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## Generation of three human induced pluripotent stem cell sublines (UCLAi004-A, UCLAi004-B, and UCLAi004-C) for reproductive science research

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### Abstract

Three induced pluripotent stem cell sublines (hiPSCs) were generated from human dermal human dermal fibroblasts (HDFs) derived from a human skin punch biopsy. The biopsy was donated from a woman with known infertility due to ovarian failure. The hiPSC sublines were created using Sendai virus vectors and were positive for markers of self-renewal including OCT4, NANOG, TRA-1-81 and SSEA-4. Pluripotency was verified using PluriTest analysis and in vitro differentiation using Taqman Real-Time PCR assays for somatic lineage markers. This participant's monozygotic twin sister also donated a skin-punch biopsy, whose resulting hiPSC lines were published previously as a resource.

### 1. Resource table

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Unique stem cell lines identifier	UCLAi004-A
	UCLAi004-B
	UCLAi004-C
Alternative names of stem cell lines	MZT06-B
	MZT06-C
	MZT06-D

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#### Declaration of Competing Interest

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.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2021.102446>.

Institution	UCLA
Contact information of distributor	Dr. Amander Clark
Type of cell lines	hiPSC
Origin	Human
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai, hOct3/4, hSox2, hKlf4, and hc-Myc
Multiline rationale	Isogenic clones
Gene modification	No
Type of modification	N/A
Associated disease	Ovarian Failure (also known as Primary Ovarian Insufficiency)
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
	N/A
Inducible/constitutive system	
Date archived/stock date	04/04/2019
Cell line repository/bank	<a href="https://hpscereg.eu/cell-line/UCLAi004-B">https://hpscereg.eu/cell-line/UCLAi004-B</a> <a href="https://hpscereg.eu/cell-line/UCLAi004-A">https://hpscereg.eu/cell-line/UCLAi004-A</a> <a href="https://hpscereg.eu/cell-line/UCLAi004-C">https://hpscereg.eu/cell-line/UCLAi004-C</a>
Ethical approval	UCLA Office of the Human Resource Protection Program- IRB#16-001176-CR and UCLA Embryonic Stem Cell Research Oversight Committee (ESCRO#2016-003)

## 2. Resource Utility

These hiPSC sublines are a valuable resource due to their dual consent for general biomedical research, and for the specific purpose of studying human fertility, infertility and differentiation of germline cells. Additionally, these sublines are a useful resource for those studying reproduction because they were derived from a woman diagnosed with ovarian failure.

## 3. Resource details

We present three hiPSC sublines derived from a woman with diagnosed infertility due to ovarian failure. The hiPSC sublines described here are to be used together with the hiPSC sublines derived from her monozygotic twin sister (MZT04) who had normal fertility. A description of the MZT04 hiPSC sublines can be found elsewhere (Pandolfi et al., 2019). One of the challenges in hiPSC studies is that genetic variability exists between unrelated individuals. The advantage of working with monozygotic twins discordant for disease is that the unaffected twin can serve as a case-control for her sister.

Furthermore, these hiPSC lines are unique in that they have been consented for biomedical research with specific consideration to germline and fertility research. Strong opinions generated by some groups for germline research and the creation of gametes using hiPSCs

illustrates the benefit of informing donors that their tissues will be used to create germ cells and gametes. In addition, receiving broader consent from research participants allows for future unanticipated research, and facilitates the sharing of generated hiPSC lines and sublines. Thus, we created three hiPSC sublines: MZT06-B (UCLAi004-A), MZT06-C (UCLAi004B), and MZT06-D (UCLAi004-C), to be ethically and responsibly used in fertility and infertility research. These sublines are not consented for the purpose of human reproduction.

