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Lab resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line (HMGUi002-A) from a healthy male individual

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A B S T R A C T

Induced pluripotent stem cells (iPSCs) can be used to generate different somatic cell types *in vitro*, including insulin-producing pancreatic β-cells. Here, we have generated iPSCs from a healthy male individual using an episomal reprogramming method. The resulting iPSCs are integration-free, have a normal karyotype and are pluripotent *in vitro* and *in vivo*. Furthermore, we show that this iPSC line can be differentiated into pancreatic lineage cells. Taken together, this iPSC line will be useful to test differentiation protocols towards β-cell as well as other cell types and will also serve as a control for drug development and disease modelling studies.

Resource utility

Integration-free human induced pluripotent stem cell (hiPSC) line from dermal fibroblasts of a healthy male individual can be used to test different differentiation protocols and will also serve as a control for drug development and disease modelling studies. (See Tables 1 and 2.)

Resource details

In order to create XM002 human induced pluripotent stem cell (hiPSC) line, primary adult skin fibroblasts were derived from a healthy male donor and then reprogrammed *via* transfection with three mixed episomal plasmids encoding human *OCT4*, *SOX2*, *NANOG*, *LIN28*, *KLF4*, and *L-MYC* (Fig. 1A). The reprogramming protocol consisted of three stages: transfection, reprogramming and expansion, was established in our laboratory (Fig. 1B). Colonies with stem cell-like characteristics started to appear around day 15 post-infection and were handpicked by day 21, when they were subjected to quality controls. XM002 iPSCs showed no integration of episomal vector and had a normal karyotype (Fig. 1C-D). Next, expression of pluripotency TFs (OCT4 and SOX2) and several pluripotency markers in XM002 iPSCs (TRA-1-81 and SSEA-4) was confirmed by immunostainings (Fig. 1E). In line with these results, FACS analysis showed that around 98% XM002 iPSCs were OCT4-positive (Fig. 1F). To thoroughly prove multi-lineage potency of the newly generated iPSC line, 2×10^6 iPSCs were injected subcutaneously into non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice to generate teratomas. The histological analysis of the induced teratomas revealed the presence of tissues from all three germ layers, confirming pluripotency of XM002 iPSCs *in vivo* (Fig. 1G). These data show that we have successfully reprogrammed human fibroblasts into pluripotent iPSCs that show expression of human pluripotent markers and display multi-lineage potency.

Next, we differentiated XM002 iPSCs into pancreatic β-cells using a previously established protocol recapitulating endocrine pancreas development (Rezania et al., 2014). Expression of markers specific to pancreatic progenitors (PDX1 and NKX6.1) and β-cells (C-PEPTIDE and PDX1) was verified by immunostaining. At the pancreatic progenitor stage, most cells co-expressed PDX1 and NKX6.1 and at the β-cell stage, we detected cells co-expressing PDX1 and C-PEPTIDE as shown by immunofluorescence (Fig. 1H).

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (*i.e.* Immunocytochemistry)	Assessed staining/expression of pluripotency markers: iPSCs are positive for OCT4, SOX2, TRA-1-81 and SSEA-4)	Fig. 1 panel E
	Quantitative analysis (*i.e.* Immunocytochemistry counting, Flow cytometry, RT-qPCR)	Around 98% of iPSCs are OCT4 positive.	Fig. 1 panel F
Genotype	Karyotype (G-banding) and resolution	46XY, XM002 iPSCs P7. Resolution 450–500	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	DNA Profiling performed 16 loci tested and all matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative	Supplemental figure1
Differentiation potential	Teratoma formation and Directed differentiation	iPSCs were differentiated into the three germ layers and into pancreatic lineage cells	Fig. 1 panel G and H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Materials and methods

Ethics statement

Selection of human donors as well as the procedures for skin biopsies, isolation, and characterization of dermal fibroblasts were done in accordance with protocols approved by the Ethics Committee of the Medical Faculty of the Eberhard Karls University, Tübingen. The study was performed according to the Declaration of Helsinki. Informed consent was given by all participants before the start of the study. Mice used in the study were housed in the animal facilities of the Helmholtz Zentrum München – German Research Center for Environmental Health (HMGU) and handled according to the German animal welfare legislation, guidelines of the Society of Laboratory Animals (GV-SOLAS) and of the Federation of Laboratory Animal Science Associations (FELASA). The teratoma formation protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of HMGU and reported to the local regulatory authority.

