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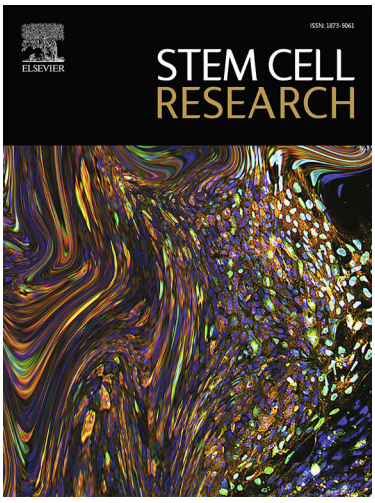

Generation and characterization of two fibroblast-derived Baboon induced pluripotent stem cell lines

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Lab Resource: Animal Multiple Cell Lines - template

Title: Generation and characterization of two fibroblast-derived Baboon induced pluripotent stem cell lines.

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Abstract:
Cross-species comparisons studying primate pluripotent stem cells and their derivatives are crucial to better understand the molecular and cellular mechanisms behind human disease and development. Within this context, Baboons (*Papio anubis*) have emerged as a prominent primate model for such investigations. Herein, we reprogrammed skin fibroblasts of one male individual and generated two induced pluripotent stem cell (iPSC) lines, which exhibit the characteristic ESC-like morphology, demonstrated robust expression of key pluripotency factors and displayed multilineage differentiation potential. Notably, both iPSC lines can be cultured under feeder-free conditions in commercially available medium, enhancing their value for cross-species comparisons.

1. Resource Table:

Unique stem cell lines identifier	MPC-PapAnu-C00001 (100A1) MPC-PapAnu-C00002 (100B1.3)
Alternative name(s) of stem cell lines	100A1 100B1.3
Institution	Faculty of Biology, Ludwig-Maximilians-Universität München
Contact information of distributor	Prof. Dr. Wolfgang Enard: enard@bio.lmu.de Jessica Jocher: jocher@bio.lmu.de
Type of cell lines	iPSCs
Origin	Baboon (*Papio anubis*)
Additional origin info	Sex: male
Cell Source	iPSCs were derived from baboon skin fibroblasts

Clonality	Clonal
Method of reprogramming	Integration-free sendai virus based OSKM vectors (CytoTune-iPSC 2.0 Sendai Reprogramming Kit, Thermo Fisher Scientific) were used for reprogramming
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR analysis for transgene detection (negative)
Associated disease	N/A
Gene/locus	N/A
Date archived/stock date	April 2021
Cell line repository/bank	N/A
Ethical approval	Fibroblasts were isolated during an autopsy in an unrelated project that was approved by the Government of Upper Bavaria, Munich, Germany (reference number 55.2-1-54-2532-184-2014, September 2015).

2. Resource utility

The utilization of two iPSC lines derived from one male Baboon skin sample enables cross-species comparisons, particularly for investigating the molecular and cellular evolution during early primate development. Additionally, these two lines offer the opportunity to evaluate clonal variation within the genetic background of one single Baboon.

3. Resource Details

Comparative analyses of human and non-human primates (NHP) can provide valuable and unique information, allowing to gain insights into evolutionary and developmental mechanisms, as well as bridge the phylogenetic gap between humans and mice (Enard, 2012). The Baboon (*Papio anubis*) is a frequently used model in biomedical research, as well as in behavioral ecology (Fischer et al., 2019). However, availability of these animals is limited and obtaining comparable cells especially during development is practically and ethically challenging for many studies. Therefore, generating induced pluripotent stem cells (iPSCs) from NHPs can aid in establishing renewable sample resources and help to overcome these challenges (Juan et al., 2023).

