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Lab resource: Stem Cell Line

Generation of a human control iPS cell line (ESi080-A) from a donor with no rheumatic diseases

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ABSTRACT

Here, we report the establishment of the human iPS cell line N1-FiPS4F#7 generated from skin cells of a patient with no rheumatic diseases, thus obtaining an appropriate control iPS cell line for researchers working in the field of rheumatic diseases. The reprogramming factors Oct4, Sox2, Klf4 and c-Myc were introduced using a non-integrating reprogramming strategy involving Sendai Virus.

Resource Table:

Unique stem cell line identifier	ESi080-A
Alternative name(s) of stem cell line	N1-FiPS4F#7
Institution	Instituto de Investigación Biomédica de A Coruña/Universidade da Coruña
Contact information of distributor	Silvia Mª Díaz-Prado. s.diaz1@udc.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 44 years old. Sex: Female Ethnicity if known: Caucasian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai Virus vectors (Cytotune reprogramming kit, ThermoFhiser Scientific).
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	N/A
Cell line repository/bank	N/A
Ethical approval	Patient informed consent obtained Ethics Committee of Research of A Coruña-Ferrol, Spain (register code 2014/405). *Comisión de Seguimiento y Control de la Donación de Células y Tejidos Humanos del Instituto de Salud Carlos III* (April 13, 2015).

1. Resource utility

Although iPSC-lines have been generated from patients with rheumatic diseases, there is still lack of appropriate control lines generated from patients with radiographic information regarding principal joints (knee, hip, hands and/or spine). Therefore, we generated a control iPS cell line from a donor with no rheumatic diseases, as proved radiographically.

2. Resource details

Dermal fibroblasts were isolated from a skin biopsy of a donor with nor radiographic signs neither symptoms of rheumatic diseases using a protocol previously described (Vangipuram et al., 2013). Sufficient

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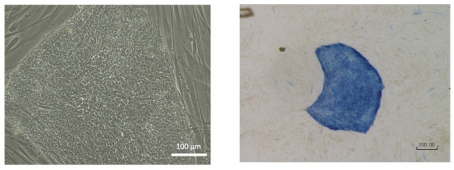
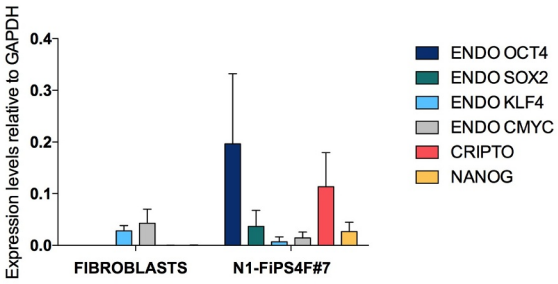
A)



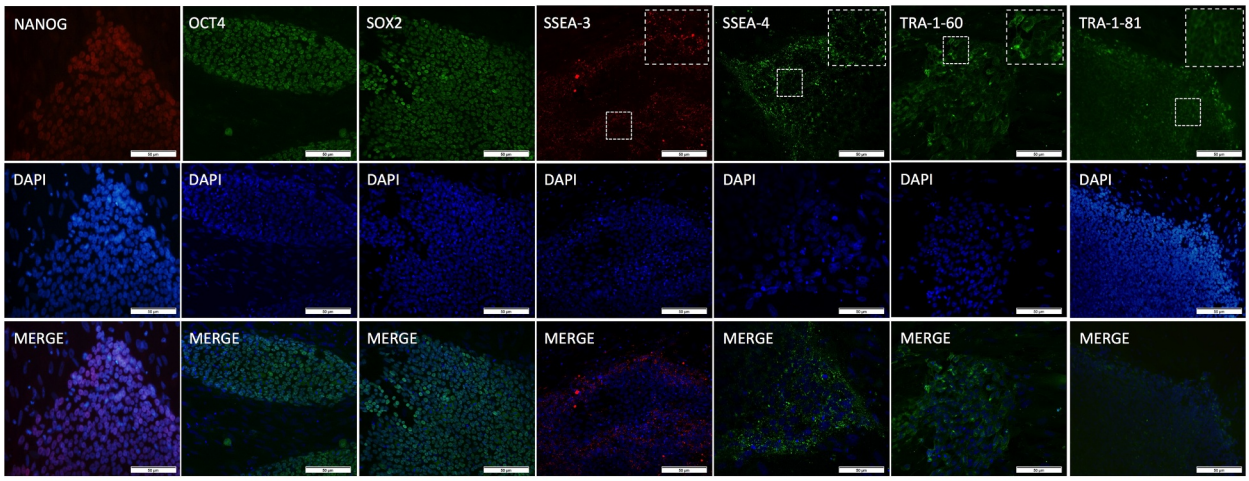
B)



