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Lab Resource: Stem Cell Line

Generation of iPSC line HEL24.3 from human neonatal foreskin fibroblasts

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Abstract
Human iPSC line HEL24.3 was generated from healthy human foreskin fibroblasts using non-integrative reprogramming method. Reprogramming factors Oct3/4, Sox2, Klf4, and cMyc were delivered using Sendai viruses.

Resource table:

Name of Stem Cell construct	CytoTuneTM-iPS Sendai Reprogramming Kit (Life Technologies)
Institution	Biomedicum Stem Cell Center, University of Helsinki
Person who created resource	Ras Trokovic
Contact person and email	Ras Trokovic, ras.trokovic@helsinki.fi
Date archived/stock date	April 30. 2015
Origin	Human foreskin cells (HFFs; CRL-2429, ATCC)
Type of resource	Biological reagent: induced pluripotent stem cell (iPS); derived from healthy human foreskin cells
Sub-type	Cell line
Key transcription factors	Oct4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature (direct URL links and full references)	http://dx.doi.org/10.1016/j.scr.2015.06.001 http://research.med.helsinki.fi/neuro/Otonkoski/core/default.html
Information in public databases	no

Resource details

To generate HEL24.3 we have used four Yamanaka reprogramming factors: Oct3/4, Sox2, Klf4, and cMyc.

To deliver reprogramming factors into fibroblasts we have used integration-free method using Sendai virus particles. The absence of reprogramming vectors has been confirmed using quantitative PCR and immunostaining against Sendai virus (SeV) epitop.

Using DNA fingerprinting we have shown that somatic cells used for reprogramming and derivative stem cells line (HEL24.3) are genetically identical.

Expression of pluripotent stem cell markers OCT4, SOX2, NANOG, and TDGF1 has been demonstrated using quantitative

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http://dx.doi.org/10.1016/j.scr.2015.05.012
1873-5061/

