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# Generation of human iPS cell line CTL07-II from human fibroblasts, under defined and xeno-free conditions

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A R T I C L E I N F O

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A B S T R A C T

CTL07-II is a healthy feeder-free and characterized human induced pluripotent stem (iPS) cell line. Cultured under xeno-free and defined conditions. The line is generated from healthy human fibroblasts with non-integrating Sendai virus vectors encoding the four Yamanaka factors, OCT4, SOX2, KLF4 and cMYC. The generated iPS cells are free from reprogramming vectors and their purity, karyotypic stability and pluripotent capacity is confirmed.

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## Resource table

| | |
|---|---|
| Name of Stem Cell line | human iPS cell line CTL07-II |
| Institution | Karolinska Institutet |
| Person who created resource | Malin Kele, Kelly Day, Harriet Rönnholm |
| Contact person and email | Anna Falk, anna.falk@ki.se |
| Date archived/stock date | 2015 |
| Origin | Human fibroblasts |
| Type of resource | Biological reagent: induced pluripotent stem cells |
| Sub-type | Male cell line, normal 46 XY. |
| Key transcription factors | OCT4, SOX2, cMYC and KLF4 |
| Authentication | Identity, karyotype, purity and pluripotency capacity confirmed, Fig. 1. |
| Link to related literature | Se references |
| Ethics | Obtained from the Ethics Review Board, Stockholm, March 28, 2012. Registration number: 2012/208-31/3. |

## Resource details

- Cell authentication analysis confirmed that human iPS cell line, CTL07-II is genetically identical to the original fibroblasts. A total of 15 alleles and gender-determining marker was used. Result in Fig. 1A.
- Human iPS cell line, CTL07-II display characteristic human iPS cell phenotype with large nucleus-to-cytoplasmic ratio, prominent nucleoli and defined bright cell borders. The iPS cells grow in monolayers with tight connections. Fig. 1B.
- Human iPS cell line, CTL07-II is free from reprogramming vectors. Confirmed with RT-PCR using Sendai-virus vector specific primers. Result in Fig. 1C.
- Human iPS cell line, CTL07-II is karyotypically normal 46 XY, analyzed by Giemsa banding, Fig. 1D.
- Human iPS cell line, CTL07-II expresses pluripotency-associated genes confirmed at mRNA level by RT-PCR and by RNA-expression array and at protein level by immunocytochemistry. Fig. 1E-H.
- Purity of the line was controlled by Cell authentication (Fig. 1A) and by immunocytochemistry demonstrating that all cells express the nuclear pluripotency associated markers OCT4 and NANOG (Fig. 1G-H).
- Human iPS cell line, CTL07-II displayed pluripotent capacity, confirmed by embryonic formation assay. RT-PCR detected presence of mesodermal, endodermal and ectodermal markers. Fig. 1I-J.
- Human iPS cell line, CTL07-II, was found pluripotent by the bioinformatics tool PluriTest. Fig. 1K-L.
- Cell line was tested for presence of Mycoplasma and found clear.

## Materials and methods

### *Culture conditions*

Fibroblast medium: IMDM, Life technologies, cat no: 2198002-032, 10% Fetal bovine serum, Invitrogen, cat no: 10270106, 1% Penicillin-streptomycin, Life Technologies, cat no: 15070-063.

iPS cell medium: Essential 8™ medium, ThermoFisher Scientific, cat nr: A1517001. iPS cell coating during derivation and expansion was human recombinant Laminin-521, Biolamina, cat no: LN521-03. iPS

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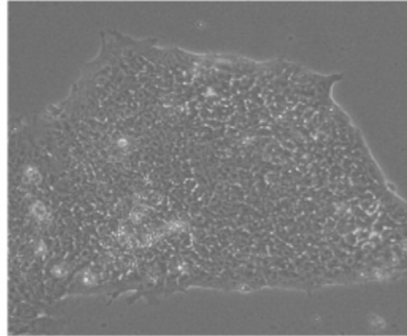
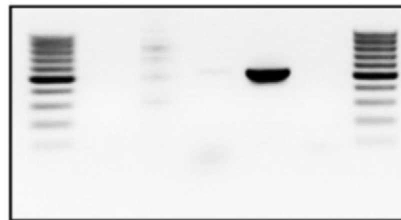
A

