



Lab resource: Stem cell line

## Lymphoblast-derived integration-free iPSC cell line from a 69-year-old male



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### ABSTRACT

Human lymphoblast cells were used to generate integration-free induced pluripotent stem (iPS) cells employing episomal plasmids expressing OCT4, SOX2, NANOG, LIN28, C-MYC and L-MYC. The derived iPS cells were defined as pluripotent based on (i) expression of pluripotent-associated markers, (ii) embryoid body-based differentiation into cell types representative of the three germ layers and (iii) the similarity between the transcriptomes of the iPS cell line and the human embryonic stem cell line H1 with a Pearson correlation of 0.95.

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### Resource table

Name of stem cell construct	pCXLE-hOCT3/4-shp53-F (ID 27077) pCSLE-hSK (ID 27078) pCXLE-hUL (ID 27080)
Institution	Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty, Heinrich-Heine-University VIB – Department of Molecular Genetics, Antwerp, Belgium
Person who characterized resource	Friederike Schröter
Contact person and email	James Adjaye <a href="mailto:james.adjaye@med.uni-duesseldorf.de">james.adjaye@med.uni-duesseldorf.de</a>
Date archived/stock date	4 June 2014
Origin	Human lymphoblast cells
Type of resource	Biological reagent: induced pluripotent stem cell, derived from human lymphoblast cells
Sub-type	Cell line
Key transcription factors	OCT4, SOX2, NANOG, LIN28, C-MYC, L-MYC
Authentication	Identity and purity of cell line confirmed as shown in Fig. 1
Link to related literature (direct URL links and full references)	Brouwers, N., van Cauwenberghe, C., Engelborghs, S., Lambert, J.-C., Bettens, K., Le Bastard, N., Pasquier, F., Montoya, A.G., Peeters, K., Mattheijssens, M., Vandenberghe, R., Deyn, P.P. de, Cruts, M., Amouyel, P., Slegers, K., van Broeckhoven, C., 2012. Alzheimer risk associated with a copy number variation in the complement receptor 1 increasing C3b/C4b binding sites. <i>Molecular psychiatry</i> 17 (2), 223–233. 10.1038/mp.2011.24.
Information in public databases	Not yet

### Resource details

Lymphoblast cells (CON8), derived from a 69-year-old individual (Brouwers et al. 2012) were reprogrammed employing oriP/EBNA-1-based episomal plasmids expressing OCT4, SOX2, KLF4, C-MYC, L-MYC, LIN28 and a p53 shRNA. Just clone B of Lymph8-iPS cell line was slightly positive for the episomal vector (at passage 10), clone A was negative for EBNA-1 and oriP but both clones were positive for the pluripotency-associated genes *OCT4*, *SOX2*, *NANOG* and *TGDF1* (Fig. 1A). Pluripotency was confirmed by (i) expression of OCT4, SOX2, NANOG, TRA-1-60 and TRA-1-81 and SSEA4 (Fig. 1B) and (ii) embryoid body (EB)-based spontaneous differentiation into cell types representative of the three germ layers, namely ectoderm (Nestin), mesoderm (SMA – smooth muscle actin) and endoderm (AFP –  $\alpha$ -feto protein) (Fig. 1C).

