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Viral-free generation and characterization of a human induced pluripotent stem cell line from dermal fibroblasts

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Abstract
Peripheral dermal fibroblasts (DF) from a healthy 56 year old female were obtained from the Centre for Healthy Brain Ageing (CHeBA) Biobank, University of New South Wales, under the material transfer agreement with the University of Wollongong. DFs were reprogrammed via mRNA-delivered transcription factors into induced pluripotent stem cells (iPSCs). The generated iPSCs were confirmed to be pluripotent, capable of three germ layer differentiation and are thus a useful resource for creating iPSC-derived healthy human cells of any lineage.

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Lab resource: Stem cell line

Viral-free generation and characterization of a human induced pluripotent stem cell line from dermal fibroblasts

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A B S T R A C T

Peripheral dermal fibroblasts (DF) from a healthy 56 year old female were obtained from the Centre for Healthy Brain Ageing (CHeBA) Biobank, University of New South Wales, under the material transfer agreement with the University of Wollongong. DFs were reprogrammed via mRNA-delivered transcription factors into induced pluripotent stem cells (iPSCs). The generated iPSCs were confirmed to be pluripotent, capable of three germ layer differentiation and are thus a useful resource for creating iPSC-derived healthy human cells of any lineage.

Resource table

Unique stem cell line identifier	UOWi001-A
Alternative name(s) of stem cell line	iPSC RB 9–8
Institution	Illawarra Health and Medical Research Institute, University of Wollongong
Contact information of distributor	Lezanne Ooi, lezanne@uow.edu.au
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 56 Sex: female
Cell Source	Dermal fibroblast
Clonality	Clonal
Method of reprogramming	Transgene-free
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	21/12/17
Cell line repository/bank	N/A
Ethical approval	HE 13/299 University of Wollongong Human Ethics Committee

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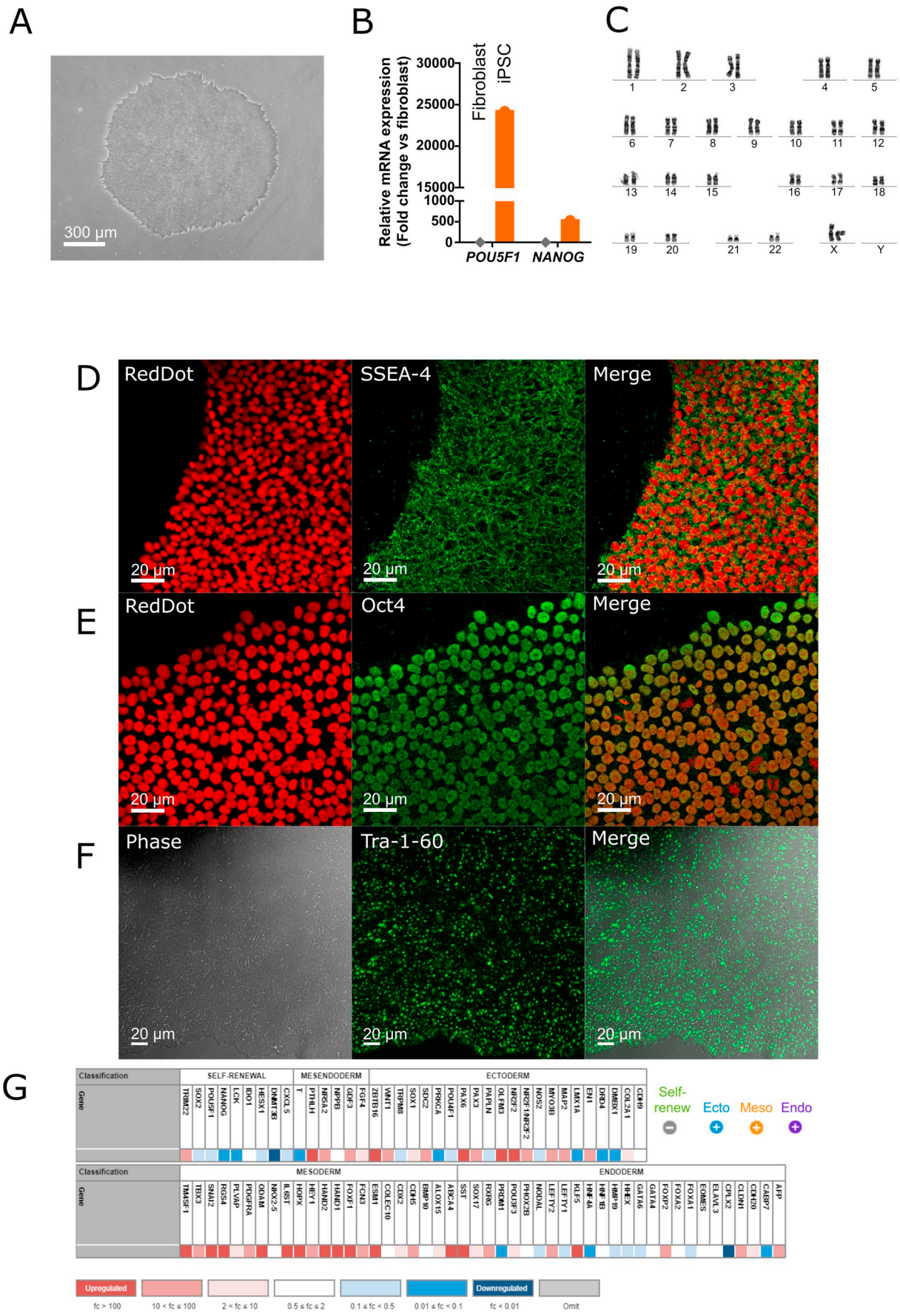