| DNA system | CTL07 Fibroblasts | CTL07-II iPS |
|---|---|---|
| AM | X,Y | X,Y |
| D3S1358 | 16,16 | 16,16 |
| D13S317 | 11,12 | 11,12 |
| D16S539 | 12,13 | 12,13 |
| D18S51 | 14,21 | 14,21 |
| D2S1338 | 19,20 | 19,20 |
| CSF1PO | 12,12 | 12,12 |
| TH01 | 7,8 | 7,8 |
| vWA | 14,14 | 14,14 |
| D21S11 | 29,30 | 29,30 |
| D7S820 | 7,8 | 7,8 |
| D5S818 | 12,12 | 12,12 |
| TPOX | 8,11 | 8,11 |
| D8S1179 | 12,13 | 12,13 |
| D19S433 | 14,14 | 14,14 |
| FGA | 21,22 | 21,22 |

B



C



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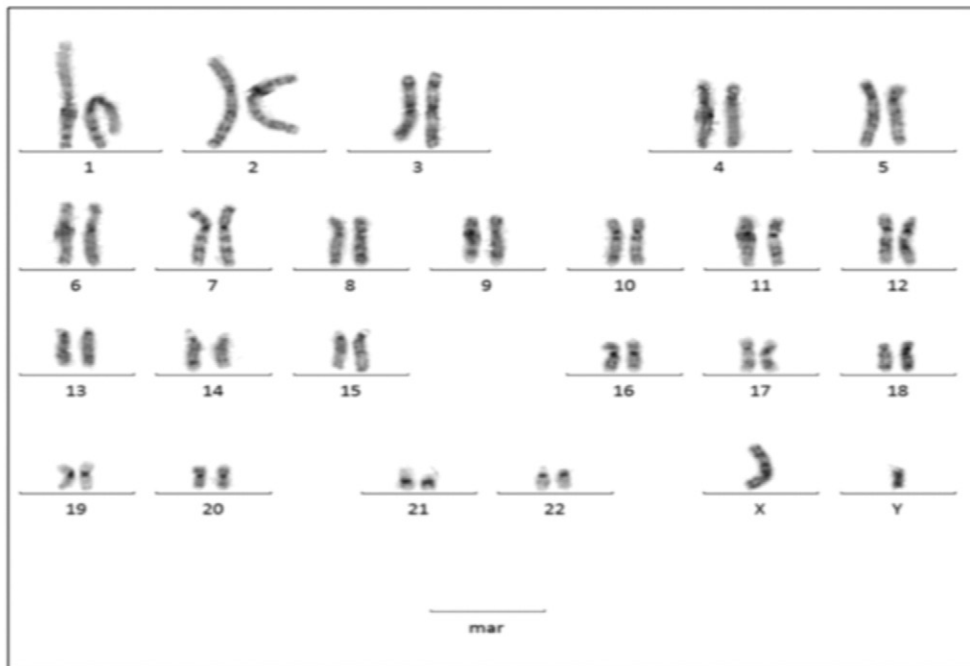


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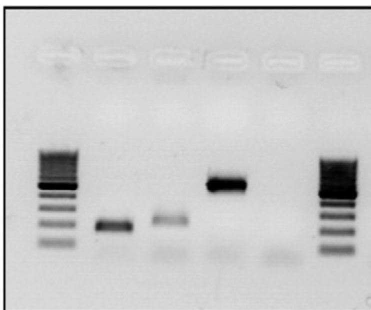

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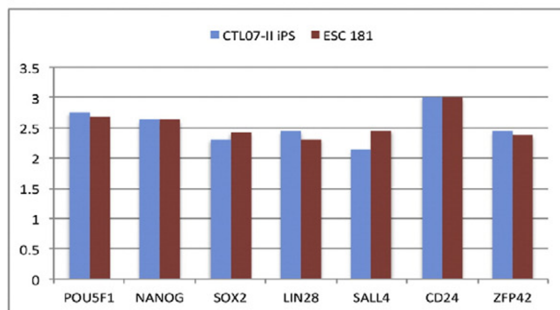