We generated three integration-free hiPSC sublines from human dermal fibroblasts (HDFs) (Table 1). Fibroblasts were derived from a skin punch biopsy from a 55-year-old woman. These fibroblasts (MZT06) were reprogrammed to hiPSCs using the non-integrating recombinant Sendai virus containing reprogramming factors OCT3/4, SOX2, KLF4 and C-MYC4. Twenty-seven days after the transduction, individual colonies were manually picked onto mouse embryonic fibroblast feeder cells to create the sublines. We selected three sublines named MZT06-B, MZT06-C, and MZT06-D and characterized them for self-renewal and pluripotency (Fig. 1 and Table 2). All hiPSC sublines exhibited typical pluripotent stem cell morphology (Fig. 1A) and markers of self-renewal, as confirmed through immunofluorescence staining for NANOG, OCT4, TRA-1-81 and SSEA-4 (Fig. 1B). The reprogrammed cells and the initial fibroblasts displayed a normal 46, XX karyotype (Fig. 1C). To evaluate pluripotency, MZT06-B, MZT06-C, MZT06-D were assessed with a PluriTest analysis (Fig. 1D) (Müller et al., 2011). Furthermore, the ability of each hiPSC subline to differentiate into the three germ layers was assessed using Taqman Real-Time PCR assays for markers of ectoderm, mesoderm, and endoderm (Fig. 1E). Averages of each hiPSCs subline were normalized to GAPDH expression for the six target genes. hESC line UCLA2 (Diaz Perez et al., 2012) was used to as a control, and delta delta CT was calculated relative this line. We also confirmed that MZT06-B, MZT06-C, and MZT06-D were negative for mycoplasma through routine mycoplasma testing (Supplemental Fig. 1A). The three hiPSC sublines did not express the exogenous reprogramming factors after continued culture (Fig. 1F) (expected band size SeV: 181 bp, c-MYC: 532 bp, klf: 410 bp, KOS: 501 bp). To confirm that the hiPSC sublines were of the same genetic background as the donated fibroblasts, short tandem repeat (STR) analysis was conducted demonstrating that each of the three hiPSC lines were identical to the original HDFs (MZT06), and to the hiPSC lines derived from the donating woman's twin sister (MZT04) (Pandolfi et al., 2019). This resource is a complement to two previous publications involving the derivation of three sublines from an unrelated woman who was fertile (Pandolfi et al., 2021a) and six sublines from a pair of monozygotic twins discordant for ovarian failure (Pandolfi et al., 2021b).

## 4. Materials and methods

### 4.1. Maintenance of hiPSC lines

Undifferentiated hiPSC subline cells were cultured on a feeder layer of mitomycin C-treated murine embryonic fibroblasts (MEFs) in hESC media (DMEM/F-12) (Life Technologies), 20% KSR (Life Technologies), 10 ng/mL bFGF (R&D Systems), 1% nonessential amino acids (Life Technologies), 2 mM L-glutamine (Life Technologies), Primocin™ (Invivogen),

and 0.1 mM  $\beta$ -mercaptoethanol (Sigma). Media was changed daily and colonies were passaged as clumps with collagenase (ThermoFisher, 17104019) 1:4–1:6 every 7 days without rock inhibitor. Cells were cultured in an incubator at 37°, 5.0% CO<sub>2</sub>.

#### 4.2. Fibroblast derivation

A 1 mm skin punch biopsy was dissected and then digested in Collagenase IV (Life Technologies) for 1 h at 37 °C, 5.0% CO<sub>2</sub>. The digested pieces were then plated down on 0.1% gelatin (Sigma) coated (Millipore) plates in human fibroblast media, 15% Fetal bovine serum (GE Healthcare), 1% Non-Essential Amino Acids (Invitrogen), 1% Glutamax, (Gibco™), 1% Penicillin-Streptomycin-Glutamine (Gibco), and Primocin (Invivogen), at 37°, 5.0% CO<sub>2</sub>. Outgrowths of fibroblasts were monitored for two weeks and the media was changed every three days. Fibroblasts were passaged using 0.05% Trypsin (Gibco) and re-plated, the derived cells were termed MZT06.

#### 4.3. Reprogramming the fibroblasts

Fibroblasts were thawed and cultivated in human fibroblast medium. When ~ 80% confluent, the MZT06 cells were transfected with Sendai virus (SeV) based non-integration CytoTune™ iPS Reprogramming Kit (Life Technologies) according to manufacturer's instructions. Colonies began to appear after 11 days and were picked after three weeks. Three colonies were manually picked and expanded onto mouse embryonic fibroblast feeder cells in hiPSC media (DMEM/F-12 (Life Technologies), 20% KSR (Life Technologies), 10 ng/mL bFGF (R & D Systems), 1% nonessential amino acids (Life Technologies), 1% Penicillin-Streptomycin-Glutamine (Gibco), Primocin™ (Invivogen), and 0.1 mM  $\beta$ -mercaptoethanol (Sigma)).

