# Generation and characterization of two Vervet monkey induced pluripotent stem cell lines derived from fibroblasts

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A B S T R A C T

Cross-species comparisons using pluripotent stem cells from primates are crucial to better understand human biology, disease, and evolution. The Vervet monkey (*Chlorocebus aethiops sabaeus*) serves as an important primate model for such studies, and therefore we reprogrammed skin fibroblasts derived from a male and a female individual, resulting in two induced pluripotent stem cell lines (iPSCs). These iPSCs display the characteristic ESC-like colony morphology, express key pluripotency markers, and possess the ability to differentiate into cells representing all three germ layers. Importantly, both generated cell lines can be maintained in feeder-free culture conditions using commercially available medium.

## 1. Resource Table

| | |
|---|---|
| Unique stem cell lines identifier | MPC-ChlSab-C00001 (76A3) <br> MPC-ChlSab-C00002 (80B1) |
| Alternative name(s) of stem cell lines | 76A3 <br> 80B1 |
| Institution | Faculty of Biology, Ludwig-Maximilians-Universität München |
| Contact information of distributor | Prof. Dr. Wolfgang Enard: enard@bio.lmu.de <br> Jessica Jocher: jocher@bio.lmu.de |
| Type of cell lines | iPSCs |
| Origin | Vervet monkey (*Chlorocebus aethiops sabaeus*) |
| Additional origin info | Sex: female (76A3) and male (80B1) |
| Cell Source | iPSCs were derived from fibroblasts established from skin biopsies collected at the Vervet Research Colony and kindly provided by the UCLA. |
| 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 | PCR analysis for transgene detection (negative) |
| Associated disease | N/A |
| Gene/locus | N/A |
| Date archived/stock date | November 2020 |
| Cell line repository/bank | N/A |

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*(continued)*

| | |
|---|---|
| Unique stem cell lines identifier | MPC-ChlSab-C00001 (76A3) <br> MPC-ChlSab-C00002 (80B1) |
| Ethical approval | Fibroblast sample collection was approved by the UCLA and VA Institutional Animal Care and Use Committees. Fibroblast import was approved by CITES (permit number: 18US12381D/9) |

## 2. Resource utility

The two iPSC lines derived from fibroblasts of a male and a female Vervet monkey, provide a valuable resource for cross-species comparisons, e.g., enabling investigations of molecular and cellular processes during early primate development. Furthermore, the two cell lines can help to assess intra-species variation within the Vervet genetic background and allow to investigate sex-related genetic factors.

## 3. Resource details

To gain insights into evolutionary and developmental mechanisms, as well as to bridge the phylogenetic gap between humans and mice, comparative analyses of human and non-human primates (NHP) can provide valuable and unique information (Enard, 2012). Among NHPs, the Vervet monkey (also called African green monkey) is, next to Rhesus macaques, the most commonly investigated NHP in biomedical

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research. One reason for this is that they are a natural host to the Simian Immunodeficiency Virus (SIV) and can be used to study adaptations to lentiviral infections. In contrast to African Vervets, the Caribbean Vervets (*Chlorocebus aethiops sabaeus*) also used here, are SIV free which allows safe and controlled studies on SIV infections and has made them a valuable source for NHP genetics (Jasinska, 2013). Generating induced pluripotent stem cells (iPSCs) from Vervets links this important NHP model to stem cell biology and comparative primate genomics (Juan et al., 2023).