Fig. 1. Characterization of iPSC line UOWi001-A.

Resource utility

This iPSC line can be used as an age and gender matched healthy control.

Resource details

Dermal fibroblast (DF) samples from a 56 year old healthy female volunteer were obtained from CHeBA Biobank, University of New South Wales. To generate the iPSC line (UOWi001-A, Fig. 1), the reprogramming factors Oct4, Sox2, Klf4, c-Myc and Lin28 were delivered via microRNA-Enhanced mRNA Reprogramming Kit (Stemgent, 00–0071) according to the manufacturer's instructions. The first iPSCs appeared after 11 days, with colonies forming within 24 h. Individual colonies (clones) were isolated after 13 days. After 6 passages, we initiated the characterization of clone 8 (UOWi001-A). The iPSC colonies had a characteristic morphology (Fig. 1A) expressed the transcripts for *POU5F1* and *NANOG*, which were not expressed in the original fibroblasts (Fig. 1B), and a normal karyotype (Fig. 1C). The iPSC colonies showed widespread expression of the pluripotency markers SSEA-4, Oct4 and Tra-1-60 (Fig. 1D, E, F). Following the confirmation of UOWi001-A identity via short tandem repeat (STR) profiling (with journal), we assessed the pluripotency potential via the hPSC Scorecard assay. Spontaneous in vitro differentiation via embryoid body formation confirmed the three-germ layer differentiation potential of UOWi001-A (Fig. 1G).

Materials and methods

Reprogramming of dermal fibroblasts

DF samples from a 56 year old female were obtained from CHeBA Biobank, University of New South Wales. The study was approved by the University of Wollongong Human Ethics Committee (HE 13/299). DFs were cultured in fibroblast medium, consisting of Dulbecco's Modified Eagle Medium F12 (DMEM/F12, ThermoFisher, 12,500–096) supplemented with 10% foetal bovine serum (Interpath SFBS-F) and 1× Non-Essential Amino Acids (ThermoFisher, 11,140,050), at 37 °C and 5% CO_2. Fibroblasts were reprogrammed using Stemgent microRNA-Enhanced mRNA Reprogramming Kit (Stemgent, 00–0071), following the manufacturer's protocol. Prior to reprogramming, Pluriton Reprogramming Medium was conditioned using newborn human foreskin fibroblasts (Globalstem, GSC3006G) as per the reprogramming protocol. Spontaneous iPSC colonies appeared on day 11 and were isolated on day 13 for expansion into individual iPSC lines (clones). Established iPSC clones were maintained in TeSR-E8 (Stemcell Technologies, 05940) on Matrigel-coated (Corning, FAL354277) plates at 37 °C in 5% O_2 and were split 1:5 using dispase (1 mg/ml, Stemcell Technologies, 07913) on reaching 60% confluency Table 1.

