Reverse Transcription Kit, AppliedBiosystems), the presence of SeV genome and transgenes in hiPS cells was analysed by PCR using the primers detailed in Table 2 and the WonderTaq polymerase (Euroclone) for cDNA amplification.

4.4. STR analysis

STR analysis was performed by the Genomics Core facility at the Instituto de Investigaciones Biomédicas (IIBM, Madrid).

4.5. In vitro directed differentiation

In order to perform directed differentiation of IMEDEAi007-A, iPSCs were first cultured on gelatin-coated plates in hiPS medium supplemented with 10 μM Rock Inhibitor and 1% of DMSO. After 75 min cells

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal appearance of packed iPS colonies. Scale bars: 100 μm	Fig. 1 panel A
Phenotype	Qualitative analysis by Immunocytochemistry	The cell line expressed the pluripotency markers: SOX2, OCT3/4, TRA-1-60 and NANOG.	Fig. 1 panel C
	Quantitative analysis by Flow Cytometry	The cell line expressed high levels of the pluripotency markers SSEA-4 and TRA-1–81	Fig. 1. Panel D
Genotype	Karyotype (G-banding) and resolution	IMEDEAi007-A: 46, XY Resolution 450–500	Fig. 1. Panel B
Identity	Microsatellite PCR analysis	Not performed	
	STR analysis	The STR profiles of cell line matched 100% with that of the parental PBMCs cells (10 loci analyzed)	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	NA	NA	NA
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR using MycoSPY Kit (Biontex).	Submitted in archive with journal
Differentiation potential	Directed differentiation	The cell line differentiated into the three germ layers including ectoderm (Nestin, Pax6 and TUJ-1), mesoderm (GATA-4, α-SMA and Vimentin) and endoderm (Sox17, AFP and FoxA2).	Fig. 1. Panel G
Donor screening (OPTIONAL)	NA	NA	NA
Genotype additional info (OPTIONAL)	NA	NA	NA

Table 2
Reagent details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-OCT4	1:50	Santa Cruz Biotech; sc-5279; RRID:AB_628051
	Rabbit anti-NANOG	1:200	Cell Signaling; D73G4; RRID:AB_10559205
	Mouse anti-SOX2	1:100	R and D Systems; MAB2018; RRID:AB_177629
	Mouse anti-TRA-1-60	1:100	Millipore; MAB4360; RRID:AB_2119183
Differentiation Markers	Rabbit anti-Nestin	1:500	Sigma-Aldrich; N5413; RRID:AB_1841032
	Rabbit anti-TUJ-1	1:500	Covance; MRB-435P-100; RRID:AB_663339
	Mouse anti-Pax6	1:100	DSHB pax6, RRID:AB_528427
	Mouse anti-GATA-4	1:300	Santa Cruz Biotech; sc-25310; RRID:AB_627667
	Mouse anti-Vimentin	1:100	Abcam; ab80667
	Mouse anti-α-SMA	1:200	RRID:AB_1603290
	Goat anti-SOX-17	1:100	Sigma-Aldrich; A2547
	Rabbit anti-AFP	1:200	RRID:AB_476701
	Rabbit anti-FoxA2	1:400	R&D Systems; AF1924; RRID:AB_355060 Dako; A0008; RRID:AB_2650473 Cell Signaling; 8186 RRID:AB_10891055
Secondary antibodies	Alexa Fluor 555 Donkey Anti-Rabbit IgG	1:500	Invitrogen; A-31572; RRID:AB_162543
	Alexa Fluor 555 Donkey Anti-Mouse IgG	1:500	Invitrogen; A-315700
	Alexa Fluor 488 Donkey Anti-Mouse IgG	1:500	RRID:AB_2536180
	Alexa Fluor 555 Donkey Anti-Goat IgG	1:500	Invitrogen; A-21202; RRID:AB_141607
	Alexa Fluor 488 Donkey Anti-Rabbit IgG	1:500	Invitrogen; A-21432, RRID: AB_2535853 Invitrogen; A-21206 RRID:AB_141708
Antibodies used for Flow Cytometry			
	Antibody	Vol. per test	Company Cat # and RRID
Pluripotency Markers	Mouse Anti TRA-1–81, APC	20 μl	BD Biosciences; 560793; RRID:AB_10550550
	Mouse Anti SSEA-4, PE	20 μl	BD Biosciences; 560,128
	Mouse IgM, k Isotype Control, APC	1 μl	RRID:AB_1645533
	Mouse IgG3, k Isotype Control PE	1 μl	BD Biosciences; 560806; RRID:AB_2034030 BD Biosciences; 559,926 RRID:AB_10050453
Primers			
	Target		Forward/Reverse primer (5′-3′)
SeV genome and transgenes (PCR)	SeV genome		GGATCACTAGGTGATATCGAGC/
	KOS transgene		ACCAGACAAGAGTTTAAGAGATATGTATC
	Klf4 transgene		ATGCACCGCTACGACGTGAGCGC/
	c-Myc transgene		ACCTTGACAATCCTGATGTGG TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG
House-Keeping Genes (PCR)	GAPDH		GCACCGTCAAGGCTGAGAAC/ AGGGATCTCGCTCCTGGAA

were passed to a p48 well plate coated with matrigel. Ectoderm differentiation was carried out according to Tchieu et al. (2017), but using 200 ng/ml Noggin (Peprotech) instead of LDN193189. Mesoderm differentiation was attained following Oldershaw et al. (2008), while the protocol of McCracken et al. (2011) was applied, with some minor modifications, for *endoderm* differentiation. After 10 days of culture cells were fixed in PFA 4% for immunocytochemistry.

4.6. Immunofluorescence staining

Undifferentiated iPSCs (for pluripotency markers assays) and differentiated cells (for differentiation potential assays) were washed with PBS, fixed for 20 min, washed again with PBS and permeabilized with 0.2% Triton X-100 and 100 mM glycine in PBS for 30 min at room temperature (RT). To block non-specific binding sites, PBS 5% BSA was added an incubated for 60 min at RT. Primary antibodies incubation was done overnight at 4 °C in PBS 2% BSA. After three washes with PBS, cells were incubated with fluorescence-conjugated secondary antibodies in PBS 2% BSA for 60 min at RT and darkness. The antibodies used are described in Table 2. After washing with PBS, cells were stained with DAPI for 5 min at RT in darkness.

4.7. Flow cytometry analysis of pluripotency marker

Cells were detached with Tryple and incubated 45 min and 4 °C with conjugated antibodies (Table 2) against cells surface markers TRA-1-81 and SSEA-4. The corresponding isotype antibodies were used as controls. Influx Cell Sorter and BD 1.0.0.650 Software were used for the cytometry analysis.

4.8. Mycoplasma detection

Mycoplasma testing was carried out using the MycoSPY Mycoplasma PCR detection kit (Biontex), which detects a wide range of bacteria from the *Mollicutes* class. An Internal Control template was included to discard the presence of PCR inhibitors and rule out false positive results.

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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.2020.101917.

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