Stem Cell Research 33 (2018) 79–82

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: Stem Cell Line

Generation of induced pluripotent stem cells (iPSCs) from human foreskin fibroblasts

Friedrich Erdlenbruch, Jürgen Rohwedel*, Philipp Ralfs, Antonia Thomitzek, Jan Kramer, Figen Cakiroglu

Institute for Virology and Cell Biology, University of Lübeck, Germany

A B S T R A C T

The human iPS cell line VUZUZLi001-A (hVH-1) was generated from human foreskin fibroblasts to be used as a control line. Reprogramming was performed by retroviral transduction of reprogramming factors OCT4, SOX2, KLF4 and c-MYC.

Resource table

Unique stem cell line identifier	VUZUZLi001-A
Alternative name(s) of stem cell line	hVH-1
Institution	Institute of Virology and Cell Biology, University of Lübeck
Contact information of distributor	Jürgen Rohwedel, juergen.rohwedel@uni-luebeck.de
Type of cell line	iPSC
Origin	human
Additional origin info	Age: unknown Sex: male Ethnicity if known: —
Cell source	foreskin fibroblasts
Clonality	mixed
Method of reprogramming	retroviral integration
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	2017-08-05
Cell line repository/bank	N/A
Ethical approval	Ethics Committee University of Lübeck; reference number: 14-100

Resource utility

The iPS cell line VUZUZLi001-A was established as a control cell line for reprogramming efficiencies and epigenetic modifications when testing single stranded RNA-based reprogramming vectors.

Resource details

Human foreskin fibroblasts were reprogrammed into induced pluripotent stem cells (iPSC) by retroviral transduction of the transcription factors *OCT4*, *SOX2*, *KLF4* and *c- MYC* using pMXs vectors. iPS cell colonies 15 days after transduction stained positive for alkaline-phosphatase (Fig. 1A). The cell colonies showed the typical ES cell-like

* Corresponding author.
E-mail address: juergen.rohwedel@uni-luebeck.de (J. Rohwedel).

https://doi.org/10.1016/j.scr.2018.10.010
Received 16 July 2018; Received in revised form 27 September 2018; Accepted 4 October 2018
Available online 06 October 2018

