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Lab resource: Stem Cell Line

Generation of human iPSCs from fetal prostate fibroblasts HPrF

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A B S T R A C T

Human induced pluripotent stem cell line was generated from commercially available primary human prostate fibroblasts HPrF derived from a fetus, aged 18–24 weeks of gestation. The fibroblast cell line was reprogrammed with Yamanaka factors (OCT4, SOX2, c-MYC, KLF4) using CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Pluripotency of the derived transgene-free iPSC cell line was confirmed both *in vitro* by detecting the expression of factors of pluripotency on a single-cell level, and *in vivo* using teratoma formation assay. This iPSC cell line will be a useful tool for studying both normal prostate development and prostate cancer disease.

Resource table

Unique stem cell line identifier	IBPi001-A
Alternative name(s) of stem cell line	HPrF hiPSCs 20A
Institution	Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic & International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic
Contact information of distributor	Karel Souček, Ph.D.; ksoucek@ibp.cz
Type of cell line	iPS cells
Origin	Human
Additional origin info	Sex: male Age: fetal donor aged between 18 and 24 weeks of gestation Ethnicity: unknown
Cell source	Prostate fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune™-iPS 2.0 Sendai Reprogramming Kit (OCT3/4, SOX2, c-MYC, KLF4)
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	January 2016–February 2017
Cell line repository/bank	N/A
Ethical approval	Animal experiments were approved by the Academy of Sciences of the Czech Republic (approval nr. 13/2015); supervised by the local ethical committee of the Institute of Biophysics of the CAS; and performed by certified individuals. Ethical statement of the provider of original HPrF cells may be found here https://www.sciencellonline.com/Documents/Ethical_Statement.pdf

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Resource utility

Generally, models for studying human prostate development and differentiation are insufficient. Therefore, we used commercially available human prostate fibroblasts HPrF for generation of an iPSC cell line to obtain a new model, useful for both *in vivo* and *in vitro* studies.

Resource details

Primary human fetal prostate fibroblasts HPrF (Fig. 1A, left) were obtained from ScienCell Research Laboratories. Fibroblast phenotype was verified previously (Kahounová et al., 2018). Reprogramming was done in feeder-free conditions using Sendai virus-based technology (CytoTune™ - iPS 2.0 Sendai Reprogramming Kit) with transcription factors OCT3/4, SOX2, c-MYC, and KLF4. The derived iPSC cell line was successfully cultivated in both feeder-free and feeder-dependent conditions (Fig. 1A, right). Derived HPrF iPSC cells were tested for mycoplasma contamination using PCR (Supplementary Fig. S1A). HPrF iPSCs (lane 6) were negative for mycoplasma-specific sequence (MYCO) when compared to positive control for mycoplasma (PC) in lane 8 and negative control (NC) in lane 10 (other lanes contain samples not relevant for this study). Lower part of the figure shows results for GDF-15, which was used as a reference gene.

Absence of reprogramming factors was confirmed using PCR detection of SeV genome and transgenes (KLF4, KOS, c-MYC) (Fig. 1B). HPrF at day 7 post reprogramming were positive for SeV and all tested transgenes, while iPSC cells at passage 33 in feeder-free (FF) conditions and passage 34 in feeder-dependent (FD) conditions were negative for all tested genes (POLR2A was used as a reference gene). Reprogramming vectors-free iPSCs were used in all following experiments. G-banding showed that parental HPrF cell line (passage 9) has karyotype 46,XY (Fig. 1C left), whereas in reprogrammed HPrF iPSC cell line cultivated in feeder-free conditions (passage 35), an aberrant karyotype with gain of chromosome 20 was found (Fig. 1C right). To confirm the identity of the extra chromosome and exclude possible translocation of 20q, FISH analysis was performed using Chromosome 20 Alpha Satellite Probe (red) and 20qter Subtelomere specific probe (green). Results showed that the extra chromosome is indeed chromosome 20 (Supplementary Fig. S1B). Since signals for both probes were detected simultaneously, the translocation of 20q was excluded. Therefore, the karyotype of derived HPrF iPSCs is 47,XY, +20. STR analysis confirmed a match in all 16 tested alleles, certifying that derived iPSC cell line and original HPrF cells are identical.

