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Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line from a healthy Caucasian male

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A B S T R A C T

The effects of genetic mutations on protein function can be studied in a physiologically relevant environment using tissue-specific cells differentiated from patient-derived induced pluripotent stem cells (iPSC). However, it is crucial to use iPSC derived from healthy individuals as control. We generated an iPS cell line from skin fibroblasts of a healthy Caucasian male by nucleofection of non-integrating episomal vectors. This cell line has normal karyotype, expresses pluripotency surface markers and pluripotency genes, and successfully differentiates into cells of the 3 germ layers. Therefore, it can be used as control for any disease of interest that is modelled using iPSC.

1. Resource Table

Unique stem cell line identifier	IDIBGIi001-A
Alternative name(s) of stem cell line	GPG1-C23
Institution	Girona Biomedical Research Institute (IDIBGI)
Contact information of distributor	Elisabet Selga, eselga@gencardio.com
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 52 Sex: Male Ethnicity if known: Caucasian
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Episomal, transgene-free
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	Copy number-PCR; qRT-PCR
Associated disease	N/A
Gene/locus	N/A
Date archived/stock date	2022
Cell line repository/bank	https://hpscreg.eu/cell-line/IDIBGIi001-A Registration ongoing at Spanish National Stem Cell Bank: https://eng.isciii.es/eng.isciii.es/QueHacemos/Servicios/BIOBANCOS/BNLC/Paginas/default.html
Ethical approval	Ethics Committee Of Clinical Research-CMRB. Catalan Authority for Stem Cell Research (Approval number 374 3071) Advisory committee for Human Tissue and Cell Donation and Use, Instituto de Salud Carlos III. Approval number P11/2015

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2. Resource utility

Tissue-specific cells differentiated from human iPSC are useful to study the effects of mutations on the function of proteins in a physiologically relevant context. We generated iPSc from a healthy caucasian 52-year-old male to be used as control in our functional studies (Selga et al., 2018; Martinez-Moreno et al., 2020; Carreras et al., 2020) (See Table 1).

3. Resource details

Dermal fibroblasts were derived from a skin biopsy of a healthy male individual. Fibroblasts were reprogrammed by nucleofection of non-integrating episomal plasmids encoding six human factors (OCT3/4, SOX2, KLF4, LIN28, L-Myc and a p53 knock down shRNA) under feeder-free conditions. The resulting iPSC lines (Fig. 1A, scale bar 1000 μm), named GPG1, were karyotypically normal (Fig. 1B). Absence of episomal plasmids was shown by determining episomal plasmid copy number in genomic DNA (gDNA) from the iPSC line by absolute quantitative real time PCR (aqRT-PCR). gDNA from control human fibroblasts 72 h post nucleofection was used as positive control. mRNA expression levels of EBNA-1 expression of control fibroblasts 72 h post nucleofection (data available upon request), and episomal plasmid-derived genes and endogenous pluripotency markers, were analysed by quantitative reverse transcriptase PCR (qPCR, Supplemental Fig. 1A) using specific primers (Table 2). Expression of pluripotency markers was confirmed by immunocytochemistry with antibodies against endogenous human OCT4, SOX2, NANOG, TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4 (Fig. 1C, scale bars 50 μm) and alkaline phosphatase activity (Supplemental Fig. 1B, scale bar 100 μm). The differentiation capacity of the lines was tested by embryoid body (EB) formation and differentiation *in vitro* towards the three germ layers, as shown by immunofluorescence analyses demonstrating the expression of definitive endoderm (AFP and FOXA2), ectoderm (TUJ1 and GFAP) and mesoderm (ASMA and ASA) markers (Fig. 1D, scale bars 50 μm). The iPSC identity was confirmed by short tandem repeat analysis (STR) and compared with the patient's fibroblasts (submitted in archive with journal). Samples were routinely tested for absence of mycoplasma contaminations by PCR (Supplemental Fig. 1C).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis – Immunocytochemistry	Presence of pluripotency markers: Oct4, Nanog, Sox2, SSEA-3, SSEA-4, Tra 1–60, Tra-1–80.	Fig. 1 panel C
	Quantitative analysis – RT-qPCR	Expression of pluripotency markers Lin28, OCT4, SOX2	Supplementary Fig. 1 panel A
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 30–500	Fig. 1 panel B
Identity	STR analysis	STR Profiling Performed 10 sites tested, all matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Negative testing by PCR	Supplementary Fig. 1 panel C
Differentiation potential	Embryoid body formation	Three germ layers formation	Fig. 1 panel D
List of recommended germ layer markers	Expression of markers demonstrated at protein (IF) level	Positive for: Ectoderm: TUJ1, GFAP Endoderm: FOXA2, AFP Mesoderm: A-SMA, ASA	Fig. 1 panel D
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

