Supporting dataset of two integration-free induced pluripotent stem cell lines from related human donors

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Integration-free induced pluripotent stem cells from related human donors’ exhibit great potential to the ongoing development of organ models. Blood cells from two different human donors were isolated, purified and reprogrammed into induced pluripotent stem cells. These induced pluripotent stem cell lines were characterized precisely for pluripotency markers (with the PluriTest and flow cytometry analysis) and their differentiation capacities into meso-, ecto- and endoderm. The induced pluripotent stem cell lines are available for commercial use and are therefore of high interest for many groups working in stem cell research. A normal karyotype of the induced pluripotent stem cells was proven with the KaryoStat assay. In total 6 human donors that belong to one family donated blood for induced pluripotent stem cell reprogramming. In this “Data in Brief” publication, we show the dataset for the two male iPSC lines HUMIMIC TISSUi006-A (StemUse106) and TISSUi007-A (StemUse107). The main characterisation was recently published by Ramme et al. in Stem Cell Research [1]. All iPSC lines were also examined negative for any mycoplasma or bacteria contamination.

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Specifications Table

| Subject | Cell Biology |
|---------|-------------|
| Specific subject area | induced pluripotent stem cells |
| Type of data | Figures and Table |
| How data were acquired | Flow Cytometry: MACSQuant® Analyzer 10 (Miltenyi Biotec), FlowLogic (Miltenyi Biotec) software |
| Description of data collection | KaryoStat®: assay was performed by Life Technologies Corporation PluriTest: assay was performed by Life Technologies Corporation Mycoplasma test: PCR by Eurofins Genomics Europe Applied Genomics GmbH Sterility: ifp (Institut für Produktqualität GmbH) with the membrane filtration method |
| Data source location | TissUse GmbH, Oudenarder Str. 16, 13347 Berlin, Deutschland |
| Data accessibility | With the article |
| Related research article | Ramme AP, Faust D, Koenig L, Nguyen T, Marx U. Generation of two additional integration-free iPSC lines from related human donors. Stem Cell Res. (2021) [1] https://doi.org/10.1016/j.scr.2021.102327 |

Value of the Data

- These supporting data provide further characterisation of the two induced pluripotent stem cell lines TISSU006-A (StemUse106) and TISSU007-A (StemUse107).
- Both iPSC lines are with donor consent for commercial use and therefore especially attractive for industrial use.
- Both human donors for the iPSC lines belong to the family from which 4 iPSC lines are already established and published [2] to study the influence of age and gender on the reprogramming into iPSCs and differentiation into specific cell types.

1. Data Description

Two healthy male humans from a Caucasian family (family tree in Fig. 2) donated blood for iPSC generation. The iPSC lines TISSU006-A (StemUse106) and TISSU007-A (StemUse107) were generated by integration-free reprogramming by transfection with episomal vectors (Epi5 Kit, ThermoFisher 15960). Four additional iPSC lines from the same family were generated before [2].

Both iPSC lines were versatility characterized for expression of pluripotency marker, sterility, normal karyotype and differentiation capacities.

The pluripotency of the iPSC lines TISSU006-A (StemUse106) and TISSU007-A (StemUse107) was proved with the transcriptomic data based bioinformatics assay PluriTest (Fig. 1A), The
Fig. 1. PluriTest® data (A) and KaryoStat™ data (B) of StemUse106 and 107 iPSCs. A: The pluripotency plot window provides a visual representation of the tested samples in the analysis. The pluripotency and novelty x/y scatter plot combines the pluripotency score on the y-axis with the novelty score on the x-axis. The red and blue background hint to the empirical distribution of the pluripotent (red) and non-pluripotent (blue) samples in the reference data set. The distribution of the StemUse 106 and 107 can be seen. A non-iPSC sample was included in this experiment to serve as a negative control for non-pluripotency. B: Whole genome view. The whole genome view displays all somatic and sex chromosomes in one frame with high level copy number. The smooth signal plot (right y-axis) is the smoothing of the log2 ratios which depict the signal intensities of probes on the microarray. A value of 2 represents a normal copy number state (CN = 2). A value of 3 represents chromosomal gain (CN = 3). A value of 1 represents a chromosomal loss (CN = 1). The pink, green and yellow colors indicate the raw signal for each individual chromosome probe, while the blue signal represents the normalized probe signal which is used to identify copy number and aberrations (if any). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Family tree of the six donors for iPSC generation. Yellow: the two additional iPSC lines SU106 and SU107. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
PluriTest, is an online bioinformatics assay comparing transcriptional profiles of a new iPSC line to 450 other profiles based on expression levels from known pluripotent and differentiated genes. A pluripotency score and novelty score is provided and can be compared to established iPSC or ESC lines [3]. The pluripotency and novelty x/y scatter plot combines the Pluripotency Score on the y-axis with the Novelty Score on the x-axis. The red and blue background hint to the empirical distribution of the pluripotent (red) and non-pluripotent samples (blue) in the reference data set.

