Stem Cell Research 51 (2021) 102193

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: Stem Cell Line

Generation of nonviral integration-free human iPS cell line KISCOi001-A from normal human fibroblasts, under defined xeno-free and feeder-free conditions

Jose Inzunza [a], Jonathan Arias-Fuenzalida [a,b], Juan Segura-Aguilar [c], Ivan Nalvarte [a], Mukesh Varshney [a,*]

[a] Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden
[b] Centre for Cancer Cell Reprogramming, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Norway
[c] Molecular and Clinical Pharmacology ICBM Faculty of Medicine, University of Chile, Chile

A B S T R A C T

KISCOi001-A is a healthy feeder-free and fully characterized human induced pluripotent stem (iPS) cell line cultured under xeno-free and defined conditions. The cell line is generated from normal human foreskin fibroblasts with non-integrating episomal plasmid vectors encoding OCT4, SOX2, KLF4, NANOG, LIN28, non-transforming L-MYC and dominant negative p53. The generated iPS cells are transgene-free and their pluripotency is confirmed by the expression of stem cell markers and capacity to differentiate into the cells of ectoderm, endoderm and mesoderm while their identity and karyotype stability is confirmed with Genomic assays.

1. Resource table

Unique stem cell line identifier	KISCOi001-A
Alternative name(s) of stem cell line	iPS-JIN-001
Institution	Karolinska Institutet, Stockholm, Sweden
Contact information of distributor	Jose Inzunza, Jose.inzunza@ki.se, Karolinska Institute Stem Cell Organoid laboratory
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: Newborn Sex: Male
Cell Source	Foreskin fibroblast
Clonality	Clonal
Method of reprogramming	Transgene free, episomal plasmid vectors
Genetic Modification	No
Type of Modification	N/A
Associated disease	N/A
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	April 2020
Cell line repository/bank	Human Pluripotent Stem Cell Registry (EU)
	https://hpscreg.eu/cell-line/KISCOi001-A
Ethical approval	Karolinska Institutet, Stockholm, Sweden Etikprövningsmyndigheten 2019-00472, Uppsala, Sweden

(continued on next column)

(continued)

2. Resource utility

Integration-free human induced pluripotent stem (iPS) cell line KISCOi001-A generated from human foreskin fibroblast can be used as reference healthy control for disease modelling and differentiation under defined xeno-free and feeder-free conditions where animal derived components are undesirable for the study.

3. Resource details

Induced pluripotent stem (iPS) cells offer great potential for disease modelling and cell therapies since they can be expanded (self-renewal) *in vitro* and differentiated into cell types of the three germ layers: such as neurons, cardiomyocytes and beta islet cells (Yamanaka, 2012). The first human embryonic stem cell (hESC) line was derived from inner cell mass cultured on mouse embryonic fibroblasts in chemically undefined media (Thomson et al., 1998) and this was later on followed for establishing

* Corresponding author.
E-mail address: mukesh.varshney@ki.se (M. Varshney).

https://doi.org/10.1016/j.scr.2021.102193
Received 19 November 2020; Received in revised form 12 January 2021; Accepted 19 January 2021
Available online 27 January 2021

iPS cell lines (Takahashi et al., 2007). Undefined conditions with animal derived components can affect the study of critical human developmental mechanisms at molecular level and can hinder clinical applications of human iPS cells (Dakhore et al., 2018). In the present study, we have generated a human iPS cell line, KISCOi001-A, from commercially available newborn foreskin fibroblast cells in chemically defined, xeno-free and feeder-free conditions except for initial fibroblast maintenance in serum supplemented medium. Dermal fibroblasts were