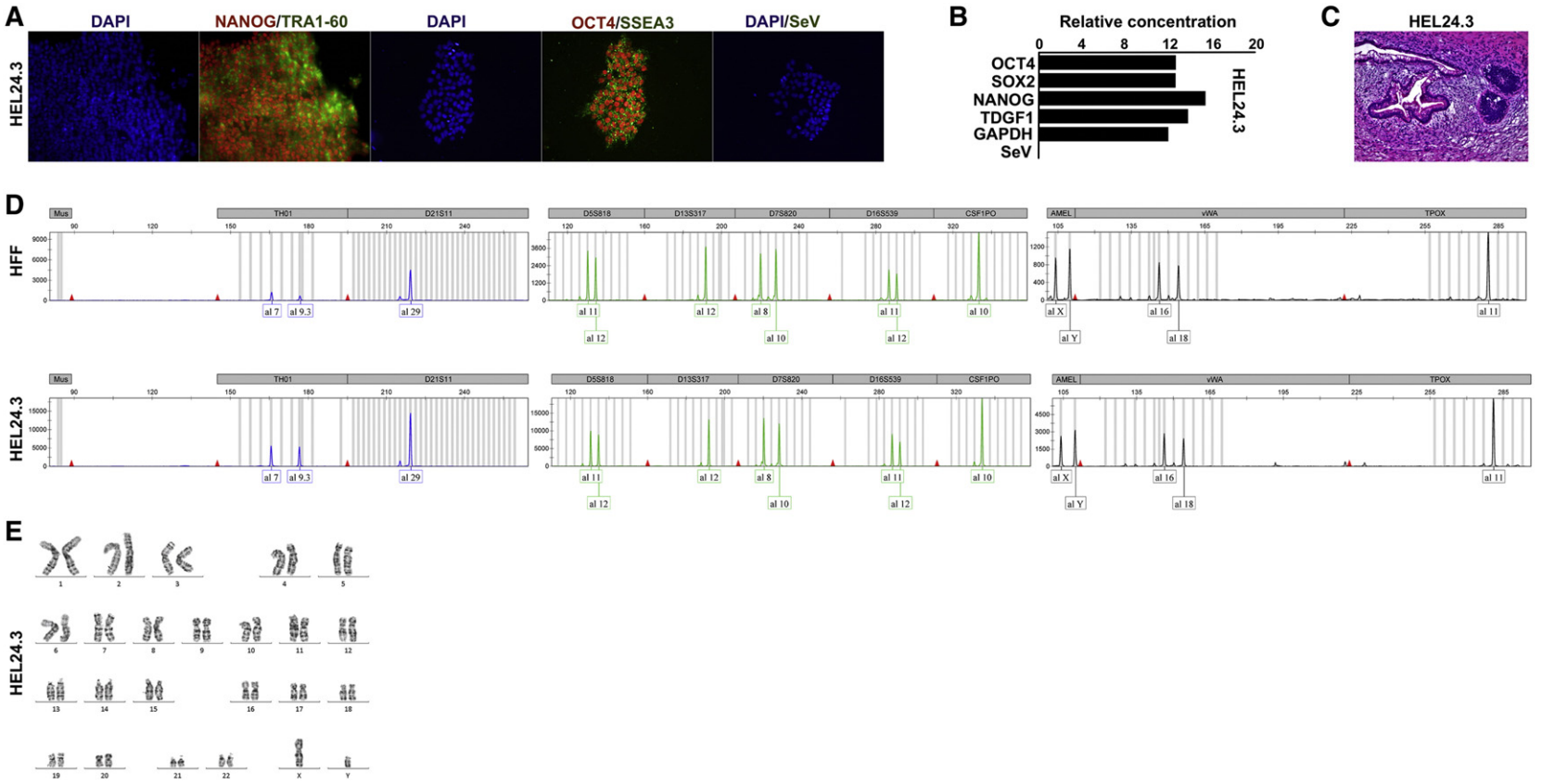

Figure 1 Characterization of iPSC line HEL24.3 generated from healthy foreskin fibroblasts. A) Representative immunofluorescent micrographs of reprogrammed fibroblasts from healthy foreskin fibroblasts (HEL24.3). Stem cells markers NANOG (red), TRA1-60 (green), OCT4 (red), and SSEA3 (green) and Sendai virus (SeV, green). Nucleus is stained with DAPI (blue). B) Quantitative PCR analysis of stem cell markers OCT4, SOX2, NANOG, TDGF1, and Sendai virus vector (SeV). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), housekeeping gene. C) Teratomas from the iPSC lines HEL24.3 showing tissues from all three germ layers. D) Authentication of HEL24.3 cell line using Promega StemEliteTM ID System. The system allows co-amplification and detection of ten human loci (9 STR loci, Amelogenin for gender identification) and one mouse locus. These loci collectively provide a genetic profile with a random match probability of 1 in 2.92×10^9, and detection of 1% fraction of mouse contaminant in a human cell line. HFF, healthy donor foreskin fibroblast line. E) Karyotype analysis. HEL24.3 had normal karyotype (46, XY).

PCR. Expression at a single cell resolution has been demonstrated using antibodies against NANOG, TRA-1-60, OCT4, and SSEA3.

Teratoma assay was used to demonstrate HEL24.3 potential to differentiate into derivatives of all three germ layers.