Here, Vervet monkey skin fibroblasts were reprogrammed to iPSCs using a commercially available Sendai virus kit to introduce OCT3/4, SOX2, KLF4 and C-MYC into the cells. Emerging colonies were picked, gradually transferred to feeder-free culture conditions, and further characterized (Table 1). The two derived clones exhibit the typical ESC-like morphology with tight cellular packaging, prominent nucleoli, and defined colony borders (Fig. 1A). Immunofluorescence (IF) staining confirmed the expression of the pluripotency associated proteins OCT3/4 and SOX2, as well as the presence of the cell surface markers TRA-1-60, SSEA4 and EpCAM (Fig. 1B). Quantification of the IF staining revealed that > 95 % of cells are expressing both pluripotency markers OCT3/4 and SOX2 (Fig. 1C). A primate-specific SINE based PCR confirmed the presence of Vervet-specific *ALU* element insertions in the iPSCs as well as the parental skin fibroblasts, confirming their derivation from the same primate species (Herke et al., 2007) (Supplementary Fig. 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 5100 and 4300 high quality SNPs with high coverage were retrieved for the cell lines 76A3 and 80B1, respectively (Supplementary Fig. S1B,C). All iPSCs were negative for mycoplasma contamination (Fig. 1E) and negative for Sendai-based reprogramming vectors (Fig. 1F). Furthermore, karyotype analysis was performed,

revealing no recurrent numerical or structural aberrations in the two iPSC lines (Fig. 1D). In addition, a detailed high resolution validation of numerical and structural chromosome integrity was performed by FISH using human chromosome specific painting probes on the 76A3 line (Supplementary Fig. S1D). To assess their differentiation capacity, an in vitro differentiation to embryoid bodies (EBs) was conducted and stainings for alpha-fetoprotein (AFP) and SOX17 were used to verify endodermal differentiation, alpha-smooth muscle actin (SMA) and pro-collagen-1 alpha-1 (COL1A1) for mesodermal differentiation, and neuron-specific beta-III tubulin and PAX6 for ectodermal differentiation (Fig. 1G). In summary, these characteristics indicate the successful establishment of two feeder-free iPSC lines from Vervet monkey (*Chlorocebus aethiops sabaeus*).

## 4. Materials and methods

### 4.1. Reprogramming of fibroblasts and iPSC maintenance

Fibroblasts were cultured on 0.2 % Gelatin coated dishes in DMEM/F12 (Fisher Scientific) supplemented with 10 % FBS and 100 U/mL Penicillin and 100 µg/mL Streptomycin (Thermo Fisher Scientific) at 37 °C and 5 % $CO_2$. The CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) was used for reprogramming following a modified protocol. Briefly, fibroblasts were incubated in suspension with the virus mix at a MOI of 5 for 1 h at 37 °C and then seeded onto a feeder layer. On day 5, medium was switched to mTesR1™ (STEMCELL Technologies). Appearing colonies were manually picked onto feeder cells and cultured in StemFit® Basic02 (Ajinomoto) supplemented with 100 ng/mL bFGF (Peprotech) and 100 U/mL Penicillin and 100 µg/mL Streptomycin. For a feeder free culture, cells were passaged using 0.5 mM EDTA on 1 % Geltrex™ (Thermo Fisher Scientific) coated wells in