Skin biopsy, isolation, and characterization of dermal fibroblasts

Punch biopsy was used to take a full thickness skin specimen from the deltoid muscle area of the upper arm. Briefly, adipose tissue remnants and larger blood vessels were removed, followed by overnight digestion at 4 °C with 10 U/mL dispase II (Roche Diagnostics, Mannheim, Germany) in 50 mM HEPES pH 7.4, 150 mM NaCl. This was followed by incubation for 30 min at 37 °C with shaking at 1200 rpm. Next, the dermis was isolated from the epidermal layer using forceps, and fibroblasts were separated from the dermis using digestion with 0.2% collagenase CLS I (Biochrom, Berlin, Germany) in DMEM, 10% BSA for 45 min at 37 °C with shaking at1200 rpm. To purify fibroblasts, the digest was filtered through a 70 µm mesh, followed by centrifugation. The pelleted cells were then re-suspended, cultured for three days in DMEM, 10% FCS, and then expanded in Medium 106 supplemented with low serum growth supplement (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Dermal fibroblasts were checked for the presence of human pathogenic viruses, *i.e.*, HBV, HCV, and HIV, using Genesig PCR-based detection kits from Primerdesign Ltd. (Chandler's Ford, UK) and for the presence of mycoplasma with a PCR test kit from PanReac AppliChem (Darmstadt, Germany) and, in parallel with, by DNA staining with DAPI. All tested cultures were found to be negative for the tested contaminants.

XM002 iPSCs generation

Using a non-integrating Episomal iPSC Reprogramming Kit (Invitrogen, Cat. no. A14703), primary fibroblasts from a healthy male donor were reprogrammed into pluripotent stem cells. This kit uses a mixture of three vectors that have an oriP/EBNA-1 (Epstein–Barr nuclear antigen-1) backbone coding for six reprogramming factors: OCT4, SOX2, NANOG, LIN28, KLF4, and L-MYC (Yu et al., 2011). Human fibroblasts were transfected using Amaxa 4D-Nucleofector system and a nucleofector kit for human dermal fibroblasts (Lonza, Cat. no. VPD-1001). Next, fibroblasts were plated onto Geltrex-coated culture dishes and incubated in supplemented fibroblast medium prepared with knockout DMEM/F-12 (Life Technologies), 10% ESC-qualified FBS (Life Technologies), 1% MEM non-essential amino acids (Life Technologies), 10 µM HA-100 (Santa Cruz) and 4 ng/mL bFGF (Life Technologies). 24 h after transfection, the medium was switched to N2B27 medium supplemented with 0.5 µM PD0325901 (Stemgent), 3 µM CHIR99021 (Stemgent), 0.5 µM A-83-01 (Stemgent), 10 µM HA-100 (Santa Cruz), 10 ng/mL hLIF (Life Technologies) and 100 ng/mL bFGF (Life Technologies). Basal N2B27 medium contained DMEM/F12 with HEPES (Life Technologies), 1 × N2 supplement (Life Technologies), 1 × B27 supplement (Life Technologies), 1% MEM non-essential amino acids, 1 × Glutamax (Life Technologies) and 1 × β-Mercaptoethanol (Life Technologies). On day 15 after transfection, the medium was switched to Essential 8 medium and cultures were assessed for emerging iPSC colonies. Undifferentiated iPSC colonies with pluripotent morphology were picked and transferred onto fresh geltrex-coated culture dishes for expansion.

