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Lab Resource: Stem Cell Line

Establishment of an induced pluripotent stem (iPS) cell line from dermal fibroblasts of an asymptomatic patient with dominant *PRPF31* mutation

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ABSTRACT

A human iPS cell line was generated from fibroblasts of a phenotypically unaffected patient from a family with PRPF31-associated retinitis pigmentosa (RP). The transgene-free iPS cells were generated with the human OSKM transcription factors using the Sendai-virus reprogramming system. iPS cells contained the expected *c.709-734dup* substitution in exon 8 of *PRPF31*, expressed the expected pluripotency markers, displayed *in vivo* differentiation potential to the three germ layers and had normal karyotype. This cellular model will provide a powerful tool to study the unusual pattern of inheritance of PRPF31-associated RP.

Resource table

Unique stem cell line identifier	*IDVi0002-A*
Alternative name(s) of stem cell line	*PRPF31-4138-iPS*
Institution	Institut de la Vision
Contact information of distributor	Olivier Goureau, olivier.goureau@inserm.fr
Type of cell line	*iPS cell line*
Origin	*Human*
Additional origin info	*Age: 75-year old* *Sex: male*
Cell Source	*Dermal fibroblasts*
Method of reprogramming	*Transgene free (Sendai Virus)*
Associated disease	*Retinitis Pigmentosa (RP) - Asymptomatic patient*
Gene/locus	*PRPF31 (c.709-734dup p.Cys247X)*
Method of modification	*N/A*
Gene correction	*NO*
Name of transgene of resistance	*N/A*
Inducible/constitutive system	*N/A*
Date archived/stock date	*May 12, 2017*
Cell line repository/bank	*Not applicable*
Ethical approval	*Approval by French regulatory agencies: CPP Ile de France (2012-A01333–40; P12-02) and the ANSM (B121362-32)*

Resource utility

This PRPF31-4138-iPS cell line constitutes a unique tool to study the pathogenesis of *PRPF31*-associated Retinitis Pigmentosa (RP). The feature of incomplete penetrance could be directly evaluated in cells affected by RP by the generation of photoreceptors and retinal pigmented epithelial cells carrying the PRPF31 mutation from this iPS cell line.

Resource details

Mutations in gene that encode pre-mRNA processing factor 31 (PRPF31) are one of the most common causes of the dominant form of Retinitis Pigmentosa (RP), a form of retinal degeneration that causes progressive visual impairment. Interestingly, mutations in ubiquitously expressed PRPF31 produce a retina-specific phenotype and are non-penetrant, with some mutation carriers being normally sighted and asymptomatic in affected families (Rose and Bhattacharya, 2016). In this study, skin fibroblasts from a 75-year-old asymptomatic carrier (Audo et al., 2010) were reprogrammed into iPS cells using non-integrative Sendai viruses containing the reprogramming factors, OCT3/4, SOX2, CMYC, KLF4. The presence of the mutation (*c.709-734dup*) in the derived PRPF31-4138-iPS cell line was confirmed by Sanger sequencing (Fig. 1A). iPS cell colonies displayed a typical ES cell-like colony morphology and growth behavior and they stained positive for alkaline phosphatase activity (Fig. 1B). We confirmed that PRPF31-4138-iPS cells were free from mycoplasma contamination (Fig. 1C) and the clearance of the vectors and the exogenous reprogramming factor genes by qPCR after ten passages