**Table 1**
Characterization and validation.

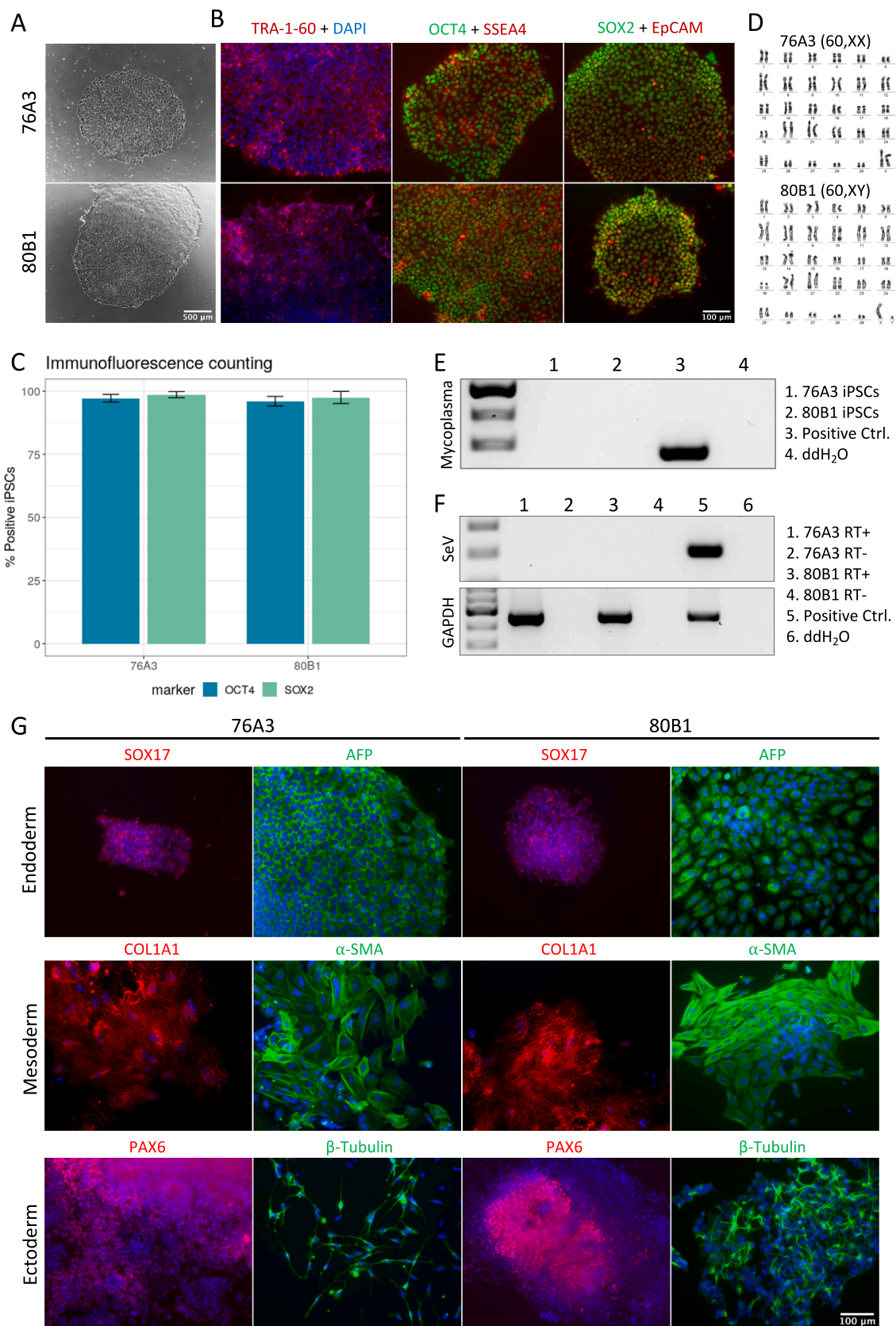| Classification | Test | Result | Data |
|---|---|---|---|
| **Morphology** | Photography phase contrast | Normal colony morphology | Fig. 1A |
| | | | Scale bar represents 500 µm |
| **Phenotype** | Qualitative analysis by immunocytochemistry | iPSCs were positively stained for OCT4, SOX2, TRA-1–60, SSEA4 and EpCAM | Fig. 1B |
| | | | Scale bar represents 100 µm |
| | Quantitative analysis by immunocytochemistry counting | % total cells positive for pluripotency markers (mean ± SD): | Fig. 1C |
| | | 76A3 | |
| | | OCT4: 97.2 % ± 1.5 % | |
| | | (2,436 cells counted) | |
| | | SOX2: 98.6 % ± 1.2 % | |
| | | (1,693 cells counted) | |
| | | 80B1 | |
| | | OCT4: 96 % ± 1.9 % | |
| | | (2,128 cells counted) | |
| | | SOX2: 97.5 % ± 2.4 % | |
| | | (1,508 cells counted) | |
| **Genotype** | Karyotype (G-banding and FISH) | 76A3: inconspicuous female karyotype, 60,XX | Fig. 1D and Supplementary |
| | | 80B1: inconspicuous male karyotype, 60,XY | Fig. S1D |
| **Identity** | SINE-based genotyping PCR | DNA profiling performed, matched between iPSCs and parental fibroblasts | Supplementary Fig. S1A |
| | SNP analysis | Variant calling performed resulting in 5100 (76A3) and 4300 (80B1) high quality SNPs | Submitted in archive with journal |
| | | | Summary: Supplementary Fig. S1B,C |
| **Mutation analysis (IF APPLICABLE)** | N/A | | |
| | N/A | | |
| **Microbiology and virology** | Mycoplasma | Mycoplasma testing by PCR: negative | Fig. 1E |
| | Sendai virus | PCR analysis for Sendai virus presence: negative | Fig. 1F |
| **Differentiation potential** | Embryoid body formation | iPSCs are capable of differentiating into the three germ layers. | Fig. 1G |
| | | Mesoderm: Smooth muscle actin (SMA) and COL1A1 | Scale bar represents 100 µm |
| | | Endoderm: α-feto protein (AFP) and SOX17 | |
| | | Ectoderm: β-III tubulin and PAX6 | |
| **Donor screening (OPTIONAL)** | N/A | | |
| **Genotype additional info (OPTIONAL)** | N/A | | |

