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Lab Resource: Multiple Stem Cell Lines

# Lymphoblast-derived hiPS cell lines generated from four individuals of a family of genetically unrelated parents and their female monozygotic twins

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A B S T R A C T

Lymphoblast cells from four individuals of a family of two genetically unrelated parents and their monozygotic twins were used to generate integration-free human induced pluripotent stem cells (hiPSCs). Reprogramming factors were delivered by co-electroporation of three episomal-based plasmids expressing OCT3/4, SOX2, KLF4, L-MYC and LIN28. The hiPSCs showed a normal karyotype, expressed pluripotency-associated markers, displayed the capacity for *in vitro* differentiation into the three germ layers and were Epstein Barr virus-free. These hiPSC lines offer the possibility to compare genetically unrelated and genetically identical tissues from different individuals and to study genotype-specific effects, which are particularly relevant for toxicology testing.

## Resource Table

| | |
|---|---|
| Unique stem cell lines identifier | LUMCi013-A<br>LUMCi014-A<br>LUMCi015-A<br>LUMCi016-A |
| Alternative names of stem cell lines | L01 (LUMCi013-A)<br>L02 (LUMCi014-A)<br>L03 (LUMCi015-A)<br>L04 (LUMCi016-A) |
| Institution | LUMC hiPSC Hotel, Department of Anatomy and Embryology, Leiden University Medical Center, Einthovenweg 20, 2333 ZC Leiden, The Netherlands |
| Contact information of distributor | Karine Raymond (k.i.Raymond@lumc.nl) |
| Type of cell lines | hiPSC |
| Origin | Human |
| Cell Source | Human lymphoblast cells |
| Clonality | Clonal |
| Method of reprogramming | Non-integrating episomal vectors<br>pCXLE-hOCT3/4 (Addgene ID 27076)<br>pCXLE-hSK (Addgene ID 27078)<br>pCXLE-hUL (Addgene ID 27080) |
| Multiline rationale | Cell lines from monozygotic twins (genetically identical) and their parents (genetically unrelated) |
| Gene modification | NO |
| Type of modification | NA |
| Associated disease | NA |
| Gene/locus | NA |
| Method of modification | NA<br>NA |
| Name of transgene or resistance | |
| Inducible/constitutive system | NA |
| Date archived/stock date | 29/10/2019 |
| Cell line repository/bank | NA |
| Ethical approval | Ethical committees and approval numbers:<br>—Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance-FWA00017598; IRB/institute codes, NTR 03-180), under the IRB reference 2003t180.<br>LUMC CME under the protocol #P13.080 |

## 1. Resource utility

The hiPSC lines here originate from transformed lymphocyte cells derived from female monozygotic twins and their parents (Willemsen et al., 2010), making it possible to generate genetically -identical and -unrelated tissues. By comparing the response of twin's, the lines are useful for studying genotype-related effects notably in toxicology.

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**Table 1**
Summary of lines.

| hiPSC line names | Abbreviation in figures | Gender | Age | Ethnicity | Genotype of locus | Disease |
|---|---|---|---|---|---|---|
| | L01 | Male | 75 | Caucasian | N/A | N/A |
| | L02 | Female | 70 | Caucasian | N/A | N/A |
| | L03 | Female | 36 | Caucasian | N/A | N/A |
| | L04 | Female | 36 | Caucasian | N/A | N/A |

### 1.1. Resource details

Lymphoblast cell lines (LCLs) are derived from donor B-lymphocytes by Epstein Bar virus (EBV) transformation and represent a useful cell type for storing genetic material and examining the influence of genetic variations on drug response (Hillger et al., 2015). In this study, LCLs derived from a family of adult monozygotic twins and their parents were used to generate hiPSCs (Table 1). The LCLs had previously been generated at the Rutgers Institute (Department of Genetics, Piscataway, NJ, USA) using a standard transformation protocol (Willemsen et al., 2010; Hillger et al., 2015).