4. Materials and methods

4.1. Reprogramming of fibroblasts

Fibroblasts were cultured in DMEM supplemented with 10% HyClone FBS and 1% penicillin–streptomycin at 37 °C and 5% CO_2. 0.5 × 10^6 fibroblasts were reprogrammed at passage 2 by nucleofection (Amaxa NHDF Nucleofector Kit and Nucleofector 2b (Lonza), U023 protocol) with Addgene episomal plasmids #27077, #27078, #27080. Seven days later, fibroblasts were seeded onto Matrigel-coated dishes in mTeSR-E8 medium (Stemcell Technologies). Approximately 20 days after nucleofection, iPS colonies were manually picked and passaged for expansion.

4.2. PCR and qPCR

Genomic DNA was extracted and aqRT-PCR with specific primers against plasmid derived EBNA1 performed. PCR signals were related to a standard curve. Genomic DNA extracted from fibroblasts 72 h post nucleofection was used as positive control. For qPCR, mRNA was isolated by Trizol-based procedure, and 1 μg of mRNA was reverse transcribed with Cloned AMV First-strand cDNA kit (Life technologies). For all reactions, SyBR green (Invitrogen) and primers listed in Table 2 were used. Ct values were normalized by % GAPDH.

4.3. Karyotype determination

Seventy percent confluent iPSC colonies were treated with colcemid (KaryoMAX colcemid, Gibco) trypsinized, incubated with hypotonic solution (KCl, Gibco), fixed in Carnoy fixative (75% methanol: 25% acetic acid) and genomic integrity of iPSCs at passage 15 was evaluated by G-banded metaphase karyotype analysis of 20 metaphase spreads at Hospital Sant Joan de Déu, Barcelona, following standard procedures.

4.4. Alkaline phosphatase (AP) staining and immunocytochemistry for pluripotency

To detect AP activity, iPSCs were fixed with 4% paraformaldehyde for 1 min, washed with PBS and incubated with AP staining solution (Sigma). Immunocytochemistry was performed with antibodies against pluripotency factors (Nanog, OCT4, SOX2, TRA-1-81, TRA-1-60, SSEA3 and SSEA4) as previously described (Kuebler et al., 2017). Primary and secondary antibodies used are listed in Table 2. Confocal images were taken using a Leica TSC SPE/SP5 microscope.

4.5. Embryoid body formation and immunocytochemistry for differentiation

In vitro differentiation was promoted by embryoid bodies (EB) formation. iPSC colonies were lifted as usual and transferred to a 96 well plate in mTeSR-1 medium (Stem Cell Technologies). The plate was centrifugated at 800 g for 10 min and incubated at 37 °C and 5% CO_2 for 24 h. Then, early EBs were transferred to an ultra-low attachment plate