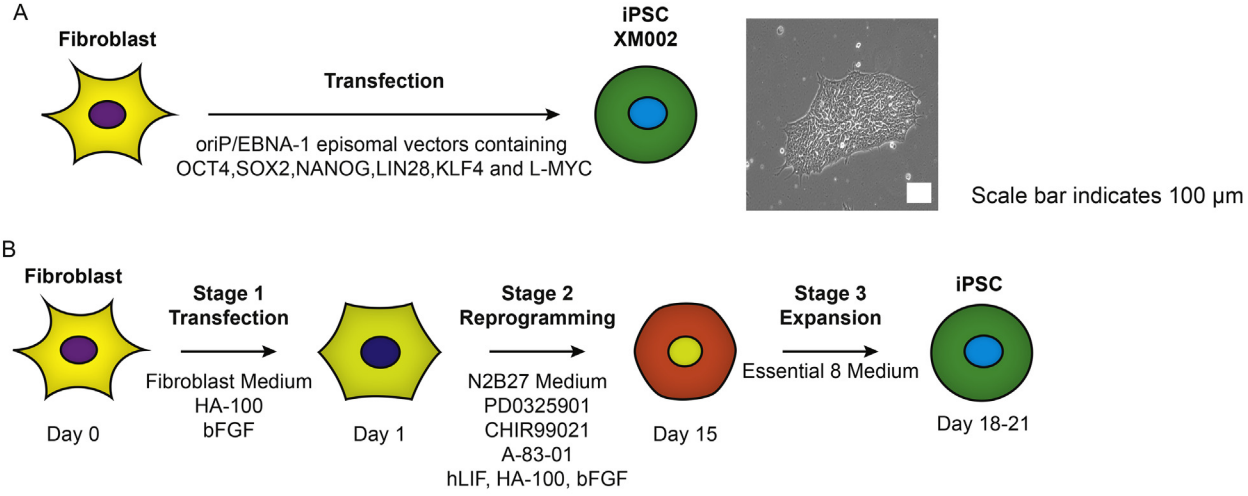
XM002 iPSCs characterization

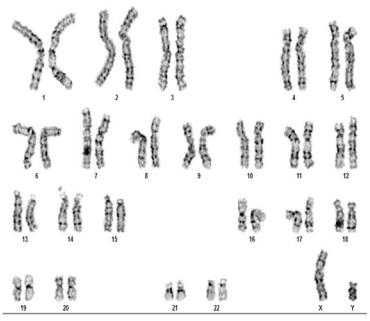
iPSC genomic DNA was extracted using a standard procedure. To exclude transgene integration, presence of the episomal backbone sequence was probed for by semi-quantitative PCR. For karyotyping cells were collected during growth in a logarithmic phase and used for karyotype analysis. Briefly, one day before incubation with colcemid (for 2 h), iPSCs were provided with fresh medium. iPSCs were trypsinized, processed with hypotonic solution (0.075 M KCl) for 20 min, and fixed with methanol:acetic acid (3:1). Metaphases of iPSCs were spread on microscope slides, and chromosomes were classified according to the International System for Human Cytogenic Nomenclature using the standard G banding technique. More than 20 metaphases were counted per cell line, and the final karyotype was stated if it was present in at least 85% of them. STR analysis was performed using the AmpFLSTR™

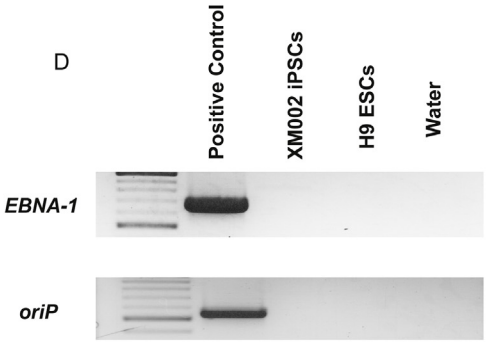
Table 2
Reagents details.

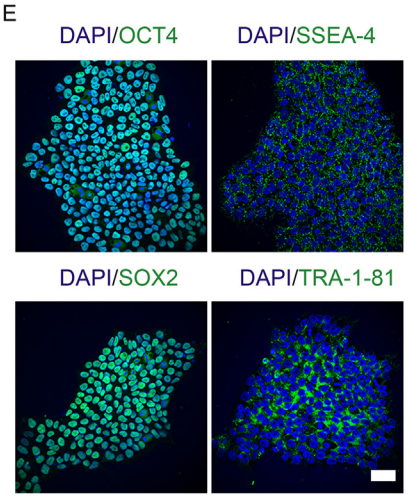
Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers (Immunofluorescence)	Goat anti-OCT4	1:500	Santa Cruz Cat #sc-8628, RRID: AB_653551
	Goat anti-SOX2	1:500	Santa Cruz Cat #sc-17,320, RRID: AB_2286684
	Mouse anti-SSEA4	1:500	Cell Signaling Cat #4755, RRID: AB_1264259
	Mouse anti-TRA-1-81	1:50	Millipore Cat #MAB4381, RRID: AB_177638
Pluripotency marker (flow cytometry)	Goat anti-OCT4	1:100	Santa Cruz Cat #sc-8628, RRID: AB_653551
Pancreatic differentiation markers	Goat anti-PDX1	1:500	R&D Systems Cat #AF2419, RRID:AB_355257
	Rabbit anti-NKX6.1	1:300	Acris/Novus Cat #NBP1–82553, RRID:AB_11023606
	Guinea pig anti-C– Peptide	1:100	Abcam Cat #ab30477, RRID:AB_726924
Secondary antibodies (Immunofluorescence)	Donkey anti-Goat IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:500	Thermo Fisher Scientific Catalog # A-11055, RRID:AB_2534102
	Donkey anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-21202, RRID:AB_141607
	Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	1:500	Thermo Fisher Scientific Cat# A-31572, RRID:AB_162543
	Alexa Fluor 488-AffiniPure Donkey Anti-Guinea Pig IgG (H + L)	1:500	Jackson ImmunoResearch Labs Cat# 706–545-148, RRID:AB_2340472
	Donkey anti-Goat IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	1:500	Thermo Fisher Scientific Cat# A-21432, RRID:AB_2535853
Secondary antibodies (flow-cytometry)	Donkey anti-Goat IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	1:500	Thermo Fisher Scientific Cat# A-21432, RRID:AB_2535853

