Stem Cell Research 33 (2018) 120–124

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Multiple Cell Lines

Generation of two human isogenic iPSC lines from fetal dermal fibroblasts

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A B S T R A C T

Two isogenic hiPSC lines, ZIPi013-B and ZIPi013-E, were generated by reprogramming fetal dermal fibroblasts with episomal vectors. Previously, the same fetal fibroblasts were reprogrammed multiple times in a study comparing other reprogramming methods. As a consequence, the genomes have been sequenced multiple times. Both new cell lines offer the opportunity to study basic stem cell biology and model human disease. They can be applied as reference cell lines for creating isogenic clones bearing disease mutations on a well-characterized genomic background, as both cell lines have demonstrated excellent differentiation capacity in multiple labs.

Resource table

Unique stem cell lines identifier	ZIPi013-B ZIPi013-E
Alternative names of stem cell lines	ZIP13K2 (ZIPi013-B) ZIP13K5 (ZIPi013-E)
Institution	Zentrum für Integrative Psychiatrie gGmbH, Kiel, Germany
Contact information of distributor	PD Dr. Franz-Josef Müller, franz-josef.mueller@uksh.de
Type of cell lines	iPSC
Origin	human
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Transgene free, episomal
Multiline rationale	Isogenic clones
Gene 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	Stock date ZIPi013-B 8th December 2017, stock date ZIPi013-E 12th December 2017
Cell line repository/bank	N/A
Ethical approval	https://www.sciencellonline.com/technical-support/ethical-statement.html Ethikkommission der medizinischen Fakultät der Christian-Albrechts-Universität zu Kiel, approval number A145/11

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https://doi.org/10.1016/j.scr.2018.10.004
Received 20 August 2018; Received in revised form 28 September 2018; Accepted 2 October 2018
Available online 12 October 2018

Resource utility

While most human embryonic stem cell studies have been conducted with reference lines, there is a lack of widely available hiPSC reference lines. We have reprogrammed commercially sourced fetal fibroblasts with a deeply sequenced genome (Bhutani et al., 2016). The resulting hiPSCs show excellent differentiation capacity and are intended for wide distribution.

Resource details

We generated ZIPi013-B and ZIPi013-E cell lines from fetal dermal fibroblasts, following the reprogramming protocol described by Okita et al., 2011 (Okita et al., 2011). Bright-field (BF) images of both lines displayed colony-like morphology of tightly packed cells with high nucleus to cytoplasm ratio, hallmarks of human pluripotent stem cells (Fig. 1A). We performed immunocytochemistry for SOX2, NANOG, OCT-3/4, SSEA-4 and TRA–1–60 (Fig. 1A, scale bar 100 µm) to demonstrate the pluripotent-like state of generated hiPSC lines. A quantitative assessment of pluripotency markers by script-based counting of immunostained cells showed more than 90% of cells in both hiPSC lines are positive for SOX2, OCT-3/4, SSEA-4 and TRA–1–60 expression (Fig. 1A, B). Both lines passed PluriTest, a bioinformatic assay based on transcriptomic assessment, further confirming their pluripotency (Supplementary Fig. 1A). We performed monolayer-based, directed differentiation of both hiPSC lines into derivatives of all three germ layers in vitro, using commercially available differentiation reagents. After differentiation into endodermal lineage, the vast majority of cells expressed the endodermal markers forkhead box A2 (FOXA2) and SRY-box 17 (SOX17) (Fig. 1B, scale bar 20 µm). Likewise, following a mesodermal differentiation protocol, both cell lines differentiated into beating cardiomyocytes and expressed actinin alpha 1 (ACTN1) and