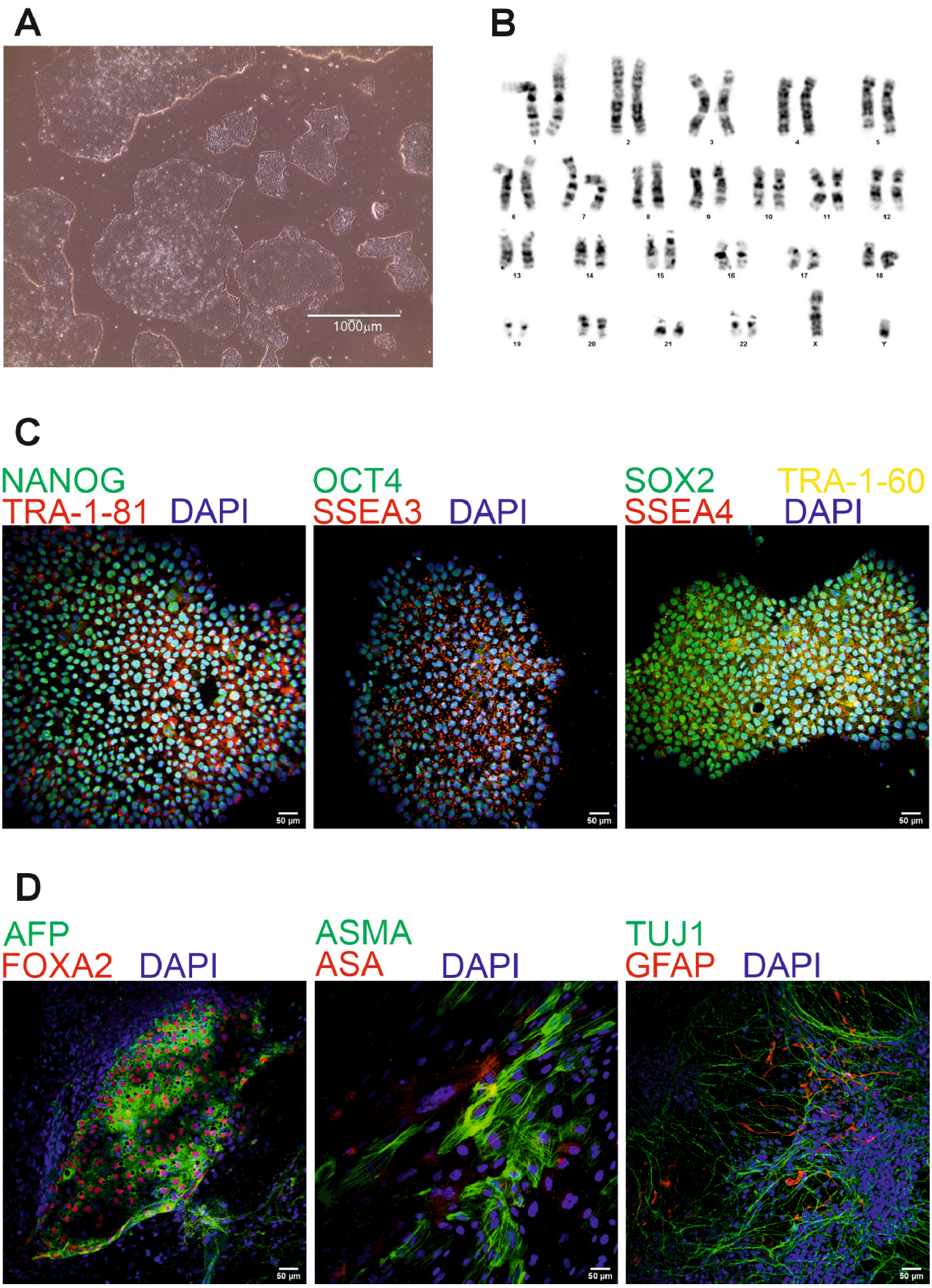

Fig. 1. Characterization of the GPG1-C23 iPS cell line.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency markers	Mouse anti-OCT4	1:2	Santa Cruz, sc-5279	AB_628051
	Goat anti-NANOG	1:5	R&D Systems, AF1997	AB_355097
	Rabbit anti-SOX2	1:100	ABR, PA1-16968	AB_2195781
	Rat anti-SSEA3	1:1	Hybridoma Bank, MC-631	AB_528476
	Mouse anti-SSEA4	1:1	Hybridoma Bank, MC-813-70	AB_528477
	Mouse anti-TRA-1-60	1:100	Millipore, MAB4360	AB_2119183
	Mouse anti-TRA-1-81	1:100	Millipore, MAB4381	AB_177638
Differentiation Markers	Mouse anti-TUJ1	1:40	Covance, MMS-435P	AB_2313773
	Rabbit anti-GFAP	1:1000	Dako, Z0334	AB_10013382
	Mouse anti-ASA	1:400	Sigma, A2172	AB_476695
	Rabbit anti-AFP	1:200	Agilent, A0008	AB_2650473
	Goat anti-FOXA2	1:50	R6D Systems, AF2400	AB_2294104
Secondary antibodies	AF488 Goat anti-Mouse	1:200	Jackson, 115-546-071	AB_2338865
	Cy3 Goat anti-Rat	1:200	Jackson, 112-165-020	AB_2338243
	AF488 Donkey anti-Rabbit	1:200	Jackson, 711-545-152	AB_2313584
	DyLight649 Goat anti-Mouse	1:200	Jackson, 115-495-075	AB_2338809
	AF488 Donkey anti-Goat	1:200	Jackson, 705-545-147	AB_2336933
	Cy3 Donkey anti-Mouse	1:200	Jackson, 715-165-140	AB_2340812
	Cy3 Donkey anti-Goat	1:200	Jackson, 705-165-147	AB_2340812
	AF488 Donkey anti-Mouse	1:200	Jackson, 715-545-151	AB_2307351
	Cy3 Donkey anti-Guinea pig	1:100	Jackson, 706-165-148	AB_2341099
	AF488 Goat anti-Mouse	1:200	Jackson, 115-546-071	AB_2338865
	Cy3 Goat anti-Mouse	1:200	Jackson, 115-165-075	AB_2338689
	Primers			
	Target	Size of band	Forward/Reverse primer (5′-3′)	
episomal plasmids (aqRT-PCR)	EBNA-1		TGGAAACCAGGGAGGCAAAT/GTCAAGGAGGTTCCAACCCG	
episomal plasmids (qPCR)	pCXLE-Oct3/4 (plasmid)		CATTCAAACTGAGGTAAGGG/	
	pCXLE-SOX2 (plasmid)		TAGCGTAAAAGGAGCAACATAG	
	pCXLE-KLF4 (plasmid)		TTCACATGTCCCAGCACTACCAGA/	
	pCXLE-LIN28 (plasmid)		TTTGTTTGACAGGAGCGACAAT	
	pCXLE-L-Myc (plasmid)		CCACCTCGCCTTACACATGAAGA/	
			TAGCGTAAAAGGAGCAACATAG	