The KaryoStat™ assay allows for digital visualization of chromosome aberrations with a resolution similar to g-banding karyotyping and was performed by Life Technologies Corporation. The size of structural aberration that can be detected and is >2 Mb for chromosomal gains and >1 Mb for chromosomal losses. The KaryoStat™ array is optimized for balanced whole-genome coverage with a low-resolution DNA copy number analysis, the assay covers all 36,000 RefSeq genes, including 14,000 OMIM® targets. The assay enables the detection of aneuploidies, submicroscopic aberrations, and mosaic events. No chromosomal aberrations were found in the iPSC lines TISSUi006-A (StemUse106) and TISSUi007-A (StemUse107) when comparing against the reference dataset (Fig. 1 B).

For flow cytometry analysis single cell suspension surface and intracellular staining was performed according to the manufacturer’s protocol, with the antibodies listed in Table 1. The raw files are provided as supplementary material.

Flow cytometry analysis for SU106 showed 98% TRA-1-60+/ SSEA1- cells (Fig. 3). SSEA1 is an early differentiation marker and, therefore, used as a negative surface marker of human iPSCs. A total of 99% of the cells were TRA-1-60+/SSEA5+ (Fig. 3); both are surface pluripotency markers. Furthermore, 99% of the cells showed double positive expression of the intracellular markers OCT3/4 and SOX2 (Fig. 3). A total of 99% of the cells were NANOG+ (Fig. 3). Isotype control staining were used as negative controls for gating the populations (Fig. 3).

Flow cytometry staining for SU107 reveals more than 98% of the cell population are positive for TRA-1-60 and negative for SSEA1, 99% are positive for SSEA5 and TRA-1-60, 93% are positive for SOX2 and 96% are positive for NANOG and OCT3/4 (Fig. 4).

We performed monolayer-based directed differentiation of the iPSC lines into ectoderm cells (neural cells), endoderm cells and mesoderm cells (cardiomyocytes). Definitive endoderm differentiation was performed using a commercial available kit and resulted into more than 98%
CXCR4 positive cells and more than 94% FOXA2 positive cells (Figs. 5 and 6). TISSUi006-A and TISSUi007-A iPSCs differentiated into beating cardiomyocytes shown by the expression of myosin heavy chain (MHC) and cardiac troponin T (cTnT) (between 6% and 47% depending on the iPSC line) (Figs. 7 and 8).

Both iPSC lines were tested negative for mycoplasma (Fig. 9) and bacterial contaminations.
Fig. 4. Flow cytometry characterization of the StemUse107 iPSCs. Gating was adjusted to fluorescence minus one controls. %P = % of live single-cell population.
Fig. 5. Definitive endoderm (DE) characterization of StemUse106 iPSCs by flow cytometry. Gating was adjusted to isotype controls. %P = % of live single-cell population.

2. Experimental Design, Materials and Methods

Cell cultures were incubated at 37°C, 5% CO₂ and 95% air humidity. All cell culture work was performed in sterile conditions under a laminar flow hood, according to good cell culture practice. All growth factors, small molecules or chemicals were diluted according to the manufacturer's instructions. Cell culture plates and components were purchased from Corning U.S. unless otherwise stated.

Isolation of peripheral blood mononuclear cells

In order to generate the TissUse iPSC lines (StemUse 106 and 107), PBMCs were isolated from peripheral blood from two human donors. Human blood samples were donated, with informed consent and ethics approval (Ethic Committee Berlin Chamber of Physicians, Germany), in compliance with the relevant laws. Around 60 mL of peripheral blood in K2EDTA vacutainers (BD) was used for PBMC isolation per donor. The blood was diluted 1:2 with phosphate-buffered saline (PBS) (without Ca²⁺ & Mg²⁺) and the solution was slowly overlaid on lymphocyte separation medium in 50-mL falcon tubes. Afterwards, the falcons, with blood overlaid on lymphocyte separation medium, were centrifuged at 20°C for 25 min at 2000 rpm without break. Subsequently, the thin white layer of mononuclear cells between the plasma and lymphocyte separation medium was transferred into a new 50-mL falcon. The plasma fraction was frozen at
Fig. 6. Definitive endoderm (DE) characterization of StemUse107 iPSCs by flow cytometry. Gating was adjusted to isotype controls. %P = % of live single-cell population.