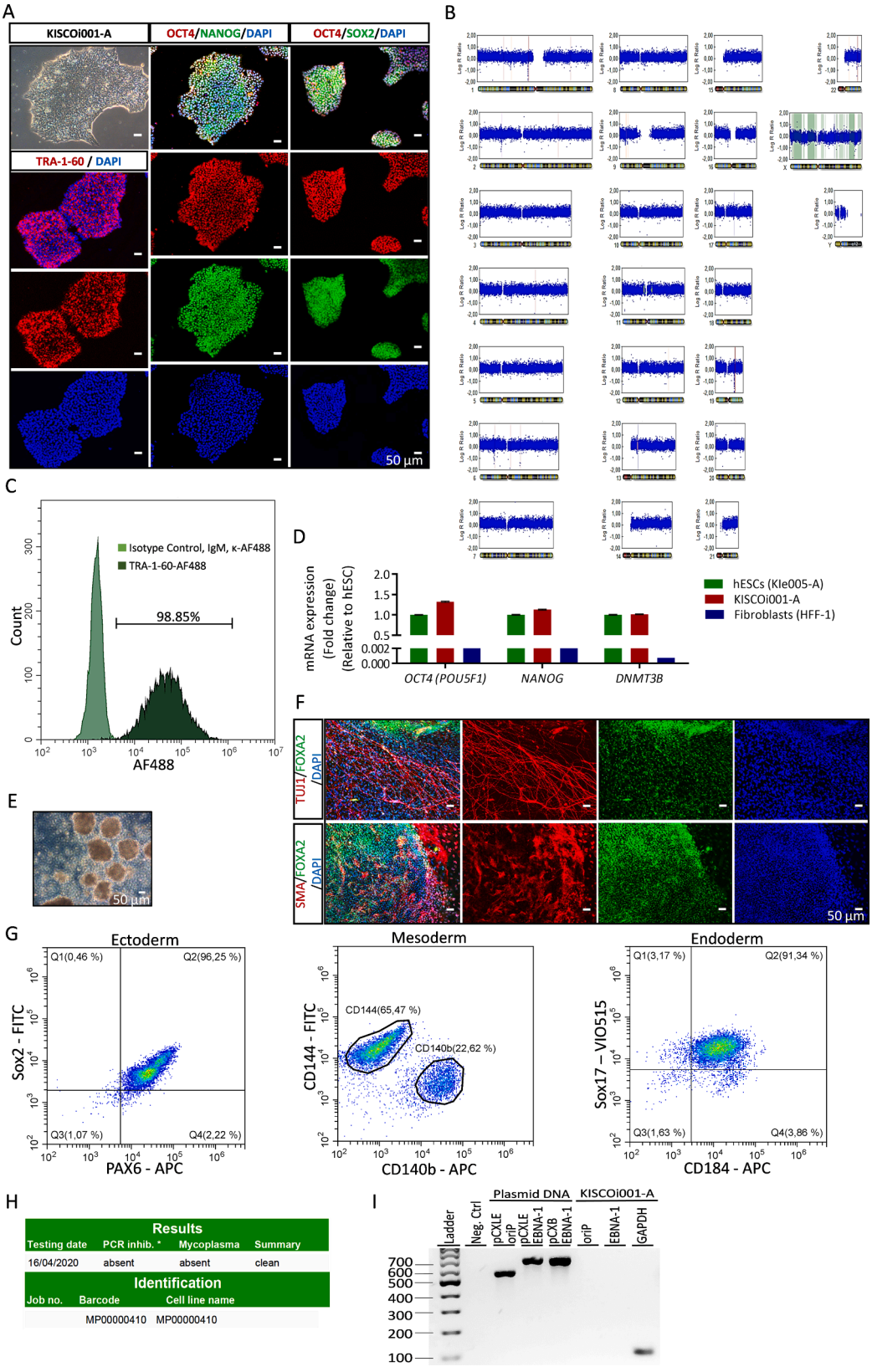

Fig. 1. Characterization of KISCOi001-A iPSC line.