Pluripotency of derived iPSC cells was confirmed both *in vitro* and *in vivo*. iPSC cells cultivated in feeder-free conditions were used in all experiments. *In vitro*, expression of pluripotency factors TRA-1-60-R, OCT 3/4, SOX2, and NANOG was verified using multicolor flow cytometry (Fig. 1D). Only viable, single cells without debris were taken into analysis and appropriate isotype controls or negative controls were used to set the gating, as shown in Supplementary Fig. S1C. Expression of NANOG, OCT 3/4, and SOX2 in iPSCs colonies was also assessed using immunofluorescence (Fig. 1E). Appropriate isotype controls for each marker after background subtraction are presented in Supplementary Fig. S1D. Both methods confirmed expression of tested pluripotency markers in derived iPSC cells. *In vivo*, pluripotency was demonstrated by a teratoma assay, where injection of feeder-free iPSC cells in testes of immunodeficient SHO mice gave rise to teratomas with identified structures from all three germ layers (ectoderm - neuroepithelium, mesoderm - cartilage, and endoderm - probably gut-like structure) (Fig. 1F). Taken together, these data show that we have generated a pluripotent iPSC cell line from human prostate fibroblasts, which, we hope, will be a new and powerful tool for further studies of human prostate development and cancer disease (Table 1). This cell line was introduced and validated in Human Pluripotent Stem Cell Registry (<https://hpscereg.eu/cell-line/IBPi001-A>).

Material and methods

Cell culture and reprogramming

HPrF (#4430, lot 4077) were cultivated in Fibroblast medium (#2301, both ScienCell Research Laboratories). Reprogramming was performed using CytoTune™ - iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) according to manufacturer's instructions in feeder-free conditions [0.5 µg/cm² vitronectin coating, Essential 8 medium kit (Thermo Fisher Scientific)]. Derived iPSCs were cultivated in feeder-free or feeder-dependent conditions [60 Gy-irradiated MEF, iPS media - KnockOut DMEM/F12, 20% KnockOut™ Serum Replacement, 1 × GlutaMAX-1, 1 × MEM Non-Essential Amino Acids Solution, 55 µM β-mercaptoethanol, 4 ng/ml bFGF (all Thermo Fisher Scientific), penicillin/streptomycin (Biosera)]. Cells were cultivated on laboratory plastic (Corning Falcon) in humidified incubator (37 °C, 5% CO₂). When thawed after cryopreservation, Y-27632 dihydrochloride (10 µM, Santa Cruz Biotechnology) was added to cultivation media for the first 24 h in both feeder-free and feeder-dependent conditions.

PCR analysis of reprogramming vectors clearance

Total RNA was isolated using High Pure RNA isolation Kit (Roche) and cDNA was synthesized from 90 ng RNA with High Capacity RNA-to-cDNA synthesis Kit (Ambion). Primers recommended in reprogramming kit were used (Table 2). PCR products were resolved on 2% agarose (Sigma-Aldrich) gel, visualized using GelRed Nucleic Acid Gel Stain (Biotium) and ChemiDoc™ MP System (Biorad).

Flow cytometry and immunocytochemistry

Single cell suspension of iPSC cells was stained with TRA-1-60-R (20 min, 4 °C) in 1% BSA/PBS/0.1% NaN₃, followed by staining with streptavidin-FITC (20 min, 4 °C), washed with PBS, and incubated with viability dye (20 min, 4 °C). Fixation, permeabilization and staining of intracellular markers was performed using Human Pluripotent Stem Cell Transcription Factor Analysis Kit (BD Biosciences) according to manufacturer's instructions. Simultaneously, isotype controls were prepared. Data were acquired with BD FACSVerser (BD Biosciences). Results were analyzed using FlowJo software (FlowJo, LLC, Data Analysis Software).