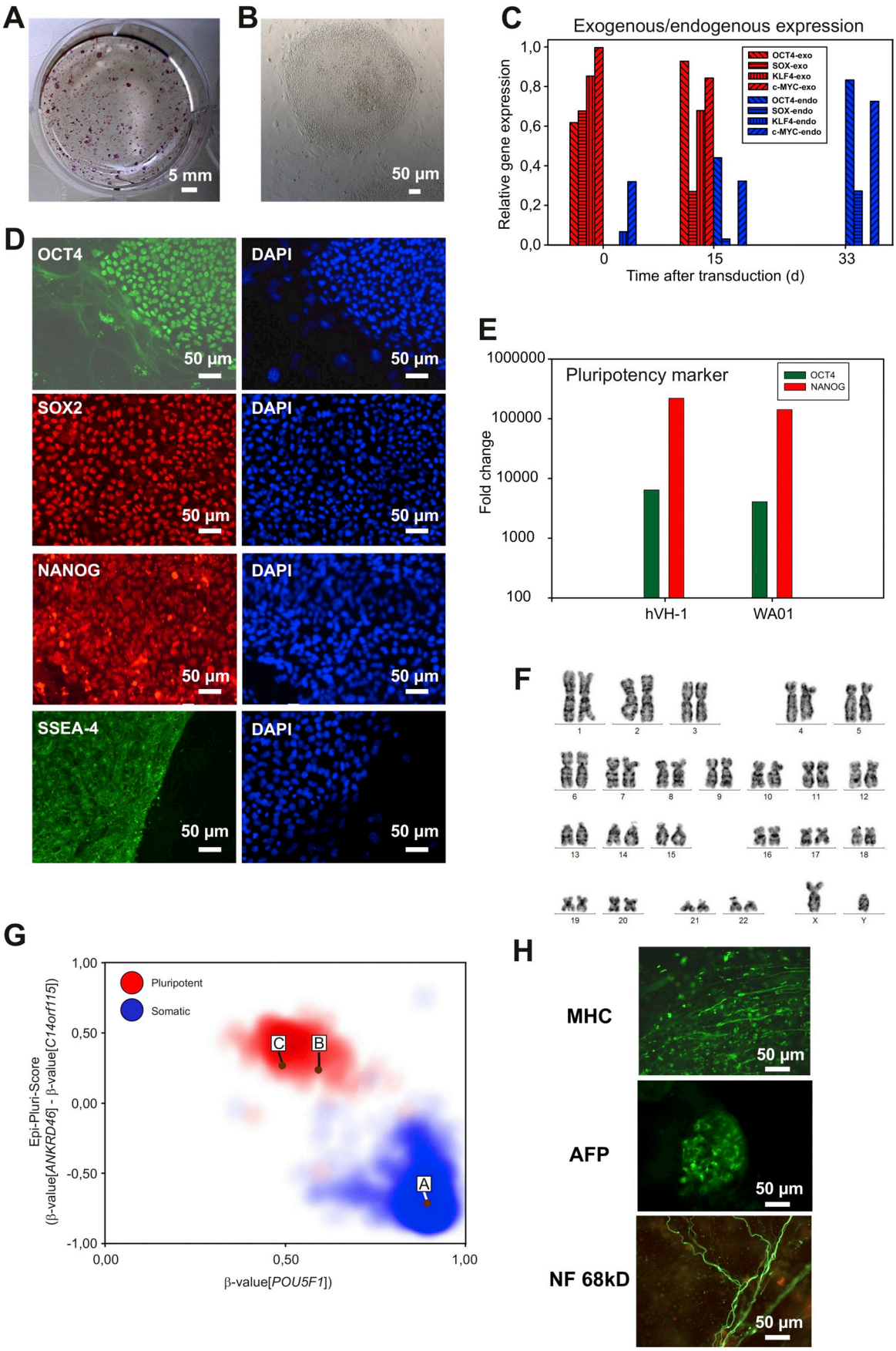

Fig. 1. Characterisation of the foreskin fibroblast-derived iPS cell line VUZUZLi001-A.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal morphology	Fig. 1B
Phenotype	Qualitative analysis: Alkaline phosphatase (AP)-staining	AP expression	Fig. 1A
	Qualitative analysis: Immunocytochemistry, EPI Pluri Score	Expression of pluripotency marker: OCT4, SOX2, NANOG, SSEA-4; Pluripotent cells	Fig. 1D Fig. 1G
	Quantitative analysis: RT-qPCR	Level of gene expression of pluripotency marker *OCT4* and *NANOG*	Fig. 1E
Genotype	Karyotype (G-banding) and resolution	46 XY, Resolution 450–500	Fig. 1F
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	21 sites tested, all matched	Submitted in archive with journal
Mutation analysis	Sequencing	N/A	N/A
(IF APPLICABLE)	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR: Negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation and immunocytochemistry	Cells expressing myosin heavy chain, α-feto protein, and 68 kDa neurofilament were detected by immunocytochemistry.	Fig. 1H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
(OPTIONAL)	HLA tissue typing	N/A	N/A

morphology (Fig. 1B). While gene expression of the exogenous factors *OCT4*, *SOX2*, *KLF4* and *c- MYC* was downregulated during reprogramming, endogenous genes were upregulated in the same time as determined by semi-quantitative RT-PCR (Fig.1C). The cells of a single isolated clone, named hVH-1, expressed the pluripotency factors OCT4, SOX2, NANOG and SSEA-4 as detected by immunostaining (Fig. 1D) and the level of expression of *OCT4* and *NANOG* in relation to the reprogrammed fibroblasts was comparable to human embryonic stem cells of line WA01 used as a control (Fig. 1E) as determined by quantitative RT-PCR. Karyotypes appeared to be normal as determined by G-banding of chromosomes (Fig. 1F). The isolated iPSCs were genetically identical to the parental fibroblasts as demonstrated by STR analysis (not shown). The pluripotent potential of the cells was verified by the EPI-pluri score test (Lenz et al., 2015), testing the methylation status at a specific CpG site localized within the gene *OCT4* (*POU5F1)* and the difference in methylation levels (β-values) of two specific CpGs in ANKRD46 and C14orf115. The combined Epi-Pluri-Score value is compared to methylation profiles of 264 pluripotent and 1951 non-pluripotent cell preparations (red and blue clouds in Fig. 1G), demonstrating that two iPS cell preparations (B and C) from line VUZUZLi001-A were pluripotent compared to non-pluripotent somatic cells (A) used as a control. The pluripotent potential of line VUZUZLi001-A was further demonstrated by EB differentiation into cells expressing myosin heavy chain (MHC), alpha fetoprotein (AFP) and the 68 kDa neurofilament (NF68kDa) (Fig.1H).