Fig. 7. Flow cytometry analysis of cardiomyocytes differentiated from StemUse106 iPSCs. Gating was adjusted to isotype controls. %P = % of live single-cell population.
Fig. 8. Flow cytometry analysis of cardiomyocytes differentiated from StemUse107 iPSCs. Gating was adjusted to unstained control. %P = % of live single-cell population.

Fig. 9. Cell culture supernatants were tested negative for mycoplasmas by PCR by Eurofins Genomics Europe Applied Genomics GmbH. The mycoplasma test was conducted for the following mycoplasma species: M. arginini, M. fermentans, M. orale, M. hyorhinis, M. hominis, M. genitalium, M. salivarium, M. synoviae, M. pirum, M. gallisepticum, M. pneumoniae, M. yeatsii, Spiroplasma citri and Acholeplasma laidlawii. Please note the test is not restricted to the mentioned species. In silico analysis has shown that more than 100 additional Mollicutes strains can be detected. Possible inhibition of the PCR reaction was verified by an internal control. Additional mycoplasma positive and negative controls were included to monitor the results. Water controls indicated the absence of PCR contaminations. Using plasmid dilutions a detection limit of 10 mycoplasma copies per test was demonstrated.

-80°C. The mononuclear cell fraction was washed three times with PBS (without Ca²⁺ & Mg²⁺), quantified and frozen in 10% DMSO in RPMI medium in cryogenic storage vials at -80°C. After one day, the vials were transferred into the nitrogen tank. Three vials of PBMCs per donor with around 3 x 10⁶ cells per vial were transferred to Phenocell SAS for reprogramming into iPSCs. Reprogramming was performed by Phenocell SAS with episomal vectors (Epi5 Kit, Thermo Fischer A15960). The iPSCs were maintained in feeder-free conditions on growth factor reduced (GFR) Matrigel-coated cell culture plates in StemMACSTM iPSC-Brew XF.

Matrigel coating of cell culture plates

Coating of cell culture plates with Matrigel, vitronectin, laminin or similar is necessary for the iPSC attachment and pluripotency maintenance. The iPSC lines SU106 and SU107 were grown on GFR Matrigel. The proteins in Matrigel polymerize and solidify at RT, therefore, cold handling at 4°C is necessary. GFR Matrigel was thawed at 4°C overnight for coating the cell culture plates. Afterwards, it was diluted 1:10 in cold KO/DMEM F12 with cold pipet tips at 4°C. Aliquots of 1 mL were prepared and stored at -20°C, the 1:10 Matrigel dilution was additionally diluted 1:10 in KO/DMEM F12 before coating the cell culture plates. Therefore, the end concentration of
the GFR Matrigel was 1:100, 1 ml of 1:100 Matrigel was used for one 6-well. The surface should be covered completely by the Matrigel solution. The coating was incubated at RT for 1 h. Before iPSC seeding, the coating was evacuated and the iPSC medium was added immediately.

Single-cell passaging of iPSCs with Accutase

The iPSCs need to be passaged every five to seven days, depending on the growth manner of the iPSC line. All StemUse iPSC lines were split every five to seven days by single-cell passaging with Accutase. The cell culture medium was discarded from the cells, the latter were washed twice with PBS (with Ca2+ & Mg2+) and Accutase was added (1 ml per 6-well) to dislodge the cells. After 5 min in the incubator, the iPSC medium with 10 μM RI was added and the cells were dislodged by pipetting. The suspension was transferred to a 50-ml falcon tube and centrifuged at 300 g for 5 min at RT. The supernatant was discarded and the cells diluted in iPSC medium with 10 μM RI. After counting the cells with the NucleoCounter®, 4,000 cells/cm² were seeded in iPSC medium with 10 μM RI on plates coated with GFR Matrigel. Cells were evenly distributed by moving the plate carefully three times forwards and backwards and side to side, before transferring them into the incubator. StemMACS™ iP-S-Brew XF medium without RI was renewed after 48 h, following a daily medium exchange.