For immunocytochemical analysis, iPSCs were cultivated on vitronectin-coated Labtek slides (BD Falcon) for 48 h, fixed (4% PFA, 15 min, RT) and washed with PBS. Permeabilization and staining was performed using similar procedure as for flow cytometry. Nuclei were stained with DAPI (1 µg/ml, 30 min, RT). Samples were washed (1% BSA/PBS) and mounted in Mowiol + 0.6% DABCO. Images were acquired in sequential mode on Leica SP5X confocal microscope (Leica Microsystems Vertrieb GmbH), and processed by background subtraction in Leica Application Suite X software (version 2.0.2.15022).

Teratoma assay

Teratoma assay was performed as published (Peterson et al., 2011). 1 × 10⁶ iPSC cells in 30 µl of PBS with bromophenol blue (0.005%) were injected into testis of Crl: SHO-Prkdc^{scid}Hr^{hr} mice (Charles River) at the age of 12.1 weeks. After 55 days, teratomas were harvested, processed for H&E staining, and analyzed with TissueFAXS scanning system (TissueGnostics) using 20× objective and TissueFAXS Viewer v4.2 software.

Karyotype and FISH analysis

IpscS cultured in E8 medium were treated with 0.11 µg/ml colcemid (Gibco) for 2–3 h, dissociated into single cells (0.1 mM EDTA, 37 °C, 3 min), treated with hypotonic solution (0.075 M KCl, 20 min),