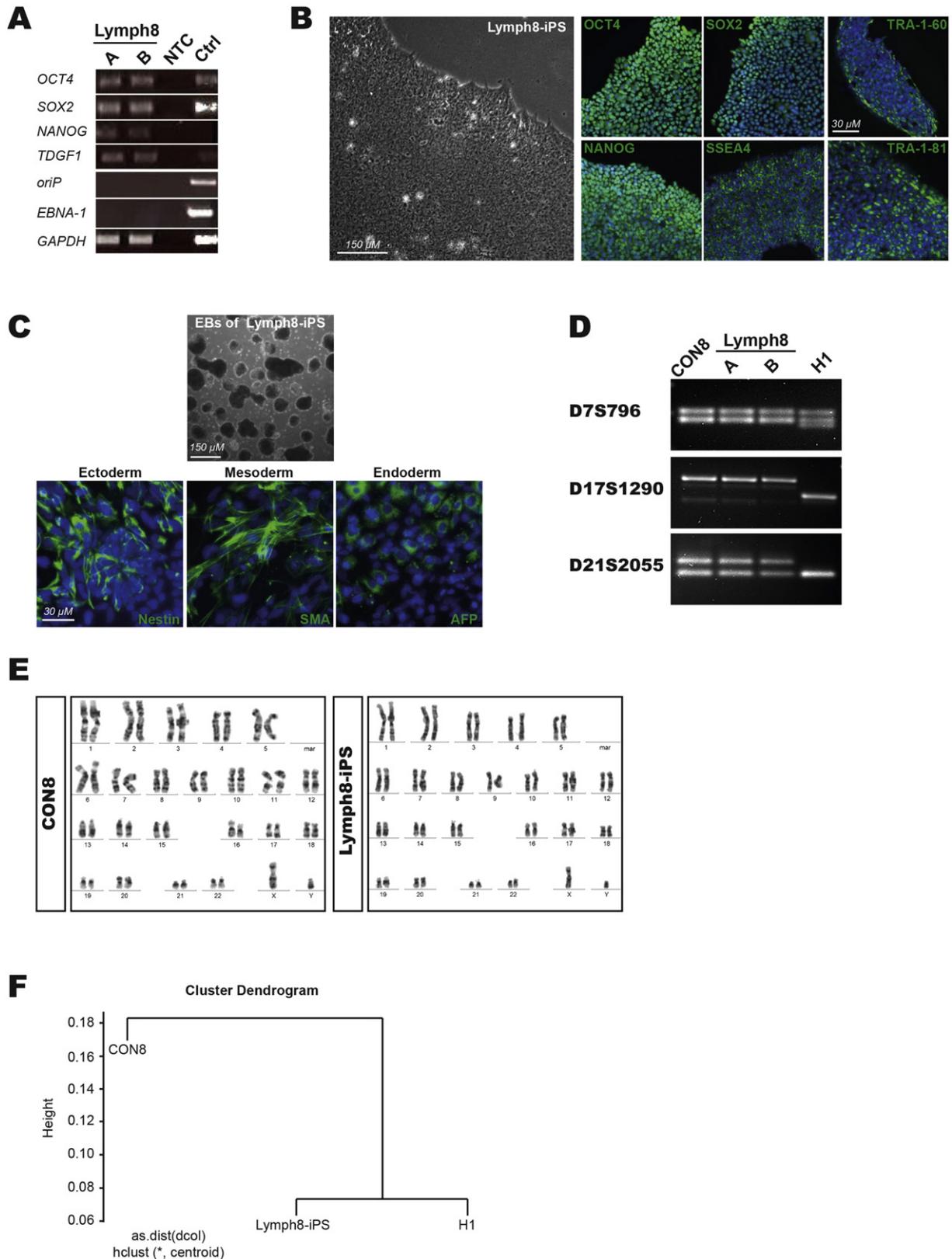
The DNA fingerprint of Lymph8-iPS cell line was identical to the parental lymphoblast line CON8 (Fig. 1D). Karyotype analysis was male (XY) and both lines exhibited a normal diploid chromosomal content (Fig. 1E). As depicted in the Dendrogram (Fig. 1F), the transcriptome of the parental lymphoblast cells is distinct from the pluripotent cell lines, Lymph8-iPS and the embryonic stem cell line H1, which cluster together with a Pearson correlation of 0.95.

### Materials and methods

#### Ethic statements

The EBV-transformed lymphoblastoid cell line, CON8, used for the generation of the iPS cell line Lymph8, was generated from peripheral blood lymphocytes of a 69-year-old male donor without any diseases.

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**Fig. 1.** Characterization of the Lymph8-iPS line. (A) Confirmation of mRNA expression of pluripotency-associated, oriP and EBNA-1 genes. NTC, non-template control; Ctrl, control line with oriP and EBNA-1 detection. (B) Immunofluorescence-based detection of human pluripotency-associated proteins OCT4, SOX1, NANOG and surface markers TRA-1-60, TRA-1-81 and SSEA4. Hoechst 33258 was used for the nuclei staining. Scale bar: big – 150  $\mu$ m and small – 30  $\mu$ m. (C) Embryoid body (EB) formation was induced in Lymph8-iPS cells in vitro and analyzed by immunofluorescence-based detection of different germ layer marker: ectoderm – Nestin mesoderm –  $\alpha$ -smooth muscle actin (SMA) and endoderm –  $\alpha$ -feto protein (AFP). Scale bar: EBs – 150  $\mu$ m and germ layer – 30  $\mu$ m. (D) Gel electrophoresis of fingerprinting PCR products. gDNA was isolated from parental lymphoblast cells CON8, Lymph8-iPS cells (separated into two clones, A and B) and embryonic stem cell line H1. DNA was amplified using PCR primers that flank different genomic regions (D7S796, D17S1290 and D21S2055). (E) Karyotyping analysis of parental lymphoblast CON8 and Lymph8-iPS line. The presence of male karyogram 46, XY. (F) Cluster Dendrogram of lymphoblast CON8, Lymph8-iPS line and embryonic stem cell line H1.