#### 4.4. PluriTest

Cryopreserved pelleted cells were sent to Life Sciences Solutions. Transcriptional profiles of the hiPSC lines were compared to an extensive reference set. The Pluripotency Score is an indication of how strongly a model-based pluripotency signature is expressed in the samples analyzed. The Novelty Score indicates the general model fit for a given sample (Müller et al., 2011). Testing was conducted on MZT06-B (p12), MZT06-C (p12), MZT06-D (P12).

#### 4.5. Taqman real-time PCR assays to evaluate differentiation

At Day 7 of self-renewal, the hiPSC sublines were trypsinized (0.05% trypsin, Life Technologies) and the MEFs were depleted by plating the cell suspension in tissue culture dishes, two times, for 5 min each. The resulting cell suspensions were pelleted and resuspended in media containing (GMEM) (Life Technologies), 15% KSR (Life Technologies), 0.1 mM nonessential amino acids (Life Technologies), penicillin/streptomycin/L-glutamine (Life Technologies), Primocin™ (Invivogen), 0.1 mM  $\beta$ -mercaptoethanol (Sigma), sodium pyruvate (Life Technologies), activin A (PeproTech), CHIR99021 (Stemgent), Y-27632 (Stemgent), filtered through a 40  $\mu$ m cell strainer (Falcon). The cell suspension was plated at a density of  $2.0 \times 10^5$  cells per well of a human plasma fibronectin (Invitrogen)-coated 12-well plate. After 24 h of incubation at 37 °C with 5.0% CO<sub>2</sub> the cells, now called incipient mesoderm-like cells (iMELCs) were trypsinized

(0.05% trypsin) and resuspended in media containing (GMEM) (Life Technologies), 15% KSR (Life Technologies), 0.1 mM nonessential amino acids (Life Technologies), penicillin/streptomycin/L-glutamine (Life Technologies), Primocin™ (Invivogen), 0.1 mM β-mercaptoethanol (Sigma), sodium pyruvate (Life Technologies), 10 ng/mL human LIF (EMD Millipore), 200 ng/mL BMP4 (R&D Systems), 50 ng/mL EGF (Fisher Scientific), 10 μM Y-27632 (Stemgent). The cells were plated at a density of  $3.0 \times 10^3$  cells per well of a low adherence spheroid forming 96-well plate (Corning) and cultured for four additional days. Testing was conducted on MZT06-B (p30), MZT06-C (p31), MZT06-D (P33).

At day 4 of differentiation in low adherence plates, the differentiated cells were harvested in RLT buffer and RNA was extracted using qiagen RNAeasy microkit (qiagen, 74004). RNA was converted to cDNA using Superscript II reverse transcriptase (Thermofisher, 18064014) and random hexamers (Thermofisher, N8080127). Taqman probes (Table 3) were used to identify two markers from each germ layer including; ectoderm: *OTX2* (72 bp product) (Thermofisher, Hs00222238\_m1), *NESTIN* (58 bp product) (Thermofisher, Hs04187831\_g1); endoderm: *SOX17* (149 bp product) (Thermofisher, Hs00751752\_s1), *FOXA2* (144 bp product) (Thermofisher, Hs00610080\_m1); mesoderm: *TBXT* (132 bp product) (Thermofisher, Hs00610080\_m1), *EOMES* (81 bp product) (Thermofisher, Hs00610080\_m1). The Taqman assays were run with the following conditions, 50° C for 2 min, 95° C for 10 min, and 50 cycles of 95° C for 15 sec followed by 60° C for 1 min. Two technical replicates were used to examine gene expression in each of the three MZT06 hiPSC sublines. qPCR was performed using CFX Connect Real-Time PCR Detection System. Averages of each hiPSCs subline were normalized to GAPDH expression for the six target genes. hESC line UCLA2 (Diaz Perez et al., 2012) was used to as a control, and delta delta CT was calculated relative this line.

#### 4.6. Karyotyping and STR analysis

The three hiPSC sublines, and the HDF primary culture that they were derived from, were karyotyped using metaphase spreads and G-banding by Cell Line Genetics (Madison, WI). Karyotyping on hiPSCs was conducted at P1 for MZT06 HDFs primary cell cultures, P4 for MZT06-B, P4 for MZT06-C, and P4 for MZT06-D. Twenty metaphase spreads were counted for each Karyotype analysis. Cell Line Genetics also performed STR analysis on the three hiPSC sublines and one HDF primary culture using the PowerPlex 16 System (cat# DC6531, Promega).