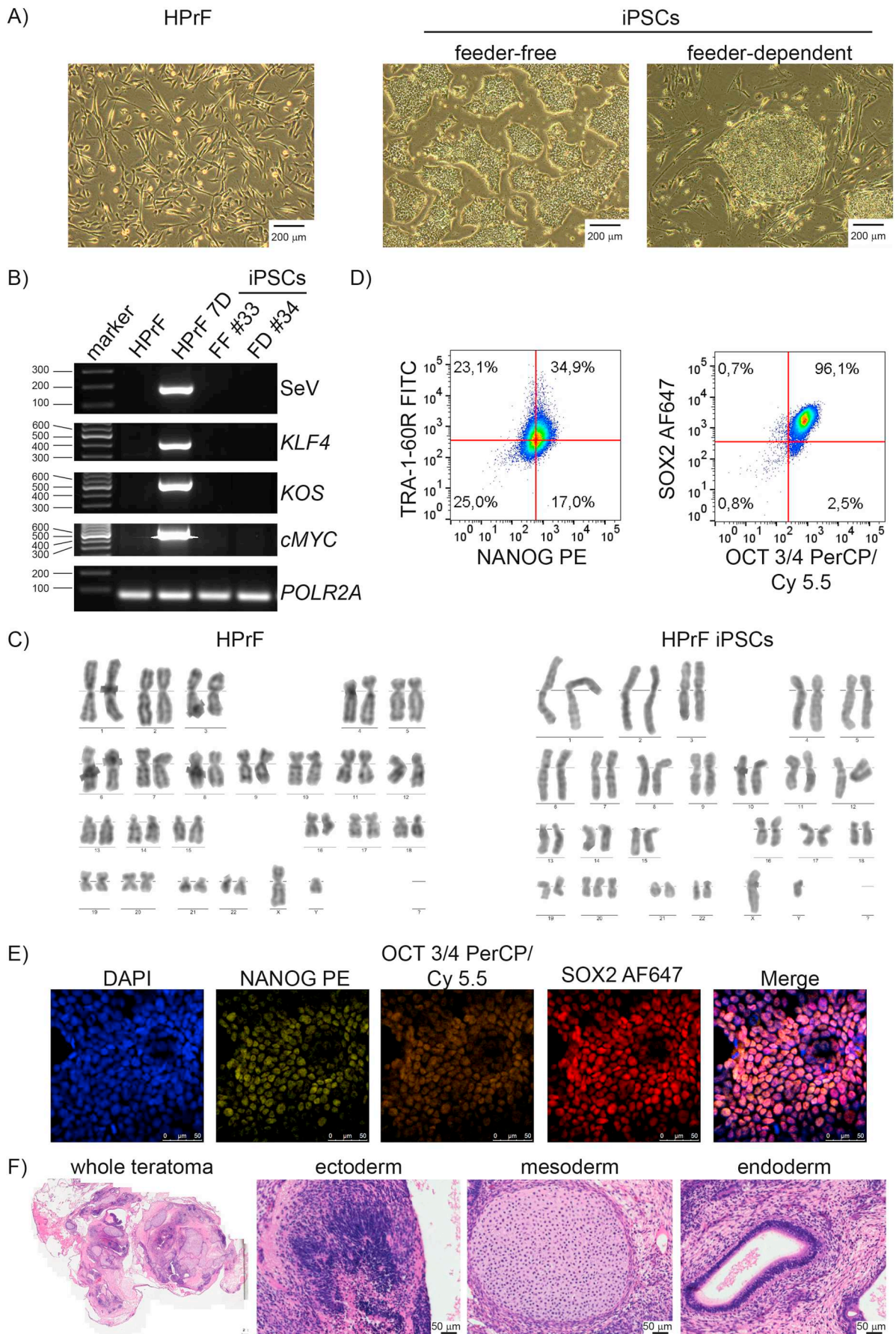


Fig. 1. Characterization and validation of iPS cell line IBPi001-A generated from human prostate fibroblasts.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Confirmed fibroblast morphology of the original HPrF cell line, and morphology of the derived iPS cell line	Fig. 1, panel A
Phenotype	Qualitative analysis - Immunocytochemistry	Expression of pluripotency markers OCT3/4, NANOG, SOX2	Fig. 1, panel E
	Quantitative analysis - Flow cytometry	Expression of pluripotency markers: OCT3/4: 98.6% SOX2: 96.8% TRA-1-60-R: 58% NANOG: 51.9%	Fig. 1, panel D
Genotype	Karyotype (G-banding)	47,XY,+20 Resolution: 300 bands	Fig. 1, panel C
Identity	STR analysis	Match between parental HPrF cell line and derived iPS cells confirmed	Available with authors
Mutation analysis	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR negative	Supplementary Fig. S1, panel A
Differentiation potential	Teratoma formation	morphological proof of pluripotency - ectodermal, mesodermal and endodermal structures identified within the developed teratoma	Fig. 1, panel F
Donor screening	HIV-1, HBV, HCV	Analyzed by vendor https://www.sciencellonline.com/products-services/primary-cells/human/cell-types/fibroblasts/human-prostate-fibroblasts.html	N/A
Genotype additional info	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 marker	TRA-1-60-R biotin	1:200	BioLegend Cat# 330603 RRID: AB_1186140
Pluripotency marker	NANOG PE	20 µl per sample	Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat. no:560589 RRID: AB_2722505
Pluripotency marker	OCT 3/4 PerCP/Cy 5.5	20 µl per sample	Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat. no:560589 RRID: AB_2722505
Pluripotency marker	SOX2 Alexa Fluor 647	20 µl per sample	Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat. no:560589 RRID: AB_2722505
Secondary detection reagent	Streptavidin FITC	1:2000	eBioscience (Thermo Fisher Scientific), cat.no 11-4317-87
Viability marker	LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit	1:500	Thermo Fisher Scientific, cat. no:L34959
Isotype control	PE Mouse IgG1, kappa	20 µl per sample	Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat. no:560589 RRID: AB_2722505
Isotype control	PerCP/Cy5.5 Mouse IgG1, kappa	20 µl per sample	Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat. no:560589 RRID: AB_2722505
Isotype control	Alexa Fluor® 647 Mouse IgG2a, kappa	20 µl per sample	Human Pluripotent Stem Cell Transcription Factor Analysis Kit BD Biosciences, cat. no:560589 RRID: AB_2722505