For reprogramming, LCLs were electroporated with oriP/EBNA-1-based episomal plasmids expressing OCT3/4, SOX2, KLF4, L-MYC and LIN28 (Okita et al., 2011). Individual hiPSC colonies were mechanically picked and the corresponding hiPSC lines were expanded at least to passage 10. hiPSCs displayed characteristic epithelial morphology with compact, dense colonies with sharp edges and containing cells with a high nucleus-to-cytoplasm ratio (Fig. 1A). The pluripotency status of the iPSC lines was confirmed by expression of the pluripotency markers OCT3/4, NANOG and SSEA4 evidenced by immunofluorescent staining (Fig. 1B) and by flow cytometry (Fig. 1C). G-banding karyotyping revealed that all lines had normal diploid karyotype (Fig. 1D). The pluripotent differentiation potential of the lines was verified by *in vitro* spontaneous differentiation towards the three germ layers, as shown by immunofluorescent staining of the ectodermal marker βIII-tubulin (B3-TUB), the endodermal marker α-fetoprotein (AFP) and the mesodermal marker platelet endothelial cell adhesion molecule-1 (PECAM-1) (Fig. 1E). The identity of the hiPSC lines was confirmed by short tandem repeat (STR) analysis, which matched that of the original LCLs. Interestingly, EBV-related latency elements necessary for the transformation of B cells to LCLs were lost from the hiPSC-derived lines as assessed by PCR (Fig. 1F). The complete characterization is summarized in Table 2.

## 2. Materials and methods

### 2.1. Cell culture

The LCLs were cultured in expansion medium (EM) composed of RPMI-1640 (Sigma-Aldrich, #R5886) supplemented with 15% fetal bovine serum (FBS) (Gibco, #10270), 2 mM L-Glutamin (Gibco#15070), 50 μg/mL streptomycin and 50 U/mL penicillin (Gibco, #25030) at 37 °C and 5% $CO_2$.

### 2.2. iPSC line generation

LCLs at passage 50 were reprogrammed by the Leiden University Medical Center (LUMC) Human iPSC Hotel, as previously described (Barret et al., 2016) with minor modifications. Briefly, $1 \times 10^6$ cells were electroporated with 1 μg of each of the episomal plasmids pCXLE-hOCT3/4, pCXLE-hSK and pCXLE-hUL (Addgene, #ID27076, #ID27078, #ID27080)) with the program E-010 of the Nucleofector™ I device and the B-cell Nucleofector Kit (VPA-1001, Lonza). Cells were subsequently plated at a density of $3.5 \times 10^4$ cells/cm$^2$ on Matrigel™-coated plates (Corning/BD Biosciences, #354277) in EM for 3 days. For the next 3 days, 1 ml of reprogramming media (RM; supplemented ReproTeSR Basal medium (Stemcell Technologies, #05921, #05922, #05923)) was added daily to the original LCL media. For the following 11 days the cells were maintained in RM with daily media replenishment and the media was changed to TeSR™-E8™ (Stemcell Technologies, #05990) after day 18. After mechanical picking, iPSCs were maintained in TeSR™-E8™ on vitronectin-coated plates (Stemcell Technologies, #07180) at 37 °C and 5% $CO_2$.

### 2.3. Immunofluorescence staining

Cells were fixed using 1% paraformadehyde for 30 min at 25 °C and permeabilized and blocked using 0.1% TritonX-100 in PBS containing 4% normal swine serum (Jackson ImmunoResearch, #014-000-121) for 1 h at 25 °C. Cells were incubated with primary and secondary antibodies (Table 3) in PBS for 1 h at 25 °C. Washes were performed with 0.05% Tween in PBS. Nuclei were stained with DAPI (Fisher Scientific, #D3571) and images were taken with a SP5 confocal microscope (Leica).