#### 4.7. Immunofluorescence staining

Immunofluorescence staining was performed by fixing the hiPSCs in 4%PFS for 15 min at room temperature, and then permeabilizing the cells with PBS plus 0.5% Triton™ X-100 (Sigma). The hiPSCs were then blocked in 10% donkey serum (Jackson ImmunoResearch) for 30 min at room temperature. Cells were incubated overnight at 4 °C with primary antibodies and then were incubated in secondary antibodies for 1 h at room temperature (Table 3). Cells were incubated with DAPI nuclear stain for 15 min. Immunofluorescence was imaged using a Zeiss LSM 880 confocal laser-scanning microscope. Immunofluorescence analysis was conducted at P11 for MZT06-B, P11 for MZT06-C, and P11 for MZT06-D.

#### 4.8. Absence of the reprogramming virus

RNA was isolated according to manufacturer's instructions (cytotune) from reprogrammed fibroblasts at P0 before hiPSCs were picked and cultured to function as the positive control. cDNA was synthesized from the RNA and RT-PCR was performed using primers provided from the manufacturer (Table 3). All hiPSC sublines were tested for absence of the reprogramming virus at P10.

#### 4.9. Mycoplasma detection

Mycoplasma was regularly tested using MycoAlert kit from Lonza - Catalog #LT07-318. We used the MycoAlert kit to calculate the presence of mycosomal enzymes within hiPSC test sample. Through measurement of ADP to ATP conversion both before and after addition of the MycoAlert™ PLUS Substrate, a ratio can be constructed that indicates presence of absence of the virus. Ratios below 1.0 indicate that the sample is negative for mycoplasma, a value over 1.2 indicates presence of mycoplasma in the sample. Mycoplasma analysis was conducted at P25 for MZT06-B, P23 for MZT06-C, and P23 for MZT06-D.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

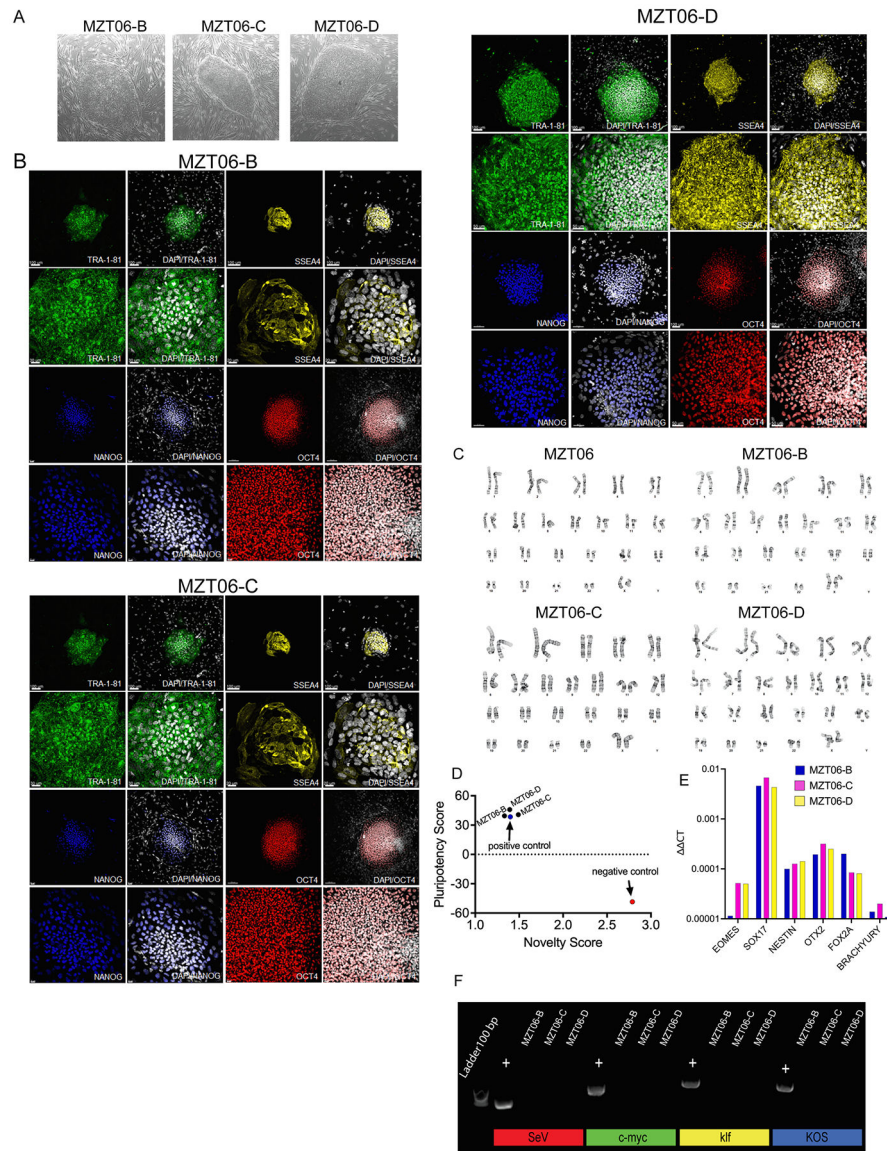
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**Fig. 1.**  
Characterization and Validation of hiPSC Sublines.