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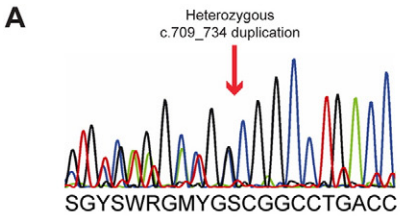
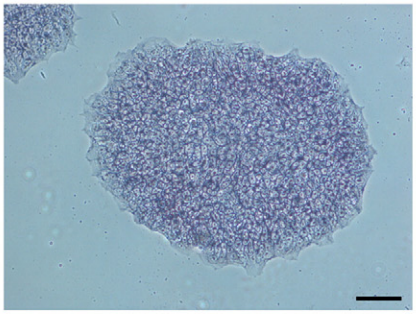
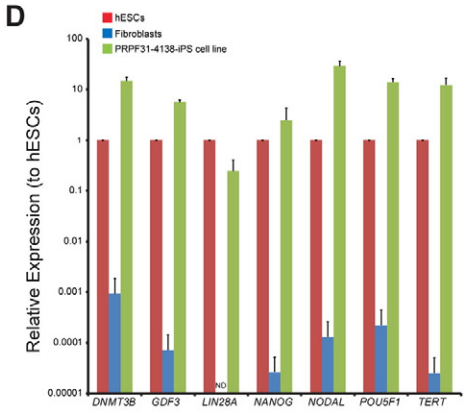
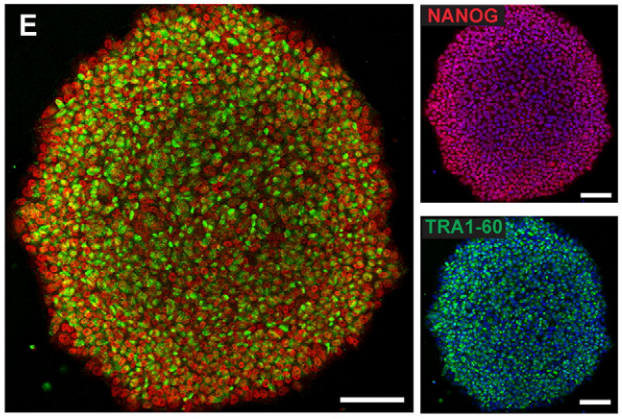


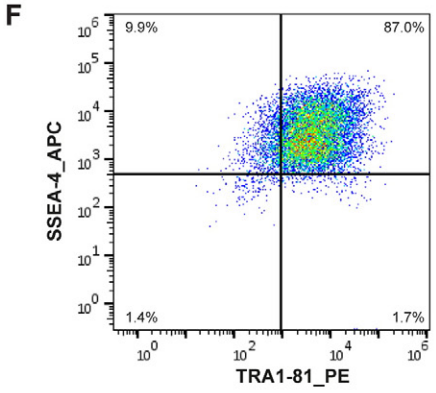


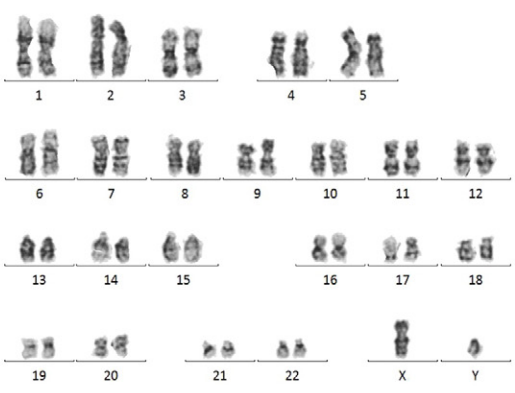
C

Sample	Ratio	Results
PRPF31-4138-iPS cell line	0.36	< 0.9 myco -
Negative sample (provided by kit)	0.09	< 0.9 myco -
Positive sample (provided by kit)	17.61	> 1.2 myco +