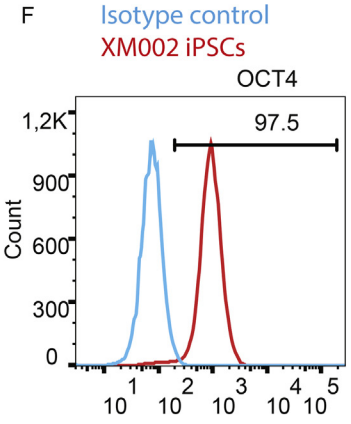
Primers		
	Target	**Forward/Reverse primer (5′-3′)**
Episomal Plasmids of genome sequencing (RT-PCR)	EBNA-1 (666 bp)	ATCGTCAAAGCTGCACACAG/ CCCAGGAGTCCCAGTAGTCA
	oriP (544 bp)	TTCCACGAGGCTAGTGAACC/ TCGGGGGTGTTAGAGACAAC

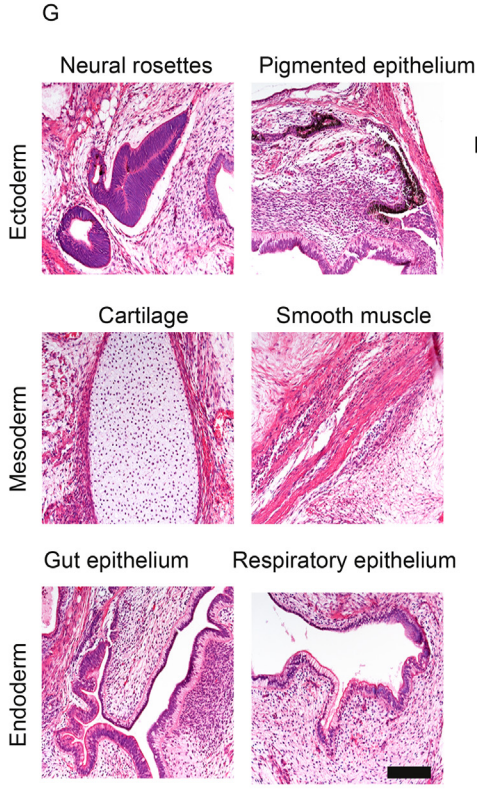












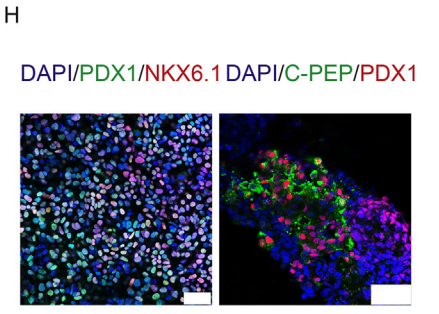


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Fig. 1. Characterization of HMGUi002-A (XM002) iPSC line. (A) Scheme showing reprogramming factors for the generation of XM002 iPSC line using episomal vectors. (B) Reprogramming protocol of skin fibroblasts into integration-free iPSCs. (C) Normal karyotype (46, XY) of the XM002 iPSC clone. (D) Semi-quantitative PCR confirms that episomal vectors did not integrate into the genomic DNA of the selected XM002 iPSC clone. H9 hESCs and water were used as negative controls, whereas transfected fibroblasts (6 days after transfection) were used as a positive control. (E) XM002 iPSCs have typical morphological hESCs characteristics and expression of several pluripotency markers. (F) Representative FACS plot of OCT4 + cells in XM002 iPSC clone. (G) Hematoxylin and eosin staining of tissue sections of teratomas generated from the XM002 iPSCs. (H) Immunofluorescence staining of PDX1 and NKX6.1 at the pancreatic progenitor stage and C-PEPTIDE and PDX1 at the β-cell stage.