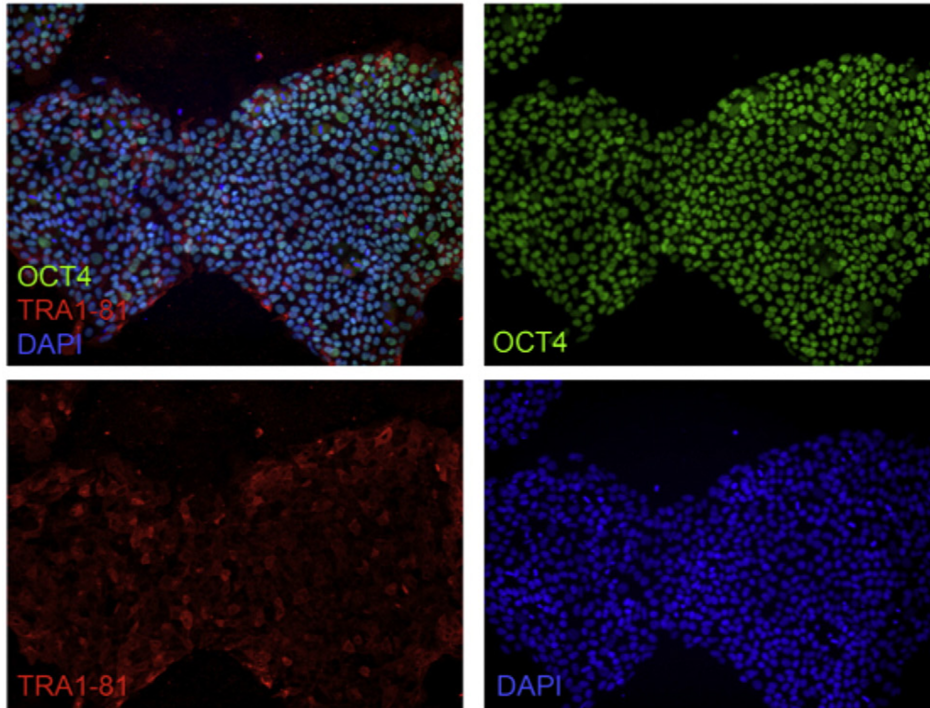


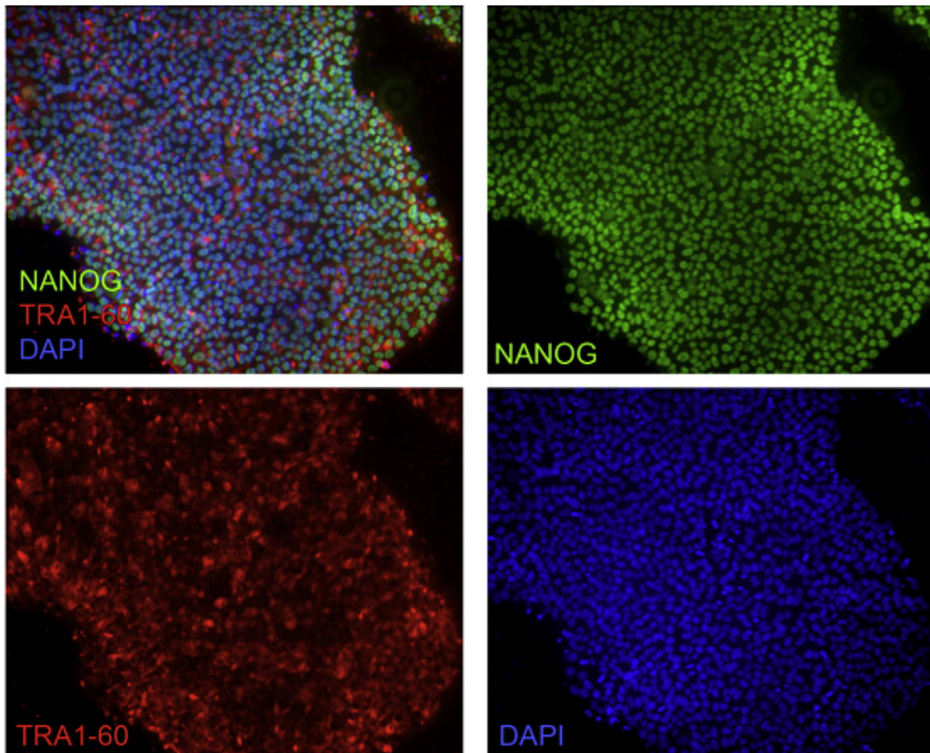


cells were manually picked for passage 1 and then chemically from passage 2 and onwards, using TrypLE Select 1×, ThermoFisher Scientific, cat no: 12563011. iPS cells were passaged with 10 μM Y27632 rho-kinase inhibitor, Millipore, cat no: SCM075.

Embryonic body formation assay: iPS cells were dissociated using TrypLE Select, and placed in 6 cm ultra low attachment wells in DMEM/F12, Life Technologies, cat no: 31331-028, with 5% KnockOut Serum, Life Technologies, cat no: 10828-028, 1% Non-essential Amino Acids 100×, Life Technologies cat no: 11140-076, 0,2% 2-mercapthoethanol 50 mM, Life Technologies cat no: 31,350-010, 1% Penicillin-Streptomycin, Life Technologies cat no: 15140-122.

*iPS cell derivation method*

CytoTune iPS Sendai Reprogramming kit, Life Technology, cat no: A1377801.