**Fig. 1.** Characterization of two Vervet monkey iPSC lines. (A) Phase contrast microscopy images of iPSC colonies. Scale bar represents 500 μm. (B) Immunofluorescence staining for pluripotency markers. Scale bar represents 100 μm. (C) Immunofluorescence couting results for OCT4 and SOX2. (D) Karyotype analysis. (E) Mycoplasma test. (F) PCR for Sendai-based reprogramming vectors. (G) Immunofluorescence staining for germ layer-specific markers. Scale bar represents 100 μm.

feeder-conditioned StemFit. The ratio of feeder-conditioned to normal StemFit was reduced by 25 % every other passage until iPSCs could be cultured under feeder-free conditions. iPSCs were passaged using 0.5 mM EDTA at a ratio of 1:10–1:40 every 5 days, changing the medium every other day.

### 4.2. Immunocytochemistry

Attached cells (passage 19–25) were fixed with 4 % PFA for 15 min at RT, permeabilized with 0.3 % Triton X-100 (Sigma Aldrich) and blocked for 30 min with 5 % FBS. Cells were incubated with primary antibodies (Table 2) diluted in staining buffer (PBS containing 1 % BSA and 0.3 % Triton X-100) overnight at 4 °C. Thereafter, cells were washed with PBS and incubated with secondary antibodies (Table 2) diluted in staining buffer for 1 h at RT. Nuclei were counterstained using DAPI at a concentration of 1 μg/mL. To obtain proportions of positively-stained cells, images of the fluorescence staining and the corresponding DAPI staining were counted using the Cell Counted plugin in ImageJ. Between 1,508 and 2,436 cells were counted for each marker and percentages were calculated based on the number of positively-stained cells divided by the number of DAPI stained nuclei. The standard deviation was calculated based on the difference between the counted images.

### 4.3. Embryoid body formation

One 6-well of iPSCs at passage 19–25 was dissociated into clumps, transferred to a sterile bacterial dish containing StemFit w/o bFGF and cultured at 37 °C with 5 % $CO_2$. During the first 8 days of floating culture, the medium was changed every other day. Then, EBs were seeded into 0.2 % Gelatin-coated 6-wells for 8 days of adherent culture. On day 16, the cells were stained with antibodies for mesoderm, endoderm, and ectoderm (Table 2) as stated above.

### 4.4. Karyotyping

Cells (passage 15–20) at 80 % confluency were incubated with 0.1 mg/mL Colcemid (Gibco) for 14 h and harvested using Accumax™ (Sigma Aldrich). Cells were treated with hypotonic Na-Citrate / NaCl for 35 min at 37 °C and then fixed with methanol / acetic acid glacial (3:1) for 20 min at -20 °C. After pelleting, cells were washed twice with methanol/acetic acid before conducting standard protocols for chromosome preparation, G-banding, and fluorescence in situ hybridization (FISH) using human chromosome specific painting probes.