Primers		
	Target	Forward/Reverse primer (5'-3')
SeV genome	SeV	GGA TCA CTA GGT GAT ATC GAG C ACC AGA CAA GAG TTT AAG AGA TAT GTA TC
Transgene	<i>KLF4</i>	TTC CTG CAT GCC AGA GGA GCC C AAT GTA TCG AAG GTG CTC AA
Transgene	<i>KOS</i>	ATG CAC CGC TAC GAC GTG AGC GC ACC TTG ACA ATC CTG ATG TGG
Transgene	<i>c-MYC</i>	TAA CTG ACT AGC AGG CTT GTC G TCC ACA TAC AGT CCT GGA TGA TGA TG
Reference gene	<i>POLR2A</i>	GCA AAT TCA CCA AGA GAG ACG CAC GTC GAC AGG AAC ATC AG
Mycoplasma	MYCO	GGC GAA TGG GTG AGT AAC ACG CGG ATA ACG CTT GCG ACC TAT G
Reference gene for mycoplasma detection	<i>GDF-15</i>	ACA CAT CAA GGT TGC CCT TC GGG CCT CAG TAT CCT CTT CC

and fixed with methanol: acetic acid (3:1). Metaphases were spread on microscope slides. G-banding karyotypic analysis was performed. More than 10 metaphase spreads were examined from iPSCs. For FISH analysis, cells were treated and samples were prepared similarly as for karyotype. Two probes were used: 20qter Subtelomere Specific Probe Green (LPT20QG, CytoCELL) and Chromosome 20 Alpha Satellite Probe Red (LPE020R, CytoCELL). Assay was performed according to manufacturer's recommendation. More than 60 mitoses were examined for HPrF iPSCs.

STR analysis

DNA was isolated from cell lines using QIAamp DNA Blood Mini Kit (Qiagen). For STR analysis 2 ng of DNA was amplified by PCR using AmpFlSTR Identifiler Plus PCR amplification kit (ThermoFisher Scientific). The PCR products of amplified STR markers were separated through capillary electrophoresis on ABI PRISM 310 Genetic Analyzer (Applied Biosystems) in POP4 gel. Amplified 0.5 µl of sample in 10 µl of Hi-Di Formamide and 0.5 µl GeneScan-500 LIZ Size Standard was used for electrophoresis after prior 3 min denaturation. Data collection settings: 60 °C temperature, 15000 V injection voltage, 10 s injection duration, 25 min run duration. The data was analyzed by GeneScan v3.1 software. The Identifiler Allelic Ladder and control DNA sample 9947A was used to calibrate fluorescently labelled STR fragments during data analysis. Sample alleles were determined and are available with authors. The analysis provided alleles commonly used for authentication by ATCC or DSMZ cell line banks (*i.e.* D5S818, D13S317, D7S820, D16S539, VWA, TH01, Amelogenin, TPOX and CSF1PO), as well as additional ones (*i.e.* D8S1179, D21S11, D3S1358, D2S1338, D19S433, D18S51 and FGA).

Detection of mycoplasma using PCR

Cell lysis was performed in lysis buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1% Triton X-100) containing proteinase K (100 µg/ml, Thermo Fisher Scientific) at 50 °C overnight. PCR reaction was performed using FastStart Taq DNA polymerase (Roche). Primer sequences were used according to publication (Persing, 1993) and are listed in

Table 2. Products were resolved in 2% agarose gel (Sigma-Aldrich) and visualized using GelRed Nucleic Acid Gel Stain (Biotium) and ChemiDoc™ MP System (Biorad).

Disclosure

There are no competing financial interests in this study.

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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.2019.101405>.

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