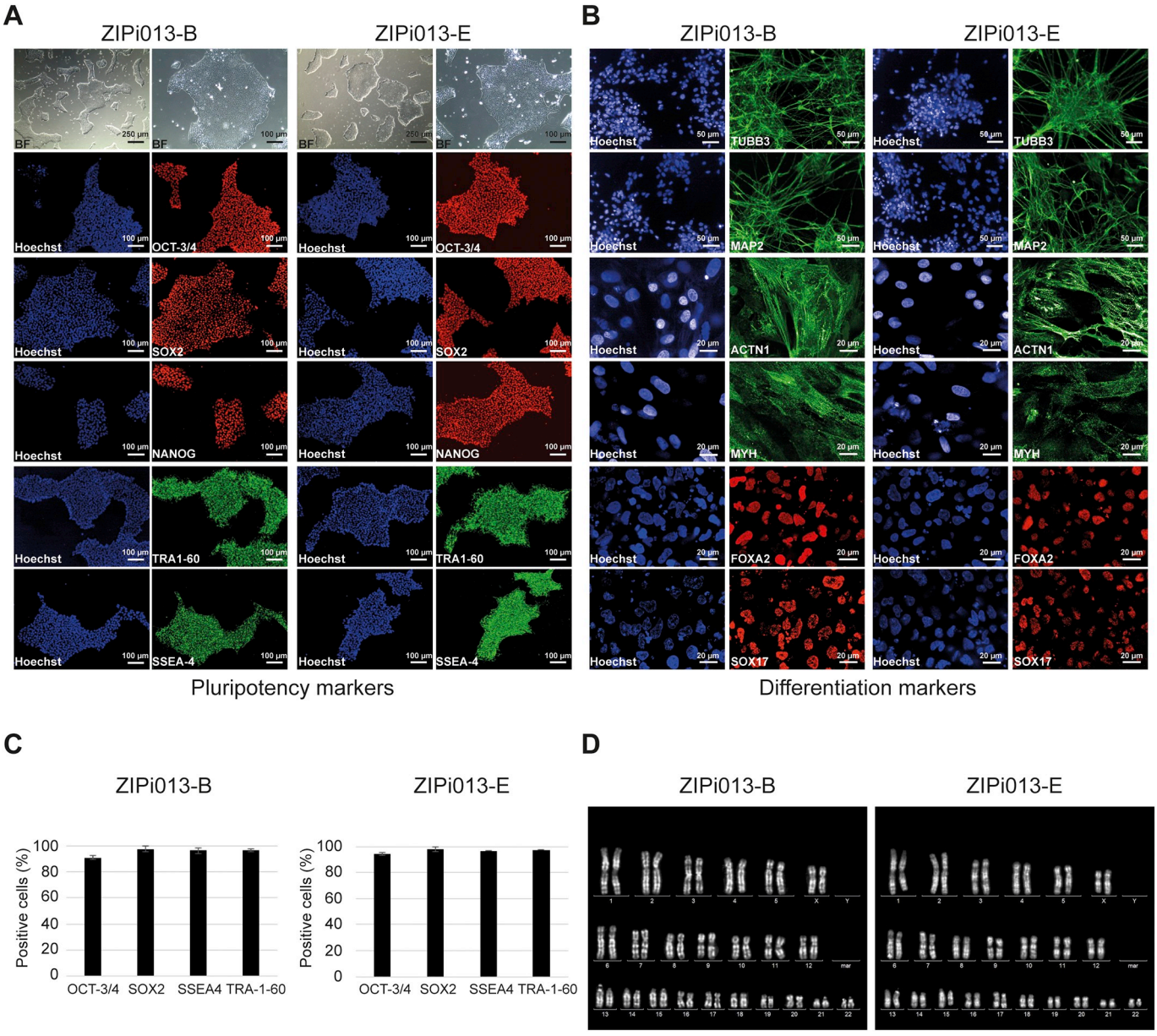

Fig. 1. Characterisation of hiPSC lines ZIPi013-B and ZIPi013-E. (A) Phase contrast images of respective hiPS cells and immunofluorescent analysis of pluripotency markers (OCT-3/4, SOX2, NANOG, TRA-1-60, SSEA-4). (B) Immunofluorescent analysis of in vitro differentiated cells from both lines indicates expression of markers associated with ectoderm (TUBB3, MAP2), mesoderm (ACTN1, MYH) and endoderm (FOXA2, SOX17). (C) Quantification of results from (A). (D) Karyotyping confirmed that cells maintained a normal euploid karyotype.