### 4.5. Mycoplasma testing

The medium of a confluent 6-well with iPSCs at passage 15–25 was collected, pelleted, and resuspended in 100 μL PBS. After incubation at 95 °C for 5 min, 1 μL was used for a screening PCR with specific primers for the Mycoplasma 16S rRNA (Table 2).

### 4.6. Genotyping PCR

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

### 4.7. SeV detection

Total RNA was isolated from iPSCs at passage 15–18 using the Direct-zol RNA Microprep Kit (Zymo Research) according to the manufacturer's instructions. After reverse transcription using the Maxima H Minus Reverse Transcriptase (Thermo Fisher Scientific), the cDNA was used to perform a PCR (36 cycles) with specific primers for SeV. The housekeeping gene GAPDH was used as positive control (Table 2).

### 4.8. Bulk RNA-sequencing and variant calling

iPSCs of both individuals 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 were used to call SNPs against the reference genome chlSab2 using GATK (Genome Analysis Tool Kit). High quality, biallelic SNPs were retained by quality filtering of the variants for high coverage (DP > 49) and quality by depth (QD > 2).

**Table 2**
Reagents details.

| | Antibodies used for immunocytochemistry/flow-cytometry | | | |
| --- | --- | --- | --- | --- |
| | Antibody | Dilution | Company Cat # | RRID |
| Pluripotency Markers | Rabbit anti-OCT4 | 1:400 | Cell Signaling Technology, Cat# 2750S | RRID: AB_823583 |
| | Mouse anti-SOX2 | 1:400 | Cell Signaling Technology, Cat# 4900S | RRID: AB_10560516 |
| | Mouse anti-SSEA4 | 1:500 | NEB, Cat# 4755S | RRID: AB_1264259 |
| | Rabbit anti-EpCAM | 1:500 | Thermo Fisher Scientific, Cat# 710524 | RRID: AB_2532731 |
| | Mouse anti-TRA-1–60 | 1:100 | Stem Cell Technologies, Cat# 60064 | RRID: AB_2686905 |
| Differentiation Markers | Mouse anti-α-Smooth Muscle Actin | 1:100 | R&D Systems, Cat# MAB1420 | RRID: AB_262054 |
| | Sheep anti-COL1A1 | 1:200 | R&D Systems, Cat# AF6220 | RRID: AB_10891543 |
| | Mouse anti- Neuron-specific beta-III Tubulin | 1:100 | R&D Systems, Cat# MAB1195 | RRID: AB_357520 |
| | Rabbit anti-PAX6 | 1:100 | Thermo Fisher Scientific, Cat# 42–6600 | RRID: AB_2533534 |
| | Mouse anti-alpha Fetoprotein | 1:100 | R&D Systems, Cat# MAB1368 | RRID: AB_357658 |
| | Rabbit anti-SOX17 | 1:500 | Bio-Techne, Cat# NBP2-24568 | RRID: AB_3075468 |
| Secondary Antibodies | Alexa Fluor 488 donkey anti-mouse IgG (H + L) | 1:500 | Thermo Fisher Scientific, Cat# A-21202 | RRID: AB_141607 |
| | Alexa Fluor 594 donkey anti-rabbit IgG (H + L) | 1:500 | Thermo Fisher Scientific, Cat# A-21207 | RRID: AB_141637 |
| | Alexa Fluor 488 donkey anti-sheep (H + L) | 1:500 | Thermo Fisher Scientific, Cat# A-11015 | 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) | 680 bp | CACAAAATACTAAAGGACTGTTAAAGG / CACAAAATACTAAAGGACTGTTAAAGG | |

*CRediT authorship contribution statement*

**Jessica Jocher:** Conceptualizing, Investigation, Methodology, Visualization, Writing – original draft, Writing - review & editing. **Fiona C. Edenhofer:** Investigation, Visualization. **Stefan Müller:** Investigation, Methodology, Visualization. **Philipp Janssen:** Data curation, Formal analysis, Visualization, Writing – review & editing. **Eva Briem:** Investigation. **Johanna Geuder:** Methodology. **Wolfgang Enard:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

**Declaration of competing interest**

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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.2024.103315.

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