reprogrammed using non-integrating episomal plasmid vectors which express the pluripotency transcription factors OCT4, SOX2, KLF-4, NANOG, LIN28, nontransforming L-MYC and dominant negative p53 suppression. iPS cell generation is greatly enhanced by p53 suppression, L-MYC and addition of EBNA-1 (episomal amplification) (Okita et al., 2011). Here, one of the clones, KISCOi001-A, was isolated and characterized (Fig. 1 and Table 1). The morphological phenotype of iPS cell line displayed a typical pluripotent colony appearance of bright borders and cells in monolayer with tight connections and large nucleus-to-cytoplasmic ratio (Fig. 1A). Immunostaining analysis revealed that the cells express high levels of pluripotency associated factors OCT4, NANOG, SOX2 and TRA-1-60 (Fig. 1A). Microarray karyotyping analysis at passage 15 revealed a normal ploidy 46XY (Fig. 1B). Flow cytometric analysis showed that more than 95% of iPS cells possess high expression of TRA-1-60 (Fig. 1C). The endogenous expression of pluripotency associated factors *OCT4* (*POU5F1*), *NANOG* and *DNMT3B* evaluated by qRT-PCR was comparable to the levels observed in hESCs (HS293/KIe005-A) (Fig. 1D). The pluripotency of iPS cell line was assessed *in vitro* by differentiation of embryoid bodies (Fig. 1E) into cells of three germ layers: ectoderm, mesoderm and endoderm. This was confirmed by immunofluorescent staining of beta-III-tubulin (TUJ1, ectoderm), α-smooth muscle actin (SMA, mesoderm) and Forkhead box protein A2 (FOXA2, endoderm) (Fig. 1F). The direct monolayer differentiation capacity of the iPS cell line was also assessed by flow cytometry. The iPS cell line KISCOi001-A was able to directly differentiate into CD184 and SOX17 double positive endoderm cells, CD140b or CD144 positive mesodermal cells, and PAX6 and SOX2 double positive ectodermal cells (Fig. 1G). Cell authentication analysis, by short tandem repeat (STR) detection, established that the human iPS cell line, KISCOi001-A is genetically identical to the original dermal fibroblasts (STR analysis). A total of 8 alleles and sex-determining markers were analyzed. Expanded iPS cell line was confirmed to be mycoplasma-free (Fig. 1H). The absence of episomal vectors was confirmed by PCR analysis after passage 15 using oriP and EBNA-1 specific primers (Fig. 1I).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase contrast microscopy	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	OCT4, NANOG, SOX2 with nuclear and TRA-1-60 with surface localization	Fig. 1 panel A
	Quantitative analysis (Flow cytometry)	Expressed high levels of pluripotency marker TRA-1-60, 98.85%	Fig. 1 panel C
	Quantitative analysis (qRT-PCR)	Expressed comparable mRNA levels of transcription factors, *OCT4 (POU5F1)*, *NANOG* and *DNMT3B* relative to the hESC (HS293/KIe005-A)	Fig. 1 panel D
Genotype	Microarray Karyotyping	46 XY	Fig. 1 panel B
Identity	STR analysis	9 STR loci matched No contamination with other cell types, same genetic identity as parental fibroblasts	STR analysis (Data available with the authors)
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR	Fig. 1 panel H
Differentiation potential	Embryoid body formation	Ectoderm (beta-III-tubulin, TUJ1)	Fig. 1 panel E and F
	Directed differentiation	Mesoderm (α-smooth muscle actin, SMA) Endoderm (Forkhead box protein A2, FOXA2) Ectoderm (SOX2+ and PAX6+): 96.25% Mesoderm (CD140b+ or CD144+): 22.62%, 65.47% Endoderm (SOX17+ and CD184+): 91.34%	Fig. 1 panel G

4. Materials and methods

4.1. Dermal fibroblasts

New-born human foreskin fibroblasts (HFF-1, ATCC, Cat. SCRC-1041) were cultured in DMEM (Thermo Fisher Cat. 41965039) with 15% FBS, 4 mM L-glutamine, 1 mM sodium pyruvate and 10 μg/ml gentamicin. Fibroblasts were dissociated with Trypsin-EDTA (Thermo Fisher cat. 25300-054) at 95% confluence.

4.2. Stem cell culture

iPS cells were propagated in standard culture conditions (37 °C and 5% CO_2) using Essential-8 (E8) medium (Thermo Fisher cat. A1517001) on 0.5 μg/cm^2 recombinant human laminin 521 (rhLN521, Biolamina cat. LN521-01) and non-enzymatically passaged in aggregates using Versene (Thermo Fisher Cat. 15040066).

4.3. Reprogramming

Fibroblasts were cultured on rhLN521 (0.5 μg/cm^2), and used at 90% confluence for the reprogramming by electroporation (100,000 cells) with the episomal plasmids indicated in Table 2 (Okita et al., 2011) in 10 μL buffer using 3 pulses of 10 ms width at 1650 V voltage using Neon transfection system (Thermo Fisher Cat. MPK5000S).