myosin heavy chain (MYH) on day 18 after inducing differentiation (Fig. 1B, scale bar 20 µm). Widespread expression of tubulin beta 3 class lll (TUBB3) and microtubule-associated protein 2 (MAP2) after 15 days in neural differentiation conditions confirmed successful lineage commitment and maturation into neuro-ectodermal derivatives (Fig. 1B, scale bar 50 µm). This result was further corroborated in ScoreCard assays which not only confirmed the cell's ability to differentiate into all three germ layers post induction of spontaneous differentiation in embroid bodies generated from ZIPi013-B and ZIPi013-E but also hinted towards a bias for mesodermal differentiation for both lines (Supplementary Fig. 1B).

The analysis of 16 short tandem repeats and Amelogenin loci proved that both clones were derived from the same parental fibroblast cell line. Karyograms obtained from Giemsa stainings of metaphase chromosomes of ZIPi013-B (at passage 14) and ZIPi013-E (at passage 18) verified that both hiPSC lines had a normal karyotype (Fig. 1D). Both clones were negative for mycoplasma at multiple time points during the characterization (Supplementary Fig. 1C). All data for the characterization of the lines are summarized in Tables 1-3.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
ZIPi013-B	N/A	Female	N/A	North American	N/A	N/A
ZIPi013-E	N/A	Female	N/A	North American	N/A	N/A

Materials and methods

Sourcing and culturing of fibroblasts

The dermal fibroblast cell line (HDF51) derived from an aborted female fetus were initially sourced from ScienCell™ (#2300). Cells used in this study were a generous gift from Jeanne Loring (The Scripps Research Institute, La Jolla, CA, USA). HDF51 were cultured in DMEM supplemented with 10% FBS and 2 mM L-glutamine.