**Table 1**

Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UCLAi004-A	MZT06-B	Female	55	White	N/A	POI
UCLAi004-B	MZT06-C	Female	55	White	N/A	POI
UCLAi004-C	MZT06-D	Female	55	White	N/A	POI

**Table 2**

Characterization and validation.

<b>Classification</b>	<b>Test</b>	<b>Result</b>	<b>Data</b>
Morphology	Phase Contrast	Normal	Fig. 1 panel A
Phenotype	Immunofluorescence	Positive for self-renewal markers: OCT4, NANOG, SSEA-4, TRA-1-81	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	46,XX	Fig. 1 panel C
Identity	STR analysis	16 sites tested, all three lines match each other, and the HDF line they were derived	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by Luminescence	Supplementary Figure 1
Differentiation potential	PluriTest In vitro Differentiation	Pluripotent Ectoderm, Mesoderm, Endoderm potential	Fig. 1 panel D Fig. 1 Panel E
Donor screening (OPTIONAL)	N/A		
Genotype additional info (OPTIONAL)	N/A		
	N/A		

**Table 3**

Reagents details.

<b>Antibodies used for immunocytochemistry/flow-citometry</b>			
	Antibody	Dilution	Company Cat # and RRID
Self-renewal markers	goat-anti-human Oct4	1:100	Santa Cruz, sc8628 RRID: AB_653551
Self-renewal markers	goat-anti-human NANOG	1:40	R&D Systems, AF1997 RRID: AB_355097
Self-renewal markers	mouse-anti-human SSEA-4	1:100	Developmental Studies Hybridoma Bank, MC-813-70 RRID: AB_528477
Self-renewal markers	mouse-anti-human TRA-1-81	1:100	eBiosciences, 14-8883-82 RRID: AB_891614
Pluripotency markers	SSEA-4-Allophycocyanin	1:30	R&D Systems, FAB1435A RRID: AB_494994
Pluripotency markers	TRA-1-85-Phycoerythrin	1:60	R&D Systems, FAB3195P RRID: AB_2066683
Pluripotency markers	TRA-1-81, Alexa Fluor 488	1:60	Stemcell Technologies, 60065AD RRID: AB_2721032
Pluripotency markers	Dapi	1:100	BioVision, B1098-25 RRID: AB_2336790
Secondary antibodies	AF488-conjugated donkey-anti-goat	1:200	Jackson Immuno Research, 705-546-147 RRID: AB_2340430
Secondary antibodies	AF488-conjugated donkey-anti-mouse	1:200	Life Technologies, A-21131 RRID: AB_2535771
<b>Primers</b>			
	Target	Forward/Reverse primer (5'-3')	
Reprogramming virus	SeV	GGA TCA CTA GGT GAT ATC GAG C/ ACC AGA CAA GAG TTT AAG AGA TAT GTA TC	
Reprogramming virus:	KOS	ATG CAC CGC TAC GAC GTG AGC GC/ ACC TTG ACA ATC CTG ATG TGG	
Reprogramming virus :	Klf4	TTC CTG CAT GCC AGA GGA GCC C/ AAT GTA TCG AAG GTG CTC AA	
Reprogramming virus :	c-Myc	TAA CTG ACT AGC AGG CTT GTC G/ TCC ACA TAC AGT CCT GGA TGA TGA TG	
Taqman assay	EOMES	Hs00610080_m1	
Taqman assay	SOX17	Hs00751752_s1	
Taqman assay	FOXA2	Hs00610080_m1	
Taqman assay	OTX2	Hs00222238_m1	
Taqman assay	TBXT	Hs00610080_m1	
Taqman assay	NESTIN	Hs04187831_g1	
Taqman assay	GAPDH	Hs02786624_g1	