Here, Baboon skin fibroblasts were reprogrammed to iPSCs using a commercially available Sendai virus kit to introduce the Yamanaka factors OCT3/4, SOX2, KLF4 and C-MYC into the cells. Following transduction, emerging colonies were picked, gradually transitioned to feeder-free culture conditions, and further characterized. The resulting iPSC clones exhibit characteristic ESC-like features, including compact cellular packaging, defined colony borders and a high nuclear / cytoplasm ratio (Figure 1A). To confirm pluripotency of the iPSCs, immunofluorescence (IF) staining was performed, affirming the expression of the pluripotency-associated proteins NANOG, OCT3/4 and SOX2, in addition to the presence of cell surface markers TRA-1-60 and SSEA4 (Figure 1B). Quantitative analysis of the IF staining demonstrated a substantial

based PCR was conducted, confirming the same Baboon-specific ALU element insertions as the parental skin fibroblasts, thereby validating their origin from the same species (Herke et al., 2007) (Supplementary Figure S1A). In addition, single nucleotide polymorphisms (SNPs) were called from bulk RNA-sequencing (bulk RNA-seq) data to profile the genotype of the cell lines. Around 7000 high quality SNPs with high coverage in both clones and parental fibroblasts were retrieved (Supplementary Figure S1B,C). All iPSCs were negative for mycoplasma contamination (Figure 1E) and the absence of Sendai-based reprogramming vectors was proven by PCR (Figure 1F). Karyotype analysis revealed no recurrent numerical or structural abnormalities (Figure 1D). In addition, a detailed high resolution analysis of numerical and structural chromosome integrity was performed by FISH using selected human whole chromosome specific painting probes to further validate one of the cell lines. All chromosomes were stained as expected, with baboon chromosome 10 being stained with two probes based on an evolutionary fusion of human chromosome 20 and 22 homologs (Best et al., 1998) (Supplementary Figure S1D). To assess the differentiation potential of iPSCs, an in vitro differentiation to embryoid bodies (EBs) was performed, and subsequent staining for alpha-fetoprotein (AFP), SOX17, alpha-smooth-muscle actin (SMA), pro-collagen-1 alpha-1 (COL1A1), PAX6 and neuron-specific beta-III tubulin confirmed differentiation into the three germ layers (Figure 1G). In summary, these characteristics suggest the successful reprogramming of Baboon fibroblasts to two feeder-free iPSC lines.

4. Materials and Methods

4.1 Reprogramming of fibroblasts and iPSC maintenance

Fibroblasts were cultured in DMEM/F12 (Fisher Scientific) supplemented with 10 % FBS, 100 U/ml Penicillin and 100 µg/ml Streptomycin (Thermo Fisher Scientific) on 0.2 % Gelatin-coated dishes at 37 °C with 5 % CO_2. For reprogramming, the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) was used at a MOI of 5 following a modified protocol. Briefly, a suspension infection with the virus mix was conducted for 1 h at 37 °C, followed by seeding on feeder cell (mitomycin-C treated mouse embryonic fibroblasts) -coated wells. The culture medium was switched to mTesR1™ (STEMCELL Technologies) on day 5. Emerging colonies were manually picked on feeder cells in StemFit® Basic02 (Ajinomoto) supplemented with 100 ng/mL bFGF (Peprotech) and PenStrep. To generate feeder-free iPSCs, cells were split using 0.5 mM EDTA on 1 % Geltrex™ (Thermo Fisher Scientific) -coated wells in feeder-conditioned StemFit. The ratio of normal to feeder-conditioned StemFit was increased by 25 % after every second passage, until iPSCs adapted to feeder-free culture conditions. Every 5-7 days, 0.5 mM EDTA was used for routine passaging at a ratio of 1:10 - 1:40, and the medium was exchanged every other day.

4.2 Immunocytochemistry

Cells at passage 20-25 were fixed with 4 % PFA for 15 min, permeabilized with 0.3 % Triton X-100 and blocked with 5 % FBS for 30 min. Cells were incubated with primary antibodies (Table 2) diluted in staining buffer (PBS containing 1 % BSA and 0.3 % Triton X-100) at 4 °C overnight. Following, cells were washed with PBS and incubated with secondary antibodies (Table 2) diluted in staining buffer for 1 h at RT. Nuclei were counterstained with 1 µg/mL DAPI. The percentage of positively-stained cells was quantified with the ImageJ software using the Cell Counter plugin. Between 2,492 and 3,725 cells were counted for each marker.