C)

MARKER	FIBROBLASTS	N1-FiPS4F#7
D5S818	11.13	11.13
D13S317	10.13	10.13
D7S820	9.11	9.11
D16S539	11.12	11.12
VWA	18	18
TH01	7.9	7.9
AMELOGENIN	X	X
TPOX	8.12	8.12
CSF	11.12	11.12
D21S11	29	29

D)



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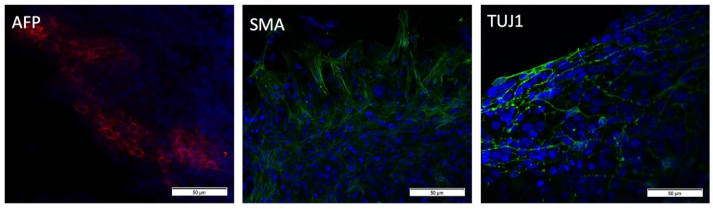
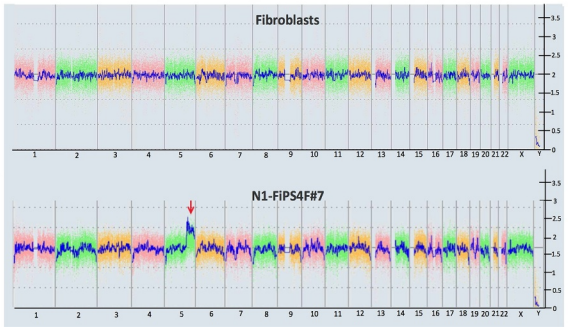


F)



Chromosome	Type	Cytoband Start	CN State	Size (kbp)
5	GainMosaic	q23.1	2.60	61,163
5	Gain	q23.3	3.00	42,579
5	Gain	q35.2	3.00	2,929

Fig. 1.

fibroblasts for reprogramming were obtained after two weeks in culture and three cell passages. These fibroblasts at the 3rd passage were reprogrammed by using Sendai virus vectors containing the human reprogramming factors Oct4, Sox2, Klf4 and c-Myc following the instructions of the manufacturer. One week after reprogramming, we found several tight, small cell colonies in feeder-culture that grew quickly and, by day 22 after transduction, showed the typical human ESC-like morphology (Fig. 1 and Table 1), which means compact colonies with defined borders, high nucleus to cytoplasm ratio and prominent nucleoli (Marti et al., 2013). When we picked 10 of these ESC-like colonies and passaged the fragments onto fresh feeder cells, most of these subcultures (clones) gave rise to new ESC-like colonies, which

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Fig. 1 panel A, left picture
Phenotype	Qualitative analysis (Immunofluorescence)	Cells showed positive staining of pluripotency markers: NANOG, OCT4, SOX2, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81	Fig. 1 panel D
	Quantitative analysis (RT-qPCR)	Cells showed expression of endogenous reprogramming factors OCT4, SOX2, KLF4 and pluripotency markers CRIPTO and NANOG. C-MYC expression was low.	Fig. 1 panel B
Genotype	KaryoStat™ assay by ThermoFhiser Scientific	Partial mosaic chromosomal gain on chromosome 5 in ~40% of the cells at passage 63. Resolution: gains >2 Mb, losses >1 Mb, absence of heterozygosity >5 Mb.	Fig. 1 panel F
Identity	STR analysis	The studied short tandem repeat locations were: D5S818, D13S317, D7S820, D16S539, VWA, TH01, AMELOGENIN, TPOX, CSF and D21S11. iPS cell line shares identity with parental fibroblasts.	Fig. 1 panel C
Microbiology and virology	Mycoplasma	Mycoplasma tested by PCR. Negative	Fig. 1, supplementary
Differentiation potential	Embryoid body formation	Sprouted cells from EBs showed positivity for muscle actin, β-tubulin and α-fetoprotein.	Fig. 1 panel D