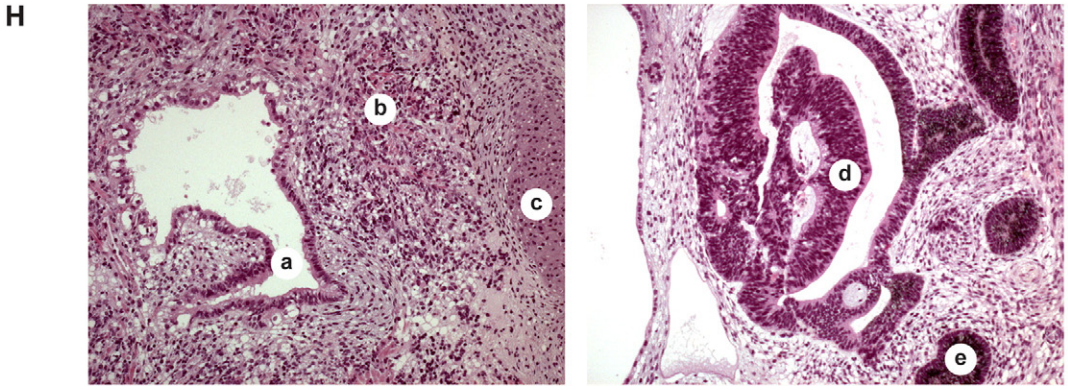


Fig. 1. Characterization of the PRPF31-4138-iPS cell line. (A) Identification of c.709-734duplication in iPS cells. (B) Positive alkaline phosphatase staining of PRPF31-4138 iPS cell colonies. (C) PRPF31-4138 iPS cell line is negative for mycoplasma contamination. (D) RT-qPCR analysis of pluripotency associated markers in PRPF31-4138 iPS cells, human ES cells and original fibroblasts. Data are normalized to human ES cells. (E) Immunohistochemistry of pluripotency markers NANOG (red) and TRA1-60 (green) in PRPF31-4138 iPS cells counterstained with DAPI (nuclei staining in blue). Scale bars = 100 μm. (F) SSEA-4 and TRA1-81 expression evaluated by flow cytometry. (G) Karyotype analysis. (H) Histological analysis of iPS cell–generated teratomas in NSG mouse. (a) neural tube; (b) gut epithelium; (c) muscle fiber and (d) cartilage.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	*hES cell-like morphology*	Fig. 1 *panel B*
	AP staining	*Positive*	
Phenotype	RT-qPCR	*Expression of pluripotency markers: DNMT3B, LIN28A, NANOG, NODAL, POU5F1, TERT, GDF3*	Fig. 1 *panel D*
	Immunohistochemistry	*Expression of pluripotency markers: NANOG and TRA1-60*	Fig. 1 *panel E*
	Flow cytometry	*SSEA-4 and TRA1-81*	Fig. 1 *panel F*
Genotype	Karyotype (G-banding) and resolution	*46XY, Resolution 450-500*	Fig. 1 *panel G*
	SNP array analysis	*Genomic integrity*	*Supplementary Fig. 1B*
Identity	SNP array analysis	*Genomic integrity and identity (parental fibroblasts and the respective iPS cell line)*	*Supplementary Fig. 1B*
Mutation analysis (IF APPLICABLE)	Sequencing	*Heterozygous (duplication)*	Fig. 1 *panel A*
Microbiology and virology	Mycoplasma	*Mycoplasma testing by luminescence: Negative*	Fig. 1 *panel C*
Differentiation potential	Teratoma formation	*Representation of all three germ layers formation*	Fig. 1 *panel H*
Donor screening (OPTIONAL)	N/A		
Genotype additional (OPTIONAL)	N/A		

(Supplementary Fig. 1A). PRPF31-4138-iPS cells exhibited typical markers of pluripotency with expression of *DNMT3B, LIN28A, NANOG, NODAL, POU5F1 (OCT4), TERT* and *GDF3* evaluated by RT-qPCR (Fig. 1D), of NANOG and TRA1-60 evaluated by immunohistochemical staining (Fig. 1E) and of SSEA-4 and TRA1-81 assessed by flow cytometry (Fig. 1F). The PRPF31-4138-iPS cell line displayed a normal karyotype (46, XY) after more than twenty culture passages (Fig. 1G). Single nucleotide polymorphism (SNP) genotyping of original fibroblasts and the generated PRPF31-4138-iPS cell line validated the identity and genomic integrity of the iPS cell line (Supplementary Fig. 1B). Teratoma assays showed the presence of normal differentiation towards endodermal, ectodermal and mesodermal layers (Fig. 1H). Taken together, we have successfully reprogrammed p.Cys247X PRPF31 dermal fibroblasts into iPS cells that can be used to generate retinal cells (Reichman et al., 2017, 2014) in order to study pathogenic mechanism underlying RP caused by mutation in *PRPF31* and the non-penetrance of the disease phenotype in affected families (Tables 1 and 2).

Materials and methods

Human fibroblast cultures and reprogramming

Human fibroblasts were cultured and reprogrammed using the CytoTune Sendai reprogramming vectors Oct4, Klf4, Sox2 and c-Myc (Thermo Fisher Scientific) as previously reported (Terray et al., 2017). The emergent iPS cell colonies were picked under a stereomicroscope and expanded on mitomycined human foreskin feeder layers. After generation of a frozen stocks, iPS cells were preferentially adapted and cultured in feeder free conditions (Reichman et al., 2017). Absence of mycoplasma contamination was verified by the MycoAlert™ Mycoplasma Detection Kit (selective biochemical test of mycoplasma enzymes) according to the manufacturer's instructions (Lonza). After ten passages, the clearance of the exogenous reprogramming factors and Sendai virus genome was confirmed by qPCR following the manufacturer's instructions (Thermo Fisher Scientific).