4.3 Embryoid body formation

One 6-well of iPSCs at passage 20-25 was dissociated to clumps and cultured in sterile bacterial dishes containing StemFit w/o bFGF at 37 °C with 5 % CO_2. Medium was changed every second day during the first 8 days of floating culture. On day 8, EBs were seeded into 6-wells coated with 0.2 % Gelatin allowing outgrowth of the EBs. On day 16, differentiated cells were stained using specific antibodies for mesoderm, endoderm, and ectoderm (Table 2).

4.4 Karyotyping

iPSCs at 80 % confluency (passage 15-20) were incubated with 0.1 mg/mL Colcemid (Gibco) for 15 h, dissociated using Accumax™ (Sigma Aldrich) and treated with hypotonic Na-Citrate / NaCl for 35 min at 37 °C. Subsequently, cells were fixed with methanol / acetic acid glacial (3:1) for 20 min at -20 °C and washed twice with methanol/acetic as stated above. A standard protocol for the preparation of differentially stained mitotic

was performed using human chromosome specific painting probes. In brief, mixtures of fluor conjugated paint probes were denatured at 75°C for 5 min, added to the metaphase slide, covered with a cover slip and sealed with rubber cement. The slide was denatured at 75°C for 2 min in a Hybrite (VYSIS, US) hybridization station and hybridized at 37°C overnight, followed by a 2 min post-hybridization wash in 0,1xSSC buffer at 60°C. Final slides were mounted in Vectashield embedding medium containing DAPI (Vector Laboratories, UK) and analyzed using an Axioplan 2 Imaging microscope (Zeiss, Germany).

4.5 Mycoplasma testing

The medium of one confluent 6-well with iPSCs at passage 19-22 was collected, pelleted, and resuspended in 100 µL PBS. After an incubation for 5 min at 95 °C, 1 µL was used for a screening PCR with specific primers for the Mycoplasma 16S rRNA (Table 2). A sample that had previously tested positive was used as an internal control.

4.6 Genotyping PCR

Total gDNA was isolated using the DirectPCR Lysis Reagent (VWR) supplemented with 20 mg/mL Proteinase K (Life Technologies), and a PCR (36 cycles) was conducted with primers for the primate-specific *Alu* SINE (Table 2).

4.7 SeV detection

Total RNA was isolated from one 6-well of iPSCs (passage 15-20) using the Direct-zol RNA Microprep Kit (Zymo Research) and cDNA was synthesized using the Maxima H Minus Reverse Transcriptase (Thermo Fisher Scientific). 50 ng cDNA were used to perform a PCR (36 cycles) with specific primers for SeV and GAPDH as housekeeping gene (Table 2).

4.8 Bulk RNA-sequencing and variant calling

iPSCs and parental fibroblasts were dissociated using Accumax, sampled in three biological replicates each and bulk RNA-seq libraries were generated using the Prime-seq workflow (https://www.protocols.io/view/prime-seq-81wgb1pw3vpk/v2). Bulk RNA-seq data of iPSCs and parental fibroblasts were used to call SNPs against the reference genome papAnu4 using GATK (Genome Analysis Tool Kit). High quality, biallelic SNPs were retained by joint genotyping of data from both iPSC lines and fibroblasts followed by quality filtering of the variants for high coverage (DP>99) and quality by depth (QD>2).

Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reporter in this paper.