Identifiler™ PCR Amplification Kit (Applied Biosystems, Cat. no. 4322288), according to manufacturer's instructions. To exclude transgene integration, presence of the episomal backbone sequence was probed for by semi-quantitative PCR. Briefly, iPSC genomic DNA was extracted using standard procedure. H9 ESCs and water were used as negative controls, whereas transfected fibroblasts (6 days after transfection) were used as positive control. Markers of the episomal backbone (oriP and EBNA-1) were amplified to exclude transgene integration. iPSCs were negative for mycoplasma as tested with the Lonza MycoAlert Mycoplasma Detection Kit (Lonza, Cat. no. LT07–418). For teratoma experiments, 2×10^6 iPSCs were injected into the right hind leg of immunocompromised NOD/SCID mice. Tumors were collected after 8 weeks, fixed, embedded in paraffin, sectioned and stained with hematoxylin/eosin (Li et al., 2012; Wang et al., 2018).

XM002 iPSCs culture

XM002 iPSCs were maintained on dishes coated with Matrigel (BD Biosciences) in mTeSR™1 medium (Stem Cell Technologies). At ~70–80% confluency, cultures were washed with 1 × DPBS without Mg^{2+} and Ca^{2+} (Invitrogen) and incubated with 1 × TrypLE Select Enzyme (Life Technology) for 3–5 min at 37 °C. Single cell suspension was prepared in mTeSR™1 medium, and spun at 1000 rpm for 3 min. Resulting cell pellet was resuspended in mTeSR™1 medium containing 10 μM Y-27632 (Sigma–Aldrich) and single cells were seeded at ~1.5×10^5 cells/cm^2 on Matrigel-coated surfaces. Cells were fed every day with mTeSR™1 medium and differentiation was started 48 h following seeding, when cultures reached ~90% confluency. The β-cell differentiation protocol was performed as previously described (Wang et al., 2019).

Immunofluorescence imaging and flow cytometry

For immunofluorescence staining, 4% paraformaldehyde was used to fix cells for 30 min. After that, cells were permeabilized in PBS containing 0.2% Triton X-100, then blocked with PBS containing 3% BSA, and incubated with primary antibodies overnight at 4 °C. Next day, the cells were incubated with the secondary antibodies for 1 h at room temperature and washed with PBS 3 times. Images were then acquired on a TCS SP5 laser-scanning microscope (Leica). For flow cytometry, iPSCs were dissociated using 1× TrypLE Select Enzyme, collected and washed with cold FACS buffer (5% FBS in 1× DPBS). iPSCs were fixed with 4% paraformaldehyde and permeabilized with donkey block solution (0.1% Tween-20, 10% FBS, 0.1% BSA and 3% donkey serum) containing 0.5% saponin. Next, iPSCs were incubated with primary antibody for 30 min at room temperature and then stained with appropriate secondary antibody for 30 min at room temperature. Flow cytometry was performed using FACS-Aria III (BD Bioscience). FACS data were analyzed using FlowJo.

Key resources table

Unique stem cell line identifier	HMGUi002-A
Alternative name(s) of stem cell line	XM002
Institution	Institute of diabetes and regeneration research
Contact information of distributor	Heiko Lickert heiko.lickert@helmholtz-muenchen.de
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 49 Sex: Male Ethnicity: Caucasian
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	A non-integrating episomal iPSC reprogramming kit, *OCT4*, *SOX2*, *NANOG*, *LIN28*, *KLF4*, and *L-MYC*
Genetic Modification	N/A
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 2014
Cell line repository/bank	N/A
Ethical approval	All experimental procedures described in the manuscript were approved by the Ethics Committee of the Eberhard Karls University Tübingen (approval # 629/2012BO2).

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Author disclosure statement

There are no competing financial interests in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101531.

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