Immunofluorescence staining

The iPSCs were cultured on Matrigel-coated glass coverslips in TeSR-E8, fixed with 4% paraformaldehyde for 10 min, permeabilised with 0.05% Triton-X for either 7 or 15 min for Oct4 and SSEA-4 respectively, and blocked with 10% goat serum for 1 h, all at room temperature (ThermoFisher, 16,210–064). Cultures were incubated with primary antibodies (Table 2) or mouse IgG control (ThermoFisher, 10400C) at +4 °C for 16 h, followed by secondary antibody incubation for 1 h at room temperature (Table 2), and nuclear staining with RedDot2 (1:200, Biotium, 40,061–1). For Tra-1-60 live stain (Table 2), cultures were incubated with TeSR-E8 and Tra-1-60 (4 µg/ml) for 30 min followed by media washing steps. *Z*-stack images were captured on a confocal microscope (Leica DMI6000B) and acquired using LAS AF (Leica Microsystems).

Karyotyping

The iPSCs were cultured on Matrigel-coated T25 flasks in TeSR-E8 until 60% confluent. Cultures were processed for karyotyping by Sullivan Nicolaides Pathology (Bowen Hills, Australia) at 400 bands per haploid set resolution.

Quantitative polymerase chain reaction (qPCR)

Fibroblast and iPSC cultures were sampled with Tri-Reagent (MRC Gene, TR 118) and RNA was extracted as per manufacturer's instructions. Sample quality was confirmed via Nanodrop (260/280 = 1.9–2.0), followed by removal of genomic DNA (Turbo DNAse, ThermoFisher, AM1907) and reverse transcription (Tetro cDNA Synthesis kit, Bioline BIO-65043). The expression of *POU5F1* and *NANOG* was assessed in triplicates via qPCR (SensiFast SYBR, Bioline, BIO-98020) on a LightCycler 480 (Roche), validated through melt curve analysis and normalized to the expression of housekeeper genes *GAPDH* and *HPRT1*. The efficiency of each sample was determined using LinRegPCR (Ruijter et al., 2009) and analysis was performed using the ΔΔCt method.

Pluripotent stem cell scorecard assessment

The iPSC colonies were grown on Matrigel-coated 60 mm dishes for 10 days from single cell passaging in mTeSR1 (Stemcell Technologies, 85,850). Colonies were transferred to non-tissue culture treated dishes following dispase incubation. One day after passaging, the iPSC colonies formed spheres and the media was changed from mTeSR1 to DMEM/F12 with 10% foetal bovine serum. After 21 days in suspension,

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1, Panel A
Phenotype	Immunocytochemistry	Expression of pluripotency markers Oct4, SSEA-4, Tra-1-60	Fig. 1, Panel D, E and F
	RT-qPCR	Cells express *POU5F1 (OCT4)*, *NANOG*	Fig. 1, Panel B
Genotype	Karyotype (G-banding) and resolution	46XX, resolution: 400	Fig. 1, Panel C
Identity	Microsatellite PCR (mPCR)	N/A	N/A
	STR analysis	18 sites tested, matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Luminescence, negative	Supplementary File 2
Differentiation potential	Scorecard	Three germline potential confirmed via hPSC Scorecard	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

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	OCT4	1:1000	Stemcell Technologies Cat# 01550, RRID: AB_1118539
	SSEA-4	1:200	Abcam Cat# ab16287, RRID: AB_778073
	Tra-1-60	1:200	Stemcell Technologies Cat# 60064A, RRID: AB_2686905
Secondary Antibodies	Alexa Fluor 488 Goat anti-mouse IgG (H + L)	1:1000	ThermoFisher Scientific Cat# A11001, RRID: AB_2534069

Primers		
	Target	Forward/Reverse primer (5′-3′)
Pluripotency Genes (qPCR)	*NANOG*	Forward: CCAGAACCAGAGAATGAAATC Reverse: TGGTGGTAGGAAGAGTAAAG
	POU5F1	Forward: GATCACCCTGGGATATACAC Reverse: GCTTTGCATATCTCCTGAAG
Housekeeping Genes (qPCR)	*GAPDH*	Forward: GAGCACAAGAGGAAGAGAGAGACCC Reverse: GTTGAGCACAGGGTACTTTATTGATGGTACATG
	HPRT1	Forward: TGACACTGGCAAAACAATGCA Reverse: GGTCCTTTTCACCAGCAAGCT

the differentiating embryoid bodies were collected, RNA extracted with Trisure (Bioline, BIO-38033), quality confirmed via Nanodrop (260/280 = 1.9–2.0), and reverse transcribed (High Capacity cDNA, ThermoFisher, 4,368,814). The hPSC Scorecard qPCR (ThermoFisher, 100,019,864) was run as per manufacturer's instruction on a Quantstudio 5 (ThermoFisher) and analysed through the Scorecard application.

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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.2018.09.014.