4.4. Episomal plasmids purge validation

The absence of residual episomal plasmids was assessed at passage 15 by endpoint PCR. Genomic DNA was extracted with the Allprep DNA/RNA kit (Qiagen Cat. 80004) and amplified with Taq DNA polymerase (Life Technologies Cat. 10342-053) using the primers indicated in Table 2.

4.5. Immunofluorescent staining and imaging

iPS cells were cultured on rhLN521 (0.5 μg/cm^2) coated 12 mm glass coverslips. Cells were washed with PBS and fixed with 4% PFA in PBS for 15 min at room temperature (RT), followed by 30 min blocking and permeabilization (3% BSA and 0.2% Triton-X in PBS). Then incubated with respective primary (4 °C overnight) and secondary (1 h at RT) antibodies (both diluted in 1% BSA in PBS) (Table 2) with PBS wash in between and nuclei stained with DAPI (Thermo Fisher Cat. D1306). Slides were observed under Axioplan-2 microscope (Carl Zeiss) equipped with Zeiss Axiocam MR scientific camera. Images were captured with Zeiss Axiovision 4.0 (Carl Zeiss) and processed in Fiji (ImageJ) (Table 2).

4.6. Embryoid body formation assay

iPS cells were dissociated in small aggregates, placed in ultra-low

Table 2
Reagents details.

Antibodies used for immunocytochemistry and flow-cytometry					
Target	Species	Dilution	Fluorophore	Company	Cat; RRID
Immunostaining antibodies					
OCT4	Mouse	1:300	Unconjugated	Thermo Fisher	MA1-104; AB_2536771
SOX2	Rabbit	1:500	Unconjugated	Abcam	ab97959; AB_2341193
NANOG	Rabbit	1:250	Unconjugated	Cell Signaling	4903; AB_10559205
TRA-1-60	Mouse	1:50	AF 488	Thermo Fisher	A25618; AB_2885001
beta-Tubulin III	Mouse	1:400	Unconjugated	Sigma-Aldrich	T8660; AB_477590
Actin, alpha-Smooth Muscle	Mouse	1:400	Unconjugated	Sigma-Aldrich	A2547; AB_476701
FOXA2	Rabbit	1:50	Unconjugated	Thermo Fisher	PA5-35097; AB_2552407
Flow cytometry antibodies					
CD144	rh IgG1	1:11	FITC 488 nm	Miltenyi	130-100-713; AB_2655151
CD140b	rh IgG1	1:11	APC 561 nm	Miltenyi	130-105-280; AB_2655085
CD184 (CXCR4)	rh IgG1	1:11	APC 561 nm	Miltenyi	130-109-844; AB_2655771
SOX17	rh IgG1	1:50	Vio515 488 nm	Miltenyi	130-111-031; AB_2653497
PAX6	rh IgG1	1:11	APC 561 nm	Miltenyi	130-107-776; AB_2653169
SOX2	rh IgG1	1:11	FITC 488 nm	Miltenyi	130-104-993; AB_2653501
Isotype IgM, κ	Mouse	1:50	AF 488	BD Biosciences	562409; AB_11153124
Secondary antibodies					
Goat Anti-Mouse IgG	Goat	1:1000	AF 568	Thermo Fisher	A-11031; AB_144696
Goat anti-Rabbit IgG	Goat	1:1000	AF 488	Thermo Fisher	A-11034; AB_257621

Primers		
Purpose	Target	Forward; Reverse primer (5′–3′)
Episomal Plasmids OriP (PCR)	OCT4, SOX2, KLF-4, L-MYC, NANOG, LIN28	TTCCACGAGGGTAGTGAACC; TCGGGGGTGTTAGAGACAAC
Episomal Plasmids EBNA-1 (PCR)	EBNA-1	ATCGTCAAAGCTGCACACAG; CCCAGGAGTCCCAGTAGTCA
Positive marker (PCR)	GAPDH	CCAAGGTCATCCATGACAACT; GATGATGTTCTGGAGAGCCCC
Pluripotency transcription factors (qRT-PCR)	*OCT4 (POU5F1)*	GAAGCTGACAACAATGAAA; GAACAAATTCTCCAGGTTG
	NANOG	AAGAACTCT CCA ACA TCC; GTCACACCATTGCTATTC
	DNMT3b	GGT TCC TGG AGT GTA ATC; TAACTTGGCTATCCTATTGTATT
Endogenous control (qRT-PCR)	*RPLP0*	GACACCCTCCAGGAAGCGA; GTGTTCGACAATGGCAGCAT
Recombinant DNA		
pCXLE-hMLN	Okita et al. (2011)	Addgene Plasmid #27079
pCXLE-hSK		Addgene Plasmid #27078
pCXLE-hOCT3/4		Addgene Plasmid #27076
pCE-mp53DD		Addgene Plasmid #41856
pCXB-EBNA1		Addgene Plasmid #41857
Software and Algorithms		
Fiji	Schindelin et al. (2012)	https://imagej.net/Fiji