### 2.4. Flow cytometry analysis

Cells were dissociated into single cells with Gentle Cell dissociation reagent (7 min at 37 °C, Stemcell Technologies, #07174), and fixed and permeabilized by using the FIX & PERM™ Cell Permeabilization kit (ThermoFisher, #GAS004), according to the manufacturer's instructions. Cells were incubated with the antibodies (Table 3) for 20 min in the dark at 25 °C and analyzed with a LSRII flow cytometer (BD) The HaCaT keratinocyte cell line was used as a negative control.

### 2.5. Karyotype analysis

G-banding analysis was conducted at the Laboratorium voor Diagnostische Genoomanalyse (LGDA), LUMC according to standard procedures. A total of 20 metaphases were analyzed for each line.

### 2.6. Induction of differentiation

The hiPSCs were passaged and maintained for three days on vitronectin-coated glass coverslips in TeSR™-E8™ and subsequently cultured in DMEM/F12 (Gibco, #31331-028) containing 20% FBS for 3 weeks with media changes every two days.

### 2.7. Genome analysis of the EBV-related latency elements

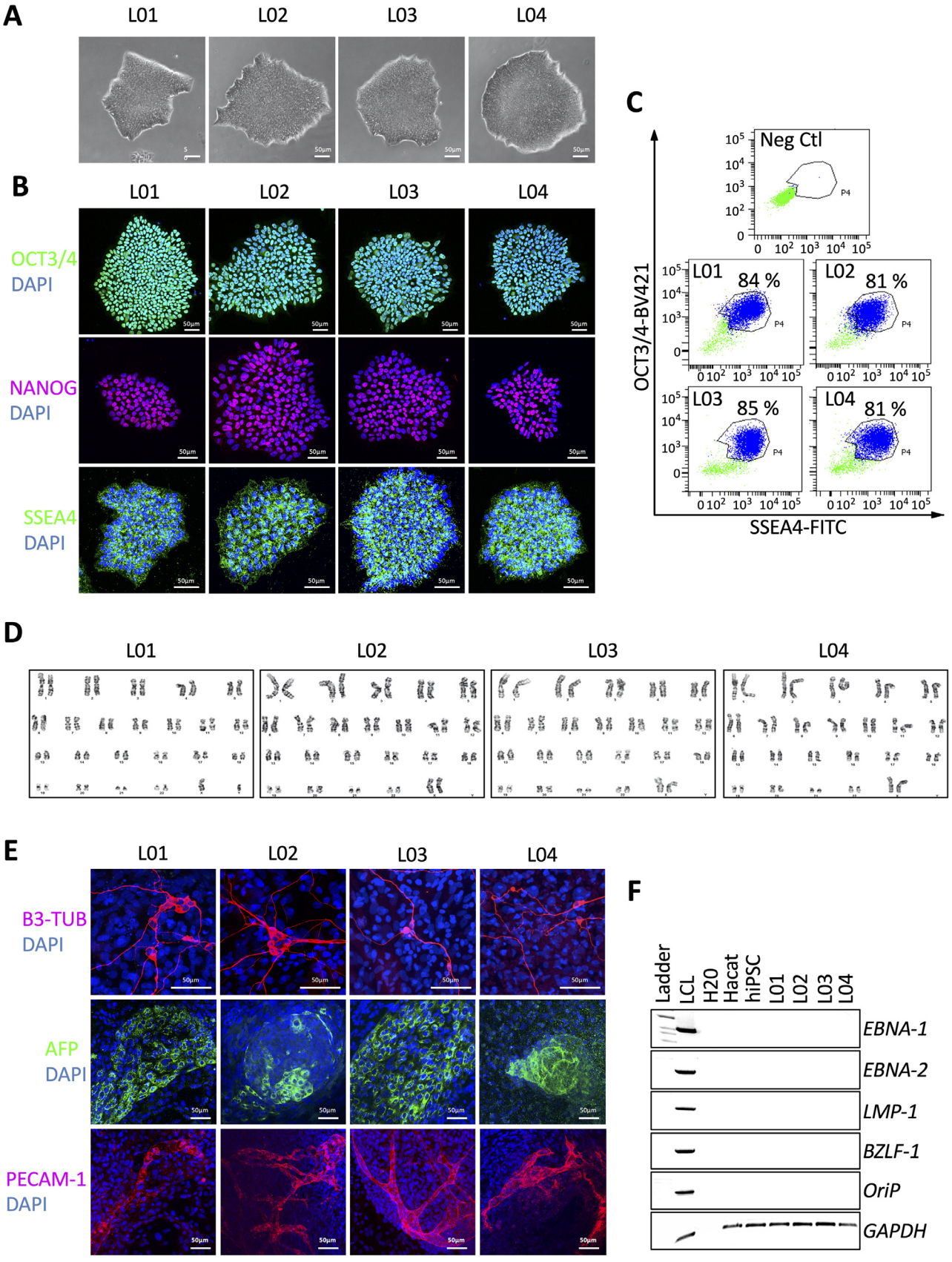
Genomic DNA was extracted from iPSCs using QuickExtract™ DNA Extraction solution (Lucigen, #QE09050) and PCR was performed by using Terra Taq polymerase (Takara, #639270) with previously described conditions (Barrett et al., 2014). Comment: Are LMP1, BZLF1 etc. described somewhere in the references? You show them in the PCR but if there is no fig legend it may be unclear what they stand for. Legend added and genes described in Barrett et al., 2014