Materials and methods

Cell culture, reprogramming and differentiation

Human foreskin fibroblasts (VHAE) were cultivated in fibroblast medium (FM) consisting of DMEM supplemented with 10% FCS, 2 mM L-glutamine, 50 µM beta-mercaptoethanol, 1% non-essential amino acids, 100 U/ml penicillin, and 100 µg/ml streptomycin. For reprogramming, the pMXs (Takahashi and Yamanaka, 2006) retroviral vectors carrying the transcription factors OCT4, SOX2, KLF4 and c- MYC were used to produce recombinant retroviruses in HEK293FT cells after polyethyleneimine transfection. VHAE cells were seeded in FM at a density of 10^4 cell per well of a six well plate and 500 µl of each virus supernatant was added. After 6–8 h the medium was changed. After 4 days the medium was changed to pluripotent cell culture (PCC) medium consisting of DMEM supplemented as described above, but FCS was replaced by 20% knock-out serum replacement and 8 ng/ml bFGF on a feeder layer of inactivated mouse fibroblasts. ES cell-like colonies appeared after additional 10 days. A single clone, designated hVH-1, was isolated and propagated by mechanical dissociation. For the EPI Pluri Score test genomic DNA was extracted from iPS cells using the Quick-DNA Miniprep kit (Zymo Research) and the test was performed by Cygenia (Aachen, Germany).

For cell differentiation, iPS cells of line hVH-1 were detached using collagenase and redissolved in PCC medium without bFGF, but with 4 mg/ml polyvinyl alcohol (Sigma-Aldrich). 5 day old EBs were transferred onto gelatine-coated chamber slides and analyzed by immunostaining 21 days later.

Semi-quantitative and quantitative RT-PCR analysis

Total RNA from transduced cells or cultures of single clones was isolated using the NucleoSpin RNA II Kit (Machery & Nagel). 500 ng of RNA was reverse transcribed using RevertAid Reverse Transcriptase (Thermo Fisher) and 1/10th from the reaction used for PCR analysis using specific primers (Table 2). For semi-quantitative analysis the PCR conditions were: Denaturation for 3 min at 95 °C, followed by amplification for 30 cycles of 30 s denaturation at 95 °C, 30 s annealing at 58 °C and 30 s elongation at 72 °C. Densitometric quantification was performed after electrophoretic separation of PCR products. Gene expression was determined relative to expression of the house-keeping gene *GAPDH*. For quantitative analysis we used TaqMan® probes specific for the gene of interest (TIB Molbiol), which were labelled with 6-FAM and had tetramethylrhodamine (TMR) as a quencher. Aliquots of 1 µl from the RT reactions were mixed with 19 µl master mix consisting of 0.4 µl TaqMan probe, 0.4 µl gene-specific primer (see Table 1), 10 µl iQ Supermix (Biorad) and 7.8 µl sterile, steam sterilised and DEPC-treated water (Roth) per probe. The thermal cycling was done using an iCycler iQ thermal cycler running with software version 3.1. (Biorad) according to the manufacturer's instructions and the following thermal conditions: 95 °C for 2 min followed by 50 cycles of 95 °C for 40 s, annealing temperature for 40 s and 72 °C for 40 s. Finally samples were heated to 95 °C for 1 min. For generation of standard curves, the PCR product was cloned into the vector pCR-TOPO (Invitrogen). Plasmid DNA was isolated using QIAGEN-tip 100 anion-exchange columns (Qiagen) and serially diluted in double-distilled water. Threshold cycles were adjusted to attain the highest possible correlation coefficient value for the standard curve provided by the manufacturer's software. According to their respective cycle numbers the concentrations of unknown samples were deduced from the standard curve. Gene expression was determined relatively to expression of the house-keeping gene *GAPDH*.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse anti-OCT-3/4 (C-10)	1:100	Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628051
	Goat anti-SOX-2 (Y-17)	1:100	Santa Cruz Biotechnology Cat# sc-17320, RRID:AB_2286684
	Goat anti-Nanog	1:100	R & D systems Cat# AF1997, RRID:AB_355097
	Mouse anti-SSEA-4 (MC813)	1:100	Santa Cruz Biotechnology Cat# sc-59368, RRID:AB_1129653
Differentiation markers	Mouse anti-myosin, sarcomeric (MF 20)	1:50	DSHB Cat# MF 20, RRID:AB_2147781
	Mouse anti-AFP (H-9)	1:50	Santa Cruz Biotechnology Cat# sc-166335, RRID:AB_2224077
	Mouse Anti-Neurofilament 68	1:50	Sigma-Aldrich Cat# N5139, RRID:AB_477276
Secondary antibodies	FITC-AffiniPure Rabbit Anti-Mouse IgG (H + L)	1:200	Jackson ImmunoResearch Labs Cat# 315-095-003, RRID:AB_2340106
	Cy3-AffiniPure Goat Anti-Mouse IgG (H + L)	1:400	Jackson ImmunoResearch Labs Cat# 115-165-003, RRID:AB_2338680
	Cy3-AffiniPure Rabbit Anti-Goat IgG (H + L)	1:400	Jackson ImmunoResearch Labs Cat# 305-165-003, RRID:AB_2339464