attachment 6 cm wells in E8 basal media. Aggregates were grown for total four days in suspension, followed by plating on gelatin (0.1%) coated coverslips in basal E8 medium for differentiation for 21 days.

4.7. Direct in vitro differentiation into the three germ layers

iPS cells were directly differentiated into the three germ layers using STemMACS trilineage differentiation kit (Miltenyi Cat. 130-115-660) in adherent monolayers.

4.8. Flow cytometry

Single cells were harvested using TRYPLE select (Thermo Fisher cat. 12563029) and filtered through 40 μm cell strainers (Corning cat. 352340). Staining of iPS and differentiated cells was performed according to the manufacturer's instructions (Table 2). Cells were analyzed using Cytoflex system (Beckman Coulter).

4.9. Mycoplasma detection, karyotyping and cell line authentication

Culture supernatant was analyzed with Mycoplasma (PCR) detection by Eurofins Genomics (Germany). Genomic DNA was analyzed by Life&Brain Genomics for karyotyping using Illumina iScan with human Global Screening Array to assess the genomic integrity (copy number variation). Cell line authentication was performed by Eurofins Genomics by STR analysis using Thermo Fisher, AmpFlSTR Identifiler Plus PCR Amplification Kit (STR analysis).

4.10. Real-time quantitative PCR analysis

Total RNA was extracted using Allprep DNA/RNA kit (Qiagen Cat. 80004) followed by cDNA synthesis using SuperScript IV VILO Master Mix (Thermo Fisher cat. 11756050). qPCR analysis was performed with Kapa SYBR fast (Roche cat. KK4604) according to the manufacturer's protocol.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This research received no external financial or non-financial support. This author has no additional relationships to disclose. This author has

no patents to disclose. J.A. declared stock ownership in Bright S.A. Luxembourg. M.V., J.I., and I.N. declared stock ownership in Joincells Nordic AB Sweden.

Acknowledgements

This work was supported by the Swedish Research Council (2017-01094), Sweden; Swedish Foundation for International Cooperation in Research and Higher Education (STINT, CS2018-7940), Sweden; Swedish Fund for Research Without Animal Experiments (N2020-0001), Sweden; Karolinska Institutet, and the Karolinska Institutet Stem Cell Organoid facility (KISCO), Sweden.

References

Yamanaka, S., 2012. Induced pluripotent stem cells: past, present, and future. Cell Stem Cell 10 (6), 678–684. https://doi.org/10.1016/j.stem.2012.05.005. PMID: 22704507.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., Jones, J.M., 1998. Embryonic stem cell lines derived from human blastocysts. Science 282, 1145–1147. https://doi.org/10.1126/science.282.5391.1145.

Schindelin, Johannes, Arganda-Carreras, Ignacio, Frise, Erwin, Kaynig, Verena, Longair, Mark, Pietzsch, Tobias, Preibisch, Stephan, Rueden, Curtis, Saalfeld, Stephan, Schmid, Benjamin, Tinevez, Jean-Yves, White, Daniel James, Hartenstein, Volker, Eliceiri, Kevin, Tomancak, Pavel, Cardona, Albert, et al., 2012. Fiji: an open-source platform for biological-image analysis. Nature Methods 9 (7), 676–682. https://doi.org/10.1038/nmeth.2019.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872.

Dakhore, S., Nayer, B., Hasegawa, K., 2018. Human pluripotent stem cell culture: current status, challenges, and advancement. Stem Cells Int. 2018, 7396905.

Okita, K., Matsumura, Y., Sato, Y., et al., 2011. A more efficient method to generate integration-free human iPS cells. Nat Methods 8, 409–412.