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Classification	Test	Result	Data
Morphology	Photography Bright field	Normal colony morphology	Figure 1A
Phenotype	Qualitative analysis by immunocytochemistry	iPSCs were positively stained for OCT3/4, NANOG, SOX2, TRA-1-60 and SSEA4	Figure 1B
	Quantitative analysis by immunocytochemistry counting	% total cells positive for pluripotency markers (mean ± SD): 100A1 OCT3/4: 97.9% ± 2.9% (3,071 cells counted) NANOG: 98.2% ± 0.3% (3,070 cells counted) SOX2: 99.1% ± 0.6% (3,362 cell counted) 100B1.3 OCT3/4: 97.8% ± 0.6% (3,725 cells counted) NANOG: 98.8% ± 1.1% (2,845 cells counted) SOX2: 98.9% ± 0.3% (2,492 cells counted)	Figure 1C
Genotype	Karyotype (G-banding) and resolution	2x inconspicuous male karyotype, 42,XY No recurrent numeric or structural aberrations, after G-banding	Figure 1D and Supplementary Figure S1D

		cell line with up to approximately 400 bphs (bands per haploid set)	
Identity	SINE-based genotyping PCR	DNA profiling performed, matched between iPSCs and parental fibroblasts	Supplementary Figure S1A
	SNP analysis	Variant calling performed resulting in 7000 high quality SNPs	Submitted in archive with journal Summary: Supplementary Figure S1B,C
Mutation analysis (IF APPLICABLE)	N/A		
	N/A		
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: negative	Figure 1E
	Sendai virus	PCR analysis for Sendai virus presence: negative	Figure 1F
Differentiation potential	Embryoid body formation	iPSCs are capable of differentiating into the three germ layers. Mesoderm: Smooth muscle actin (SMA) and COL1A1 Endoderm: α-feto protein (AFP) and SOX17 Ectoderm: β-III Tubulin and PAX6	Figure 1G
Donor screening (OPTIONAL)	N/A		
Genotype additional info (OPTIONAL)	N/A		
	N/A		

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Rabbit anti-OCT4 Mouse anti-SOX2 Rabbit anti-NANOG Mouse anti-SSEA4 Mouse anti-TRA-1-60	1:400 1:400 1:400 1:500 1:100	Cell Signaling Technology, Cat# 2750S Cell Signaling Technology, Cat# 4900S Cell Signaling Technology, Cat# 4903S NEB, Cat# 4755S Stem Cell Technologies, Cat# 60064	RRID: AB_823583 RRID: AB_10560516 RRID: AB_10559205 RRID: AB_1264259 RRID: AB_2686905
Differentiation Markers	Mouse anti- α-Smooth Muscle Actin Sheep anti-COL1A1 Mouse anti- Neuron-specific beta-III Tubulin Rabbit anti-PAX6 Mouse anti-alpha Fetoprotein Rabbit anti-SOX17	1:100 1:200 1:100 1:100 1:100 1:500	R&D Systems, Cat# MAB1420 R&D Systems, Cat# AF6220 R&D Systems, Cat# MAB1195 Thermo Fisher Scientific, Cat# 42-6600 R&D Systems, Cat# MAB1368 Bio-Techne, Cat# NBP2-24568	RRID: AB_262054 RRID: AB_10891543 RRID: AB_357520 RRID: AB_2533534 RRID: AB_357658 RRID: AB_3075468
Secondary Antibodies	Alexa Fluor 488 donkey anti-mouse IgG (H+L) Alexa Fluor 594 donkey anti-rabbit IgG (H+L) Alexa Fluor 488 donkey anti-sheep (H+L)	1:500 1:500 1:500	Thermo Fisher Scientific, Cat# A-21202 Thermo Fisher Scientific, Cat# A-21207 Thermo Fisher Scientific, Cat# A-11015	RRID: AB_141607 RRID: AB_141637 RRID: AB_2534082

	Primers		
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Reprogramming factor clearance	Sendai Virus	180 bp	GGATCACTAGGTGATATCGAGC / ACCAGACAAGAGTTTAAGAGATATGTATC
	GAPDH (housekeeping gene)	450 bp	ACCACAGTCCATGCCATCAC / TCCACCACCCTGTTGCTGTA
Mycoplasma testing	Mycoplasma 16S	270 bp	TGCACCATCTGTCACTCTGTTAACCTC / GGGAGCAAACAGGATTAGATACCCT
Genotyping PCR	*Alu* (primate-specific SINE)	666 bp	TCTAAGGCAGCCATTGAGTG / CCAGGTTTGCCTCTGACTCC

Declaration of interests

☐ 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.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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