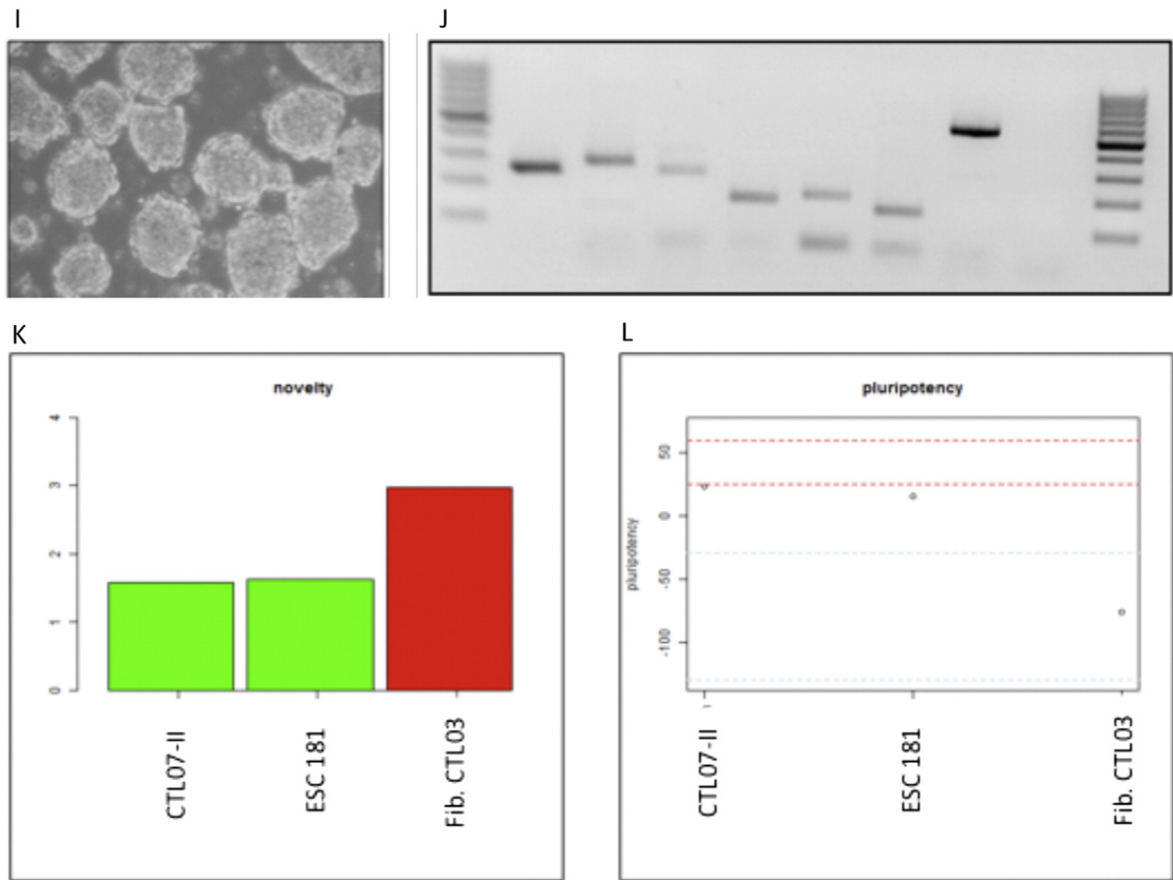


**Fig. 1.** A) Cell authentication performed on 15 specific alleles with gender determining marker (AM). Cell authentication is performed on CTL07 fibroblasts and established iPS cells. The established iPS cells and fibroblasts are identical and no cross-contamination from other cells have occurred. B) Bright field picture of iPS cell colony. The iPS cells display characteristic stem cell morphology with distinct and bright edges, high nucleus-to-cytoplasm ratio and pronounced nucleoli. C) Loss of Sendai virus based reprogramming vectors analyzed by RT-PCR. From left, DNA ladder, Sendai virus specific backbone, Sendai virus specific KLF4, Sendai virus specific cMYC, GAPDH, GAPDH without template, DNA ladder. D) Karyotyping by Giemsa banding. Human iPS cell line, CTL07-II was found to have normal 46 XY karyotype. E) Expression of pluripotency-associated genes for iPS cell line, CTL07-II by RT-PCR. From left; DNA ladder, NANOG, OCT4, GAPDH and GAPDH without template, DNA ladder. F) Expression of pluripotency-associated genes for iPS cell line, CTL07-II in comparison to established human embryonic stem cell line 181 (hESC 181) (Hovatta, 2003). Result from expression array Illumina HumanHT-12 v4 BeadChip. G) Immunocytochemistry on CTL07-II for pluripotnecy markers, OCT4 and TRA1-81. H) Immunocytochemistry on CTL07-II for pluripotnecy markers, NANOG and TRA1-60. I) Bright field picture of free-floating embryonic bodies at day 5 of differentiation. J) RT-PCRanalysis of embryonic bodies at day 21 of