showed positivity for alkaline phosphatase activity (AP) (Fig. 1). The clearance of the virus and the exogenous reprogramming factor genes were confirmed by quantitative real time PCR (qRT-PCR) after ten cell culture passages. The endogenous expression of the pluripotency associated transcription factors OCT4, SOX2, KLF4, NANOG and CRIPTO as well as c-MYC was also evaluated by qRT-PCR (Fig. 1 and Table 1). According to these above-mentioned parameters all together with the low expression levels of c-MYC, one clone was selected to establish the iPS cell line. We confirmed by DNA fingerprinting analysis that the selected line shared identity with parental fibroblasts (Fig. 1 and Table 1). Immunofluorescence analysis showed that the line is positive for the intracellular self-renewal makers NANOG, OCT4 and SOX2, and the surface pluripotency-related markers SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81 (Fig. 1 and Table 1). The differentiation potential of the selected iPS cell line was confirmed according to the immunofluorescence-based detection of the endoderm marker alpha-Fetoprotein (AFP), the mesoderm marker smooth muscle actin (SMA) and the ectoderm marker beta-Tubulin III (TUJ1) (Fig. 1) after the pluripotent differentiation protocols based on embryoid bodies (EBs) formation. Interestingly, after 2–3 weeks of mesodermal differentiation, spontaneously beating cardiomyocytes were observed in cell culture dishes. The selected line has been adapted to feeder-free culture conditions and karyotype has been checked, showing a partial mosaic chromosomal gain on chromosome 5 in ~40% of the cells at passage 63 (Fig. 1, red arrow and suppl figure 2 and Table 1). Absence of mycoplasma contamination was checked regularly by PCR (suppl Fig. 1 and Table 1).

3. Materials and methods

3.1. Isolation and characterization of dermal fibroblasts

A 44 years old woman with no symptoms of rheumatic diseases was selected for the study. Absence of joint damage was confirmed radiographically in hip, knee, hand and spine. Dermal fibroblasts were obtained from a skin biopsy as previously described (Vangipuram et al., 2013).

3.2. Non-integrative reprogramming of fibroblasts

The reprogramming process was conducted introducing the factors Oct4, Sox2, Klf4 and c-Myc (CytoTune-iPS Sendai reprogramming Kit; ThermoFisher Scientific) in patient's fibroblasts at the 3rd passage. Colonies were cultured onto feeder cells (75 Gy-γ-irradiated human foreskin fibroblasts; HFF-1, ATCC) and using hES culture medium [DMEM Knockout without L-glutamine, 20% knockout serum replacement, 1% MEM non-essential aminoacids, 1% Glutamax 100X, 1% Penicilin/Steptomicin, 0.1 mM β-mercaptoethanol and 100 μg/ml basic fibroblast growth factor (all from Gibco)], which was changed daily. Cells were kept at 37 °C in a humidified atmosphere with 5% CO_2. The clonal iPS cell line was established by manually picking human ESC-like colonies. Subsequently, iPS cell clones in passages 10–20 were adapted and cultured in feeder-free conditions on rh-laminin-521 with Stemflex medium (all from Gibco). The first 3-4 passages were performed manually as described above. Further cell propagation was performed with Versene solution 1X (Gibco).

3.3. Alkaline phosphatase analysis

The iPS cell line N1-FiPS4F#7 with more than twenty passages was seeded on a feeder layer plate. When colonies appeared, AP activity was studied using alkaline phosphatase blue membrane substrate solution kit (AB03000, Sigma-Aldrich).

3.4. RNA extraction and qRT-PCR analyses

Total RNA from parental fibroblasts at the 4th passage, the iPS cell line at the 10th passage, and parental fibroblasts immediately after reprogramming (positive control for Sendai virus) was extracted using the RNeasy Mini Kit (Qiagen). cDNA synthesis was performed using SuperScriptTM Vilo™ master mix (ThermoFisher Scientific). Expression levels of the endogenous pluripotency associated genes (OCT4, SOX2, KLF4, NANOG and CRIPTO), the oncogene c-MYC, and the silencing of the exogenous reprogramming factor genes and Sendai virus genome were analyzed, in duplicate, by qRT-PCR on the LightCycler 480 Instrument (Roche) using LightCycler 480 SYBR Green I Master (Roche). Primers used for the amplification were previously described (Aasen et al., 2008).

3.5. EB formation and in vitro differentiation

Pluripotent differentiation assay was performed through the EB formation protocol. For EB formation, hanging drop method was used (Vangipuram et al., 2013). After 48h, formed EBs were transferred independently to 0.1% gelatin (Millipore)-coated 8 well chamber-slides (Merk), and cultured at 37 °C in a humidified atmosphere with 5% CO_2 and in specific differentiation media (Galera et al., 2016) to stimulate differentiation towards endodermal, mesodermal and ectodermal lineages.