Mutation analysis

Genomic DNA from human iPS cells was extracted with Nucleospin Tissue Kit (Macherey-Nagel) according to the manufacturer instruction. PCR amplification flanking exon 8 of *PRPF31* (Table S2) was performed using HOT FIRE Pol DNA Polymerase (Solis BioDyne). PCR products were sequenced using BigDye® Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific) on a 3730 DNA analyzer (Applied Biosystems).

Karyotype analyses

Conventional cytogenetic analysis was performed as described previously (Reichman et al., 2014). Molecular karyotype was analyzed by SNP genotyping using Illumina's Infinium HumanCore-24 Bead Chips (Illumina, Inc., San Diego, USA) at Integragen (Evry, France). Processing was performed on genomic DNA following the manufacturer's procedures. LogR ratio and B allele plots were generated in GenomeStudio software (Illumina, Inc.).

In vivo pluripotency analysis by teratoma formation assay

Teratoma assays were performed as described previously (Reichman et al., 2014).

Real-time PCR analysis

Total RNAs were extracted using Nucleospin RNA II kit (Macherey-Nagel) and cDNA synthesized using the QuantiTect reverse transcription kit (Qiagen) following manufacturer's recommendations. qPCR analysis was performed in three minimum independent biological experiments with custom TaqMan® Array 96-Well Fast plates (Thermo Fisher Scientific) according to the manufacturer's protocol as described previously (Reichman et al., 2014).

Flow cytometry

Cells were detached with Accutase solution and harvested for quantitative analysis by flow cytometry (Cytomics FC500 MCL; Beckman Coulter) by staining the TRA-1-81, and SSEA-4 antibodies and data were analyzed with FlowJo software.

Alkaline phosphatase and immunofluorescence staining

Staining of fixed hiPS cells was performed as described previously (Reichman et al., 2014).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2017.10.007.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow cytometry			
	Antibody	Dilution	Company Cat # and RRID
NANOG	Rabbit mAb anti-NANOG	1:300	Cell Signaling Technology Cat#D73G4, RRID:AB_4903
TRA1-60	Mouse mAb anti-TRA1-60(S)	1:300	Cell Signaling Technology Cat#TRA-1-60(S), RRID:AB_4746
TRA1-81 PE- conjugated	Mouse IgM anti Human TRA-1-81	1:50	R&D Systems Cat#TRA-1-81, RRID:AB_FAB8495P-025
SSEA4 APC-conjugated	Mouse IgG_3 anti Human/Mouse SSEA-4	1:50	R&D Systems Cat#MC-813-70, RRID:AB_FAB1435A-025
Secondary antibodies	Alexa Fluor 594-conjugated Donkey anti-Rabbit IgG (H + L),	1:600	Thermo Fisher Scientific Cat# A-21207, RRID: AB_141637
	Alexa Fluor 488-conjugated Donkey anti-Mouse IgG (H + L)	1:600	Thermo Fisher Scientific Cat# A-21202, RRID: AB_141607

Primers		
	Target	Forward/reverse primer (5′–3′)
Targeted mutation sequencing	PRPF31	CTCTCTGCTTTCTTCTGACC/TGAGTGCTACCGTCAGCT
Elimination of Sendai virus transgenes	*SeV*	Assay ID: Mr04269880_mr
(qPCR - TaqMan)	*SeV-OCT4*	Assay ID: Mr04269878_mr
	Sev-KLF4	Assay ID: Mr04269879_mr
	Sev-SOX2	Assay ID: Mr04269881_mr
	Sev-CMYC	Assay ID: Mr04269876_mr
Pluripotency Markers	*DNMT3B*	Assay ID: DNMT3B-Hs00171876_m1
(qPCR - TaqMan)	*GDF3*	Assay ID: GDF3-Hs00220998_m1
	LIN28	Assay ID: LIN28A-Hs00702808_s1
	NANOG	Assay ID: NANOG-Hs02387400_g1
	NODAL	Assay ID: NODAL-Hs00415443_m1
	POU5F1 (OCT4)	Assay ID: POU5F1-Hs00999632_g1
	TERT	Assay ID: TERT-Hs00972656_m1
House-Keeping Genes	*18S*	Assay ID: 18S-Hs99999901_s1
(qPCR - TaqMan)		

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Author disclosure statement

There are no competing financial interests in this study.

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