Reprogramming

HDF51 were reprogrammed as previously published (Okita et al., 2011). A total of 1×10^6 cells were transfected with 1.5 µg of each of the following episomal plasmids pCXLE-hUL, pCXLE-hSK and pCXLE-hOCT3/4-shp53F (Addgene, 27,080, 27,078 27,077, respectively). The Neon™ Transfection System (ThermoFischer Scientific, MPK500) in combination with the Neon™ Transfection System 100 µl Kit (ThermoFischer Scientific, MPK10025) and Resuspension Buffer R for adherent cells was used to electroporate the cells at 1650 V, 3 time pulses for 10 ms. Following electroporation, cells were resuspended in fibroblast medium without antibiotics and seeded onto Mitomycin C-treated and thereby mitotically inactivated mouse embryonic fibroblast feeder cells. Two days post transfection, medium was switched to TeSR-E7 (StemCell Technologies, 05910) and emerging colonies were manually picked between day 25–40 and transferred onto Matrigel coated plates (Corning, 354,234). Subsequent feeder-free culturing was performed using EDTA-based passaging after every 4–5 days with a 1:4 split ratio. Cells were cultured in a 5% CO_2 atmosphere at 37 °C.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	normal	Fig. 1A
Phenotype	Qualitative analysis Immunocytochemistry	OCT-3/4, SOX2, NANOG, SSEA-4 and TRA-1-60	Fig. 1A
	Quantitative analysis	OCT-3/4	Fig. 1C
	Script-based cell counting	91.1 ± 1.3% (ZIPi013-B) 94.3 ± 1.4% (ZIPi013-E) SOX2 97.8 ± 2.0% (ZIPi013-B) 97.7 ± 1.8% (ZIPi013-E) SSEA-4 96.5 ± 2.0% (ZIPi013-B) 96.8 ± 0.2% (ZIPi013-E) TRA-1-60 96.7 ± 0.8% (ZIPi013-B) 97.4 ± 0.1% (ZIPi013-E)	
Genotype	Karyotype (G-banding) and resolution	Both lines 46XX, 450–500 bphs (band per haploid set)	Fig. 1D
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 16 sites tested and all matched	N/A Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	PCR-based Negative	Supplementary Fig. 1C
Differentiation potential	Directed differentiation	Ectodermal derivative: TUBB3 and MAP2	Fig. 1B
		Mesodermal derivative: ACTN1 and MYH	Fig. 1B
		Definitive endoderm: FOXA2 and SOX17	Fig. 1B
	PluriTest	Pluripotency and Novelty Score passed	Supplementary Fig. 1A
	ScoreCard	Indicates pluripotency and differentiation potential into three germ layers	Supplementary Fig. 1B
Donor screening (OPTIONAL)	HIV 1 + 2, Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
Description	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Mouse anti-SOX2	1:200	Thermo Fisher Scientific, Cat # MA1–014, AB_2536667
Pluripotency marker	Rabbit anti-NANOG	1:50	Abcam, Cat # ab21624, AB_446437
Pluripotency marker	Rabbit anti-OCT-3/4	1:200	Cell Signalling Technology, Cat # 2750, AB_823583
Pluripotency marker	Mouse anti-SSEA-4	1:100	Merck Millipore, Cat # MAB4304, AB_177629
Pluripotency marker	Mouse anti-TRA-1-60	1:100	Merck Millipore, Cat # MAB4360, AB_2119183
Differentiation marker	Mouse anti-SOX17	1:100	R&D SYSTEMS, Cat # MAB1924, AB_2195646
Differentiation marker	Rabbit anti-FOXA2	1:100	Abcam, Cat # ab108422, AB_11157157
Differentiation marker	Rabbit anti-ACTN1	1:25	Thermo Fisher Scientific, Cat # PA5–17308, AB_10986146
Differentiation marker	Mouse anti-MYH	1:50	Abcam, Cat # ab50967, AB_942084
Differentiation marker	Mouse anti-TUBB3	1:500	Biolegend, Cat # MMS-435P, AB_2313773
Differentiation marker	Mouse anti-MAP2	1:200	Abcam, Cat # ab 11,267, AB_297885
Secondary antibody	Goat anti-rabbit IgG Alexa Fluor 488 conjugated	1:1000	Thermo Fisher Scientific, Cat # A-11008, AB_143165
Secondary antibody	Goat anti-mouse IgG Alexa Fluor 488 conjugated	1:1000	Thermo Fisher Scientific, Cat # A-11001, AB_2534069
Secondary antibody	Goat anti-mouse IgM Alexa Fluor 488 conjugated	1:1000	Thermo Fisher Scientific, Cat # A-21042, AB_2535711

Primers		
	Target	Forward/Reverse primer (5′-3′)
e.g. Episomal Plasmids (qPCR)	N/A	N/A
e.g. Pluripotency Markers (qPCR)	N/A	N/A
e.g. House-Keeping Genes (qPCR)	N/A	N/A
e.g. Genotyping	N/A	N/A
e.g. Targeted mutation analysis/sequencing	N/A	N/A

In vitro differentiation into germ layers

STEMdiff Definitive Endoderm Kit (StemCell Technologies, 05110) was used according to the manufacturer's instructions to differentiate hiPSCs into endodermal cells. Differentiation into mesodermal derivatives was achieved using Pluricyte Cardiomyocyte kit (Ncardia, PM-CDK805) resulting in beating cardiomyocytes after day 8. To induce ectodermal lineage, we differentiated hiPSCs into Neural Stem Cells (NSCs) using Neural Induction Kit (Thermo, A1647801). Seven days post induction, NSCs were seeded for terminal differentiation into mixed neuronal cultures. Briefly, 3×10^4 NSCs/cm^2 were seeded onto Laminin (Thermo, 23,017,015) and Poly-L-Ornithine (Sigma, P4957) coated dishes using neural differentiation medium composed of Neurobasal medium supplemented with 1× GlutaMAX, 200 µM ascorbic acid, 1× B-27 and 1× CultureOne supplement for additional 10 days with partial media change every other day.