Primers		
	Target	Forward/Reverse primer (5′-3′)
Transgenes (RT-PCR)	*OCT4*	CCTCACTTCACTGCACTGTA/CCTTGAGGTACCAGAGATCT
	SOX2	CCCAGCAGACTTCACATGT/CCTTGAGGTACCAGAGATCT
	KLF4	GATGAACTGACCAGGCACTA/CCTTGAGGTACCAGAGATCT
	c-MYC	TGCCTCAAATTGGACTTTGG/CGCTCGAGGTTAACGAATT
Pluripotency marker (RT-PCR)	*OCT4*	CCTCACTTCACTGCACTGTA/CAGGTTTTCTTTCCCTAGCT
	SOX2	CCCAGCAGACTTCACATGT/CCTCCCATTTCCCTCGTTTT
	KLF4	GATGAACTGACCAGGCACTA/GTGGGTCATATCCACTGTCT
	c-MYC	TGCCTCAAATTGGACTTTGG/GATTGAAATTCTGTGTAACTGC
Pluripotency marker (RT-qPCR)	*OCT4*	GAGATATGCAAAGCAGAAACCCT/CACACTCGGACCACATCCTT
	NANOG	GCCTGAAGAAAACTATCCATCCTTG/TGTCTTCCTTTTTTGCGACACTC
House-Keeping Gene (RT-PCR and RT-qPCR)	*GAPDH*	GAAGGTGAAGGTCGGAGTC/GAAGATGGTGATGGGATTTC

STR analysis

Genomic DNA was extracted using the Quick-DNA Miniprep kit (Zymo Research). PCR amplification of 21 distinct STRs was carried out using the Powerplex 21 (Promega). PCR products were separated on an ABI Genetic Analyser 3500 xl and analyzed by GeneMapper ID-X version 1.3 (Applied Biosystems).

Karyotyping

iPS cells of line hVH-1 were treated for 3 h at 37 °C with 0.1 µg/ml Karyomax COLCEMID solution (Life Technologies). Cells were trypsinized and resuspended in hESC medium. Metaphases were obtained by adding 75 mM KCl dropwise, incubation at 37 °C for 6 min and by fixation, using cold fresh-made 3:1 methanol:acetic acid. Karyotype analysis was carried out on GTG-banded metaphases.

Immunostaining

iPS cells or EBs were washed with PBS, fixed with cold methanol:acetone (7:10) for 5 min, washed three times with PBS and blocked with 7.5% bovine serum albumin for 30 min at room temperature. Specimens were then incubated with the primary antibodies (Table 2) for 1 h at 37 °C. After washing with PBS, slides were incubated for 1 h at 37 °C with secondary antibodies (Table 2) and DAPI was added. Specimens were washed with PBS and distilled water, embedded in Vectashield mounting medium (Vector) and analyzed with a fluorescence microscope.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.10.010.

References

Lenz, M., Goetzke, R., Schenk, A., Schubert, C., Veeck, J., Hemeda, H., Koschmieder, S., Zenke, M., Schuppert, A., Wagner, W., 2015. Epigenetic biomarker to support classification into pluripotent and non-pluripotent cells. Sci. Rep. 5, 8973.
Takahashi, K., Yamanaka, S., 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676.