



Lab Resource: Stem Cell Line

Generation of iPSC line HEL47.2 from healthy human adult fibroblasts



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Abstract

Human iPSC line HEL47.2 was generated from healthy 83-year old male dermal fibroblasts using non-integrative reprogramming method. Reprogramming factors Oct3/4, Sox2, Klf4, and cMyc were delivered using Sendai viruses.

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Resource table.

Name of stem cell construct	CytoTune™-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	Healthy 83-year old male dermal fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cell (iPS); derived from 83-year old healthy dermal fibroblasts
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

Primary fibroblasts were grown from the skin biopsy donated by a healthy 83-year old male volunteer donor.

To generate HEL47.2 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) epitope.

We did conduct chromosomal G-band analysis on HEL47.2 and its donor fibroblast, and found the same karyotype 46,X,abn(Y), showing that both lines are genetically identical.

Expression of pluripotent stem cell markers OCT4, SOX2, NANOG, and TDGF1 has been demonstrated using quantitative 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 HEL47.2 potential to differentiate into derivatives of all three germ layers.

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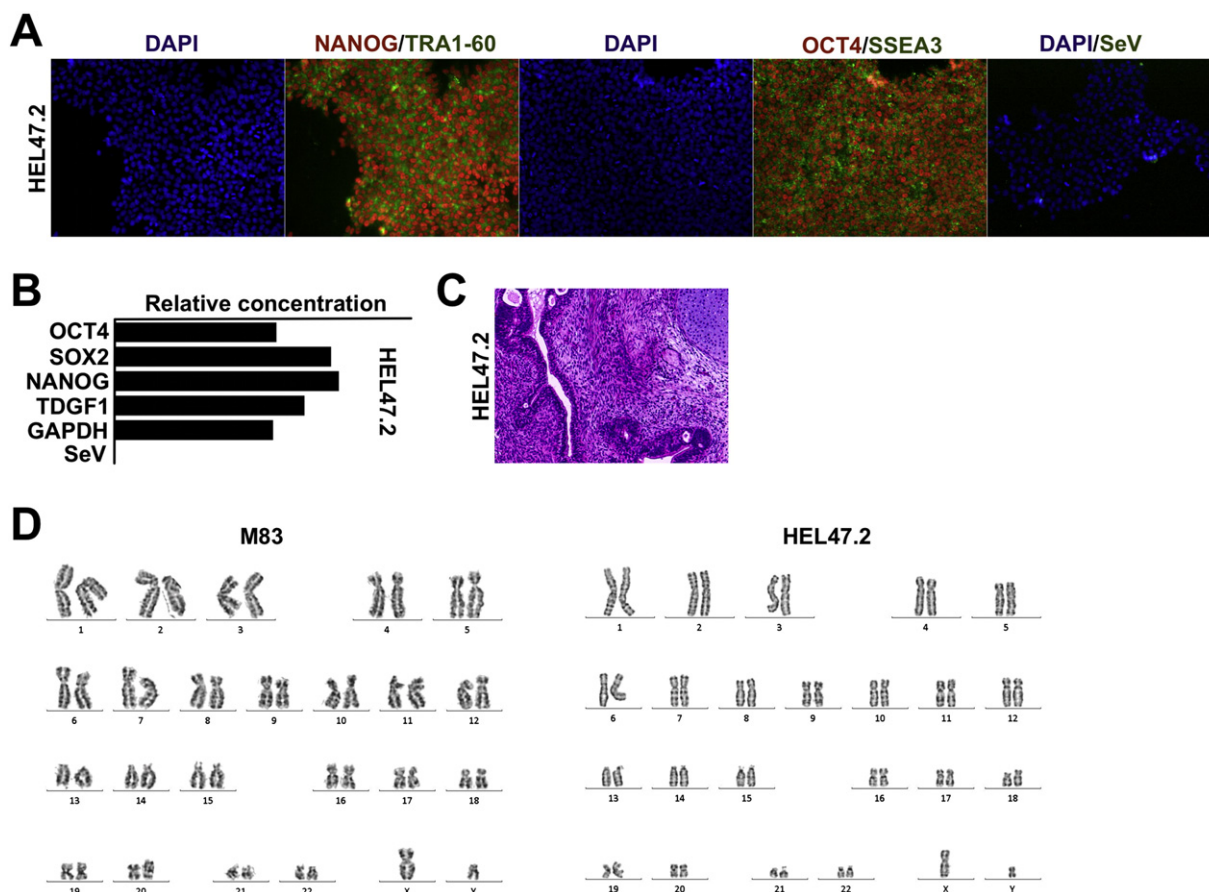


Figure 1 Characterization of iPSC line HEL47.2 generated from healthy foreskin fibroblasts. A) Representative immunofluorescent micrographs of reprogrammed fibroblasts from healthy 83-year old male donor (HEL47.2). Stem cells markers NANOG (red), TRA1-60 (green), OCT4 (red), and SSEA3 (green) and Sendai 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 HEL47.2 showing tissues from all three germ layers. D) Karyotype analysis. HEL47.2 and its parental fibroblast line (M83) showed the same 46,X, abn (Y) karyotype.

Materials and methods

Cell culture and reprogramming. Dermal fibroblasts from healthy 83-year old male donor were derived from a skin biopsy and cultured in DMEM (Sigma) supplemented with 10% FBS (Life Technologies) and GlutaMAX (Life Technologies). Protocol for fibroblast derivation can be found <http://research.med.helsinki.fi/neuro/Otonkoski/protocols>. Fibroblasts were reprogrammed using CytoTune™-iPS Sendai Reprogramming Kit (<https://www.lifetechnologies.com/order/catalog/product/A1378001>) using the method described (Trokovic et al., in press-a,b). To enhance the reprogramming of fibroblasts 0.25 mM sodium butyrate (NaB; Sigma, B5887) was added to all reprogramming experiments. HEL47.2 was cultured in hESC medium: DMEM/F12 with GlutaMAX (Life Technologies), 10% Knock-Out 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. HEL47.2 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), 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 μ l mixture containing 10 μ l of SYBR green-Taq mixed solution (Sigma) and 5 μ l of 2 μ M-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' \rightarrow 3') were as follows: OCT4_endoF: TTGGGCTCGAGAAGGATGTG; OCT4_endoR: TCCTCTCGTTGTGCATAGTCG; SOX2_endoF: GCCCTGCAGTACAACTCCATSOX2_endoR: TGCCCTGCTGCGAGTAGGA; NANOG-F: CTCAGCCTCCAGCAGATGC; NANOG-R: TAGATTTTCATTCTCTGGTCTGG; TDGF1-F: TCAGAGATGACAGCATTTGGC; TDGF1-R: TTCAGGCAGCAGGTTCTGTTTA; SeV-F: GGATCACTAGGTGATATCGAGC; SeV-R: ACCAGACAAGAGTTTAAGAGATATGATC; 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/>). Authentications, HEL47.2 and its parental fibroblast line (M83) showed the same 46,X, abn (Y) karyotype.

References

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