### 2.8. Short tandem repeat (STR) analysis

Cell line authentication was performed by the Department of Human Genetics, LUMC, by using the PowerPlex® Fusion System 5C autosomal STR kit (Promega) as previously described (Westen et al., 2014).

### 2.9. Mycoplasma detection

The mycoplasma status was assessed using the MycoAlert™ mycoplasma detection kit (Lonza, #LT07-418) following the manufacturer's protocol.




*(caption on next page)*

**Fig. Characterization of the LUMCi013-A (L01), LUMCi014-A (L02), LUMCi015-A (L03) and LUMCi016-A (L04) hiPSC lines**. A, Colonies display typical morphology of hiPSCs maintained in TeSR™ E8™ on vitronectin-coated plates. B, Pluripotency markers detected by immunofluorescent staining. hiPSC colonies are positive for OCT3/4 (green), NANOG (red) and SSEA4 (green) pluripotency markers. C, Pluripotency markers analyzed by flow cytometry. More than 80% of the cells maintained in TeSR™ E8™ on vitronectin-coated plates, are positive for the OCT3/4 and SSEA4 pluripotency markers. D, G-banding karyotyping indicate normal diploid karyotypes. E, Spontaneous differentiation *in vitro*. Cells can differentiate towards the three germ layers, as shown by immunofluorescent staining of the ectodermal marker B3-TUB (red), the endodermal marker AFP (green) and the mesodermal marker PECAM-1 (red). F, Analysis of Epstein Barr virus-related genes (EBNA-1, EBNA-2, LMP-1, BZLF-1, and OriP) by PCR of genomic DNA obtained from parental LCL (LCL), from daughter iPSC lines (L01, L02, L03 and L04), and from negative control lines (human keratinocyte cell lines (Hacat) and independent hiPSC line (hiPSC)). GAPDH is used as a loading control.