differentiation. From left; DNA ladder, alpha-feto protein, GATA4 (mesoderm), HAND1, RUNX1 (mesoderm) and N-CAM, NESTIN (ectoderm), GAPDH and GAPDH without template, DNA ladder. K) PluriTest result, Novelty score. From left: human iPS cell line CTL07-II, human ES cell line hESC 181, Fibroblasts CTL03. Green indicates that the expression pattern is similar to pluripotent cells and red indicates mismatch in expression pattern. Both human iPS cell line CTL07-II and the human ES cell line hESC 181 were found pluripotent. Novelty score for CTL07-II was: 1,58. L) Model-Based multi-Class Pluripotency score. Based on all samples in the stem cell model matrix. From left human iPS cell line CTL07-II, human ES cell line hESC 181, human fibroblasts CTL03. Pluripotency score for CTL07-II was: 23,27.

iPS cell clones appeared around 3 weeks post transduction, these were manually picked and expanded clonally in defined and xeno-free conditions see culture conditions se culture conditions.

*Cell authentication*

AmpFISTER@Identifiler, Applied Biosystems, cat no: 4322288.

Performed at the Forensic department in Linköping, Sweden. Cell pellets were collected at fibroblast stages and at established iPS cell stages.

*RT-PCR*

mRNA extraction using Qiagen RNeasy mini kit cat no: 74,106 with additional DNase I treatment, RNase-Free DNase set, Qiagen, cat no: 79,254. Total cDNA was produced by SuperScript III First Strand kit containing RNaseH, Invitrogen cat no: 18080051, on 500 ng of total mRNA.

GAPDH F AGGTCGGAGTCAACGGATTTG, GAPDH R GTGATGGCATG GACTGTGGT.