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.1% Triton X-100 for 20 min and blocked with 5% FBS for 60 min at room temperature (RT). Incubation of the primary antibodies was carried out overnight at 4 °C followed by 60 min incubation of secondary antibodies at RT. Nuclei were stained with 1 µg/ml of Hoechst (Sigma, H6024) for 20 min at RT. Cells were then visualized using an Opera High Content Imaging System (PerkinElmer).

Assessment of pluripotency

Expression of pluripotency-associated markers (SOX2, OCT-3/4, SSEA-4 and TRA1–60) was quantified by image-based scripting algorithms as described in (De Sousa et al., 2017) using Columbus Analysis Software (PerkinElmer). PluriTest and ScoreCard assay were performed following the procedures outlined in the original publications (Müller et al., 2011; Tsankov et al., 2015), respectively.

Karyotyping and STR analysis

Karyotyping was performed using standard G-banding techniques on metaphase chromosomes. Metaphase arrest was induced by incubating the cells with Colcemide solution (0.1 µg/ml) for 1 h at 37 °C. Chromosomal content was released by incubation with hypotonic solution (0.075 M KCl) for 20 min at 37 °C. Chromosomes were fixed with methanol:glacial acetic acid = 3:1 and stained with 5% Giemsa solution (phosphate buffer, pH 6.8) for 10 min at room temperature. 22 metaphase spreads were analysed for both ZIPi013-B and ZIPi013-E. PowerPlex 16 multiplex STR analysis (Promega, DC6531) was performed according to manufacturer's instructions for the DNA typing of parental fibroblasts, ZIPi013-B and ZIPi013-E.

Mycoplasma testing

Universal Mycoplasma Detection Kit (ATCC, 30-1012 K) was performed according to manufacturer's instructions to confirm the absence of mycoplasma in HDF51 just prior to reprogramming and after every 5 passages in hiPSC culture.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.10.004.

Acknowledgement

We are very grateful to PD Dr. Almuth Caliebe from the Institute of Human Genetics, University Hospital Schleswig-Holstein (Kiel) and PD Dr. Susanne Bens from the Institute of Human Genetics, University Hospital Ulm for providing karyotype analysis. The study was supported by a bilateral grant from the Bundesministerium für Bildung und Forschung (BMBF, Germany) and the California Institute for Regenerative Medicine (CIRM, United States) (13GW0128A to FJM and 13GW0128B to OP). In addition, FJM received funding from the BMBF (01GM1513D) and from the Deutsche Forschungsgemeinschaft (DFG, Germany) (MU 3231/3-1 and Schleswig-Holstein Cluster of Excellence, EXC 306 “Inflammation at Interfaces”).

References

Bhutani, K., Nazor, K.L., Williams, R., Tran, H., Dai, H., Džakula, Ž., ... Loring, J.F., 2016. Whole-genome mutational burden analysis of three pluripotency induction methods. Nat. Commun. 7, 10536.

De Sousa, P.A., Steeg, R., Wachter, E., Bruce, K., King, J., Hoeve, M., ... Allsopp, T.E., 2017. Rapid establishment of the European bank for induced Pluripotent Stem Cells (EBISC)- the *Hot Start* experience. Stem Cell Res. 20, 105–114.

Müller, F.-J., Schuldt, B.M., Williams, R., Mason, D., Altun, G., Papapetrou, E., ... Loring, J.F., 2011. A bioinformatics assay for pluripoteny in human cells. Nat. Methods 8 (4), 315–317.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., ... Yamanaka, S., 2011. A more efficient method to generate integration-free human iPS cells. Nat. Methods 8 (5), 409–412.

Tsankov, A.M., Akopian, V., Pop, R., Chetty, S., Gifford, C.A., Daheron, L., ... Meissner, A., 2015. An improved ScoreCard to assess the differentiation potential of human pluripotent stem cells. Nat. Biotechnol. 33 (11), 1182–1192.