**Table 2**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Transmission light microscopy | Normal | Fig. 1A |
| Phenotype | Pluripotency status, qualitative analysis: Immunofluorescent staining | All the lines showed positive staining of pluripotency markers: Oct3/4, NANOG, SSEA4. | Fig. 1B |
| | Pluripotency status, quantitative analysis: flow cytometry | Percentage of cells positives for Oct3/4 and SSEA-4: L01 (84%); L02 (81%); L03 (85%) and L04 (81%) | Fig. 1C |
| Genotype | Karyotype (G-banding) Resolution 450–500 | L01 (46,XY); L02 (46,XX); L03 (46,XX) and L04 (46,XX) | Fig. 1D |
| Identity | Microsatellite PCR (mPCR) OR STR analysis | Not performed<br>22 sites tested, all sites matched | N/A<br>Submitted in archive with journal |
| Mutation analysis (IF APPLICABLE) | Sequencing | N/A | N/A |
| | Southern Blot OR WGS | N/A | N/A |
| Microbiology and virology | Luminescence-based mycoplasma testing. | Negative. | Submitted in archive with journal |
| | Detection of Epstein Barr virus-related latency elements by PCR. | Negative. | Fig. 1F |
| Differentiation potential | Pluripotency function; spontaneous differentiation | Expression of ectodermal marker b3-tubulin (B3-TUB), endodermal marker alpha-fetoprotein (AFP) and mesodermal marker platelet endothelial cell adhesion molecule-1 (PECAM-1) was detected. | Fig. 1E |
| Donor screening (OPTIONAL) | HIV 1 + 2 Hepatitis B, Hepatitis C | Not performed | N/A |
| Genotype additional info (OPTIONAL) | Blood group genotyping | Not performed | N/A |
| | HLA tissue typing | Not performed | N/A |

**Table 3**
Reagents details.

| Antibodies used for immunocytochemistry/flow-cytometry | | | |
|---|---|---|---|
| | Antibody | Dilution | Company Cat # and RRID |
| Pluripotency Markers | mouse IgG2b anti-OCT3/4 | 1:100 | Santa Cruz, Sc-5279 |
| Pluripotency Markers | mouse IgG1 anti-NANOG | 1:150 | Santa Cruz, Sc-293,121 |
| Pluripotency Markers | mouse IgG3 anti-SSEA4 | 1:30 | Biolegend, #330402 |
| Pluripotency Markers | BV421 Mouse IgG1 anti-OCT3/4 | 1:50 | BDBioscience, AB#565644 |
| Pluripotency Markers | FITC Human IgG1 anti-SSEA4 | 1:25 | Miltenyi, #130-098-371 |
| Differentiation Markers | mouse IgG2ba anti-b3-tubulin | 1:4 000 | Covance, #MMS-435P |
| Differentiation Markers | rabbit anti-AFP | 1:25 | Quartett, #2011200530 |
| Differentiation Markers | mouse IgG1 anti-PECAM-1 | 1:100 | DAKO, #M0823 |
| Secondary antibodies | Alexa 647 Goat Anti-Mouse IgG2b | 1:250 | Invitrogen Cat# 21242 |
| Secondary antibodies | Alexa 488 Goat Anti-Mouse IgG3 | 1:250 | Invitrogen Cat# 21151 |
| Secondary antibodies | Alexa 568 Goat Anti-Mouse IgG1 | 1:250 | Invitrogen Cat# 21124 |
| Secondary antibodies | Alexa 568 Goat Anti-Mouse IgG | 1:500 | Invitrogen Cat# A11031 |
| Secondary antibodies | Alexa 488 Donkey Anti-rb IgG | 1:500 | Invitrogen Cat# A21206 |
| Primers | | | |
| | Target | Forward/Reverse primer (5′-3′) | |
| EBV-related latency elements (genomic PCR) | EBNA-1 | ATCAGGGCCAAGACATAGAGA/ GCCAATGCAACTTGGACGTT | |
| EBV-related latency elements (genomic PCR) | EBNA-2 | CATAGAAGAAGAAGAGGATGAAGA/ GTAGGGATTCGAGGGAATTACTGA | |
| EBV-related latency elements (genomic PCR) | BZLF-1 | CACCTCAACCTGGAGACAAT/ TGAAGCAGGCGTGGTTTCAA | |
| EBV-related latency elements (genomic PCR) | LMP-1, | ATGGAACACGACCTTGAGA/ TGAGCAGGATGAGGTCTAGG | |
| EBV-related latency elements (genomic PCR) | OriP | TCGGGGGTGTTAGAGACAAC/ TTCCACGAGGGTAGTGAACC | |
| House keeping gene (genomic PCR) | GAPDH | ACCACAGTCCATGCCATCAC/ TCCACCACCCTGTTGCTGTA | |

## Declaration of Competing Interest

The authors declare no conflict of interest.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101654.

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