3.6. Immunofluorescence analyses

Undifferentiated iPS cells at passages 40–60, and cells sprouted from EBs during pluripotent differentiation were fixed, and immunofluorescence was performed as previously described (Borestrom et al., 2014). Antibodies used are listed in Table 2.

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	Rabbit anti-NANOG	1:100	Abcam Cat# ab109250, RRID: AB_10863442
	Mouse anti-Oct-3/4	1:50	Santa Cruz Biotechnology Cat# sc- 5279, RRID: AB_628051
	Mouse anti-Sox-2	1:500	Santa Cruz Biotechnology Cat# sc-365823, RRID: AB_10842165
	Mouse anti-SSEA-4	1:500	Santa Cruz Biotechnology Cat# sc-21704, RRID: AB_628289
	Rat anti-SSEA-3	1:500	Invitrogen Cat# MA1-020, RRID: AB_2536682
	Mouse anti-TRA-1-60	1:100	Invitrogen Cat#MA1-023, RRID: AB_2536699
	Mouse anti-TRA-1-81	1:100	Abcam Cat# ab16289, RRID: AB_2165986
Differentiation Markers	Rabbit anti-alpha 1 fetoprotein	1:500	Abcam Cat# ab46799, RRID: AB_867622
	Mouse anti-alpha smooth muscle actin	1:100	Abcam Cat# ab7817, RRID: AB_262054
	Mouse anti-beta-Tubulin III	1:500	Sigma-Aldrich Cat# T8660, RRID: AB_477590
Secondary antibodies			
	Goat anti-rabbit IgG-PE	1:200	Santa Cruz Biotechnology Cat# sc-3739, RRID: AB_649004
	Rabbit Anti-Mouse Immunoglobulins, FITC Conjugated	1:200	Agilent Cat# F031302, RRID: AB_578651
	Goat anti-rat IgG, Alexa Fluor 568 Conjugated	1:200	Thermo Fisher Scientific Cat# A-11077, RRID: AB_2534121
Primers			
	Target	**Forward/Reverse primer (5′-3′)**	
Sendai virus vector (qPCR)	OCT4 Sendai virus	CCCGAAAGAGAAAGCGAACCAG/ AATGTATCGAAGGTGCTCAA	
	SOX2 Sendai virus	ATGCACCGCTACGACGTGAGCGC/ AATGTATCGAAGGTGCTCAA	
	KLF4 Sendai virus	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA	
	C-MYC Sendai virus	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG	
	Sendai virus	TGGTGCATTTTCGGTTGTTG/ ACCAGACAAGAGTTTAAGA	
Endogenous reprogramming factors (qPCR)	OCT4	GGAGGAAGCTGACAACAATGAAA/ GGCCTGCACGAGGGTTT	
	SOX2	TGCGAGCGCTGCACAT/ TCATGAGCGTCTTGGTTTTC	
	KLF4	CGAACCCACACAGGTGAGA/ GAGCGGGCGAATTTCCAT	
	C-MYC	AGGGTCAAGTTGGACAGTGT/ TGGTGCATTTTCGGTTGTTG	
Pluripotency markers (qPCR)	CRIPTO	CGGAACTGTGAGCACGATGT/ GGGCAGCCAAGGTGTCATG	
	NANOG	ACAACTGGCCGAAGAATAG/ GGTTCCCAGTCGGGTTCAC	
Housekeeping gene (qPCR)	GAPDH	GCACCGTCAAGGCTGAGAAC/ AGGGATCTCGCTCCTGGAA	

3.7. Karyotype analysis, authentication and mycoplasma testing

Karyotype analysis of the iPS cell line at passage 63 was conducted by using the KaryoStat™ service (ThermoFisher Scientific), which allows for digital visualization of chromosome aberrations.

For STR evaluation, genomic DNA was extracted from the iPS line and parental fibroblasts using the DNeasy Blood and Tissue Kit (Qiagen), and sent to the genomic service at the *Alberto Sols* Institute of Biomedical Research for analysis.

Absence of mycoplasma contamination in the iPSCs was checked by the Genomic platform of the Instituto de Investigación Biomédica de A Coruña. (Table 1).

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101683.

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