Primers for loss of Sendai vectors: SeV F GGATCACTAGGTGATATCG AGC, SeV R ACCAGACAAGAGTTTAAGAGATATGTATC, Klf4 SeV F TTCC TGCATGCCAGAGGAGCCC, Klf4 SeV R AATGTATCGAAGGTGCTCAA, cMyc SeV F TAACTGACTAGCAGGCTTGTCG, cMyc SeV R TCCACATACAG TCCTGGATGATGATG.

Primers for pluripotency factors: NANOG F CATGAGTGTGGATCCA GCTTG, NANOG R CCTGAATAAGCAGATCCATGG, OCT4 F CTCACCCTG GGGGTTCTATT, OCT4 R CTCCAGGTTGCCTCTCACTC.

RT-PCR for detection of remaining Sendai vectors was performed on 500 ng of total cDNA and at 32 PCR cycles. RT-PCR for pluripotency factors and germ layer specific markers were performed on 500 ng of total cDNA and a total of 30 PCR cycles. Control was GAPDH for all the RT-PCR analyses.

Test for Loss-of Sendai vector and expression of pluripotency markers was performed at passage 12.

Primers for mesoderm: HAND1 F CATCACCAATGAACCTCGTG, HAND1 R GCAGGGAAGTCAGGACCATAG, RUNX1 F CCCTAGGGGATGTT CCAGAT, RUNX1 R TGAAGCTTTTCCCTCTTCCA, Primers for ectoderm: N-CAM F ATGGAAACTCTATTAAAGTGAACCTG, N-CAM R TAGACCTCA TACTCAGCATTCCAGT, NESTIN F CTGCTACCCTTGAGACACCTG, NESTIN R GGGCTCTGATCTCTGCATCTAC, primers for endoderm: AFP F AGCTTG GTGGTGGATGAAAC, AFP R CCCTCTTCAGCAAAGCAGAC, GATA4 F CTAG ACCGTGGGTTTTGCAT, GATA4 R TGGGTTAAGTGCCCCTGTAG.

DNA ladder: GeneRuler™ 100 bp DNA ladder, Invitrogen cat no: 150,628,050.

*Immunocytochemistry*

Cells were fixed in 4% formaldehyde for 10 min and blocked with 10% bovine serum in PBS + 0,2% Triton at room temperature for 1 h. Primary antibodies: rabbit *anti*-OCT4, Cell Signaling, cat no: C30A3, rabbit anti-NANOG, Cell Signaling, cat no: 4903S, mouse-*anti*-TRA1-81, BD Pharmingen cat no: 560072, mouse-anti-TRA1-60, R&D Systems cat no: 560072. All primary antibodies were diluted 1:200 in blocking buffer and incubated at 4 °C overnight.

Secondary antibodies: donkey anti-rabbit A488, Invitrogen, cat no: A21206 (OCT4), goat anti-rabbit biotin conjugated, Life Technologies, cat no: A24535, TSA plus, Perkin Elmer cat no: NEL741001KT (NANOG), goat anti-mouse Cy3, Life Technologies, cat no: M30010 (TRA1-81 and TRA1-60). All secondary antibodies were diluted 1:500 in PBS and incubated for 1 h at room temperature.

Nuclear marker, DAPI was diluted 1:5000 in PBS and incubated at room temperature for 30 min, Life Technologies, cat no: D1306. Vectashield Vector Laboratories, H1000, was used for mounting onto microscope slides.

Microscope: Zeiss, Axioskop 2 and software: Axiovision.

*Expression array*

mRNA extraction, se Material and methods RT-PCR. mRNA quality tested by Bioanalyzer. cDNA and hybridization performed according to Illumina protocols for expression array: Illumina HumanHT-12 v4 BeadChip, cat no: BD-103-0204.

PluriTest a bioinformatics tool for predicting pluripotency home page at: wwww.pluritest.org, (Müller et al., 2011).

## References

Hovatta, O., 2003. A culture system using human foreskin fibroblasts as feeder cells allows production of human embryonic stem cells. Hum. Reprod. 18 (7), 1404–1409.
Müller, F.J., et al., 2011. A bioinformatics assay for pluripotency in human cells. Nat. Methods 8 (4), 315–317.