Materials and methods

Cell culture and reprogramming.Healthy human foreskin fibroblasts (http://www.lgcstandards-atcc.org/products/all/CRL-2429) were cultured in DMEM (Sigma) supplemented with 10% FBS (Life Technologies) and GlutaMAX (Life Technologies). Fibroblasts were reprogrammed using CytoTuneTM-iPS Sendai Reprogramming Kit (https://www.lifetechnologies.com/order/catalog/product/A1378001) using the method described (REFF: SCR-D-14-00142). To enhance the reprogramming of fibroblasts 0.25 mM sodium butyrate (NaB; Sigma, B5887) was added to all reprogramming experiments. HEL24.3 was cultured in hESC medium: DMEM/F12 with GlutaMAX (Life Technologies), 10% KnockOut Serum Replacement (Life Technologies), 0.1 mM 2-mercaptoethanol (Life Technologies), 1× Non-Essential Amino Acids (Life Technologies), and 6 ng/ml bFGF (Sigma) and routinely propagated with combination of collagenase IV treatment and mechanic dissociation. HEL24.3 was subsequently adapted and culture in a feeder free conditions on matrigel in the presence of E8 medium (Life Technologies). iPSC lines were routinely split using 0.5 mM EDTA and thawed in the presence of Rho-kinase inhibitor (Y-27632, Sigma) during 24 h after thawing.

Immunocytochemistry. Cells were fixed at room temperature with 4% paraformaldehyde for 10 min. Non-specific proteins were blocked by ultra V block (Thermo). The cells were then treated with primary antibodies overnight at 4 °C. Primary antibodies were against TRA-1-60 (1:500, MA1-023, Thermo Fisher), OCT4 (1:500, C30A3, Cell Signalling), and SSEA3 (1:100, MAB4303, Millipore). After washing with PBS, the cells were incubated with fluorescence-conjugated secondary antibody AlexaFluor 488: donkey anti-goat (1:500, A11055, Life Technologies), goat anti-mouse (1:500, A21042, Life Technologies), and goat anti-rat (1:500, A21212, Life Technologies) for 45 min, and finally mounted to cover-slip with Vectashield mounting medium with DAPI (Vectorlabs).

Quantitative polymerase chain reaction (qPCR). Total RNA was extracted using RNA Spin II (Macherey-Nagel) by following the manufacturer's instructions. Briefly, first-strand cDNA was synthesized from 2 μg total RNA by SuperScript III reverse transcriptase (Invitrogen) with oligo dT primer (Invitrogen) in 20 μl volume. 1% of above cDNA was used for each qPCR reaction in a 20 μl mixture containing 10 μl of SYBR green-Taq mixed solution (Sigma) and 5 μl of 2 μM-primer mix. PCR reactions were carried out in a Corbette thermal cycler (Qiagen) for 40 cycles and each cycle contained 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. RNA without reverse transcription was used as a negative control. The relative expression level of genes was calculated by calibrating their CT values with that of the housekeeping gene Cyclophilin G. Primer sequences (5′→3′) were as follows: OCT4_endoF: TTGGGCTCGAGAAGGATGTG; OCT4_endoR: TCCTCTCGTTGTGCATAGTCG; SOX2_endoF: GCCCTGCAGTACAACTCCAT SOX2_endoR: TGCCCTGCTGCGAGTAGGA; NANOG-F: CTCAGCCTCCAGCAGATGC; NANOG-R: TAGATTTCATTCTCTGGTTCTGG; TDGF1-F: TCAGAGATGACAGCATTTGGC; TDGF1-R: TTCAGGCAGCAGGTTCTGTTTA; SeV-F: GGATCACTAGGTGATATCGAGC; SeV-R: ACCAGACAAGAGTTTAAGAGATATGTATC; GAPDH-F: GGTCATCCATGACAACTTTGG; GAPDH-R: TGAGCTTCCCGTTCAGCTC

Demonstration of three germ layer differentiation capacity. About 200,000 morphologically intact iPSC were intratesticularly injected into male NMRI nude mice (Scanbur). The resulting tumors were collected 8 weeks after injection, fixed with 10% formalin, and hematoxylin and eosin stained. The experimental animal welfare committee of the District Government of Southern Finland approved the animal experiments.

Verification and authentication. Chromosomal G-band analyses were performed at the Yhtyneet Medix laboratoriot, Finland (http://www.yml.fi/). Cell line authentication has been done with StemEliteTM ID system (Promega) by Genomic Unit of Technology Centre, Institute for Molecular medicine Finland (FIMM).

References

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