The research protocol was approved by the Ethics Committee of the University Hospital Antwerp and the University of Antwerp, Belgium.

#### Cell culture

The lymphoblast cell line CON8 (Brouwers et al. 2012) was cultured in RPMI 1640 supplemented with 15% fetal bovine serum (Invitrogen™), 1% Glutamax (Invitrogen™), 1% Sodium pyruvate (Invitrogen™) and 1% Penicillin/Streptomycin (Invitrogen™) at 37 °C and 5% CO<sub>2</sub>.

#### Derivation of the iPS cell line

Lymphoblast cells (CON8) were reprogrammed by nucleofection of oriP/EBNA-1-based episomal plasmids (expressing OCT4, SOX2, KLF4, L-MYC, LIN28 and a p53 shRNA) (Okita et al. 2011) at the Biomedicum Stem Cell Center, University of Helsinki, Finland, (<http://research.med.helsinki.fi/neuro/Otonkoski/core/default.html>) as a service. The iPS cells were cultured under feeder-free conditions on Matrigel®-coated plates in E8 medium (Invitrogen™).

#### Polymerase chain reaction

RT-PCR to assess the expression levels of the transgene and endogenous stem cell markers were carried out by the Biomedicum Stem Cell Center, University of Helsinki, Finland.

#### Embryoid body formation

Embryoid body (EB) formation was carried out as described in (Matz and Adjaye 2015). In brief, after culturing of the iPS cells in ultra-low attachment flask (Corning) in FDTA medium, the EBs were replated onto gelatin-coated plates, again in FDTA medium lacking bFGF and Dorsomorphin (Frank et al. 2012).

#### Immunocytochemistry

iPS cells and differentiated EB cells were fixed with 4% paraformaldehyde for 15 min at room temperature. After blocking with 5% normal goat serum in 0.5% Triton-X100 PBS solution, cells were incubated with the primary antibody overnight at 4 °C. The following antibodies were used: for the iPS characterization – rabbit anti-OCT4, rabbit anti-SOX2, rabbit anti-NANOG (all from the Cell Signaling iPS Cell Reprogramming Kit; all 1:800) and mouse anti-SSEA4, mouse anti-TRA-1-60 or mouse anti-TRA-1-81 (Cell Signaling Pluripotency Surface Marker Kit, all 1:1000); for the germ layer differentiation – rabbit anti-Nestin (Sigma Aldrich; 1:1000), mouse anti-SMA (Cell Signaling; 1:1000) and rabbit anti-AFP (Sigma Aldrich; 1:500). Cy2- or Cy3-conjugated secondary antibodies (Invitrogen; 1:500) were used to visualize the signal. Hoechst 33258 (1:5000; Sigma Aldrich) was added during secondary antibody incubation. The wells were covered with PBS solution and the fluorescent images obtained by an inverse fluorescence microscope LSM 700 (Carl Zeiss) and analyzed employing Adobe Photoshop software (Adobe, USA).

#### Karyotype analysis

The karyotype analysis was evaluated and performed at the Institute of Human Genetics and Anthropology, Heinrich-Heine-University, Düsseldorf. Thirty and twenty metaphases were counted for the parental lymphoblast line CON8 and the iPS cell line Lymph8, respectively.

#### DNA fingerprinting

Genomic DNA was isolated according to the manufacturer protocol from the lymphoblast line CON8, the Lymph8-iPS line, separated in

two independent lines A and B, and the human embryonic stem cell line (H1). The STR analysis was performed by PCR amplification with specific primers (Prigione et al. 2011; Wang and Adjaye 2011).

#### RNA-based microarray analysis

Total RNA from cell lysate was isolated using Direct-zol RNA Mini Prep (Zymo Research) in combination with peqGold TriFast (PeqLab Biotechnology) according to the manufacturer's protocol. Microarray analysis was outsourced to the Genomics/Transcriptomic Laboratory of the BMFZ, Heinrich-Heine-University, Düsseldorf. Affymetrix raw data in form of CEL files was read into the R/Bioconductor environment (Gentleman et al. 2004) using the package affy (Gautier et al. 2004). Probesets were normalized with the *rma* method and probesets with a coefficient of variation greater than 0.1 were filtered for the cluster analysis. Cluster analysis was performed employing the function *hclust* parameterized with Pearson correlation as similarity measure and centroid linkage as agglomeration method.

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