			AGCCATATGGTAGCCTCATGTCCGC/	
			TAGCGTAAAAGGAGCAACATAG	
			GGCTGAGAAGAGGATGGCTAC/	
			TTTGTTTGACAGGAGCGACAAT	
Endogenous pluripotency genes (qPCR)	endogenous Oct3/4 (cds) endogenous SOX2 (cds)		CCCCAGGGCCCCATTTTGGTACC/	
	endogenous LIN28 (cds)		ACCTCAGTTTGAATGCATGGGAGAGC	
	endogenous L-Myc (cds)		TTCACATGTCCCAGCACTACCAGA/	
			TCACATGTGTGAGAGGGGCAGTGTGC	
			AGCCATATGGTAGCCTCATGTCCGC/	
			TCAATTCTGTGCCTCCGGGAGCAGGGTAGG	
			GCGAACCCAAGACCCAGGCCTGCTCC/	
			CAGGGGGTCTGCTCGCACCGTGATG	
Controls (qPCR)	EBNA-1		ATCAGGGCCAAGACATAGAGATG/	
			GCCAATGCAACTTGGACGTT	
House-Keeping Gene (qPCR)	GAPDH		GCACCGTCAAGGCTGAGAAC/AGGGATCTCGCTCCTGGAA	
e.g. Targeted mutation analysis/sequencing	N/A			

in mTeSR-1 for additional 24 h. After this time, EBs were transferred to matrigel-coated slide flasks and cultured in differentiation media for 21–28 days. Ectoderm medium: 50% Neurobasal medium, 50% DMEM/F12, 1% N2, 1% B27, 1% Glutamax and 1% Penicillin-Streptomycin; Endoderm medium: Knockout-DMEM, 10% FBS-Hyclone, 1% MEM-NEAA, 0.1% β-mercaptoethanol, 1% Glutamax and 1% Penicillin-Streptomycin (all Gibco); Mesoderm medium: Endoderm medium supplemented with 0.5 mM L-Ascorbic acid (Sigma). Cells were analysed by immunocytochemistry with specific antibodies (Table 2) against endodermal markers AFP and FOXA2, ectodermal markers TUJ1 and GFAP and mesodermal markers ASMA and ASA as previously described (Kuebler et al., 2017). Confocal images were taken using a Leica TSC SPE/SP5 microscope.

4.6. Authentication and mycoplasma testing

To confirm line identity, genomic DNA was obtained from fibroblasts and from iPSCs and used for STR analysis.

Samples were routinely tested for absence of mycoplasma contaminations by PCR.

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.

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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.2022.102717.

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