Direct differentiation into definitive endoderm

Both iPSC lines were differentiated into the definitive endoderm (DE) using the STEMdiff™ DE Kit (TeSR™-E8™ Optimized, STEMCELL Technologies), according to the manufacturer’s instructions, with minor modifications. The iPSCs were split with Accutase and seeded with 33,000 cells/cm² cells on GFR Matrigel in iPSC medium supplemented with 10 μM RI and STEMdiff™ DE TeSR™-E8™ Supplement (1:20). Following medium changes were performed according to the manufacturer’s instructions.

Direct differentiation into cardiomyocytes

Both iPSC lines were differentiated successfully into beating cardiomyocytes. SU106 iPSCs were grown until 50% confluent, the cells were washed gently with PBS and the medium was replaced with cardiac differentiation medium (RPMI 1640 with L-glutamine, 2% B-27™ supplement minus insulin, 50 μg/ml L-ascorbic acid 2-phosphate magnesium salt hydrate, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol and 1% penicillin-streptomycin) and the addition of 5 μM CHIR-99021 (LC Laboratories). After 48 h, the medium was replaced with cardiac differentiation medium with 5 μM Wnt-C59 (Cayman). After an additional 48 h, the cells were washed gently with PBS and the medium was replaced with differentiation medium with 2% normal B-27™ supplement instead of B-27™ supplement minus insulin. The latter was changed every two to three days from then on. SU107 iPSCs were grown until 90% confluent, following the medium was replaced with cardiac differentiation medium (RPMI 1640 with L-glutamine, 2% B-27™ supplement without insulin, 50 μg/ml L-ascorbic acid 2-phosphate magnesium salt hydrate, 2 mM L-glutamine and 1% penicillin-streptomycin) and the addition of 10 μM CHIR-99021 (Sigma). After one day, the medium was changed to cardiac differentiation medium without CHIR-99021. After 24 h, the medium was replaced with cardiac differentiation medium with 3 μg/ml IWP-4 (Cayman). After an additional 3 days, the medium was replaced with cardiac differentiation medium without IWP-4. After an additional 48 h, the medium was replaced with cardiac differentiation medium with 2% normal B-27™ supplement instead of B-27™ supplement minus insulin. The latter was changed every two to three days from then on. Flow cytometry analysis of cardiomyocytes was performed on day 9 for StemUse106 and on day 17 for StemUse107.

Flow cytometry

Suspension surface staining was performed according to the manufacturer’s protocol (Miltenyi Biotec). Intracellular staining was performed with the Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher), according to the manufacturer’s protocol. Automatic compensation with fluorescent beads was performed according to the manufacturer’s protocol (Miltenyi Biotec). Live cell discrimination was performed at the beginning of the panel setup of all different flow cytometry analyses to analyse where the living cells, dead cells and debris populations are in the FSC/SSC plot. Data was acquired on a MACSQuant® Analyzer 10 (Miltenyi Biotec) flow cytometer and analysed with the FlowLogic (Miltenyi Biotec) software. Gates were adjusted
according to the respective isotype controls for StemUse106 and to the fluorescence minus one controls for StemUse107 iPSCs considering only the viable single-cell population.

Karyotyping

The KaryoStat™ assay was performed by Life Technologies Corporation with iPSCs of both lines in passage 17. Cells were prepared according to the Genomic DNA Purification Kit (Catalog #: K0512) and quantified using the Qubit™ dsDNA BR Assay Kit (Catalog #: Q32850). 250 ng total gDNA was used to prepare the GeneChip® for KaryoStat™ according to the manual, and is an array that looks for copy number variants and single nucleotide polymorphisms across the genome.

Mycoplasma and bacterial testing

Cell culture supernatants were tested negative for mycoplasmas by PCR by Eurofins Genomics Europe Applied Genomics GmbH. The sterility of cell culture supernatants was tested by the contract lab ifp (Institut für Produktqualität GmbH) with the membrane filtration method.

Ethics Statement

Human blood samples were used with ethical approval by Ethikkomission der Ärztekammer Berlin; Eth 25/16.

CRediT Author Statement

Anja Patricia Ramme: Conceptualization, Data curation, Writing – original draft; Daniel Faust: Data curtion; Leopold Koenig: Data curtion; Nhutuyen Nguyen: Data curtion; Uwe Marx: Conceptualization, Supervision.

Associated Research Article

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Uwe Marx is a founder of TissUse GmbH, which commercializes MPS platforms. Anja Ramme, Daniel Faust, Leopold Koenig and Nhutuyen Nguyen are employed by TissUse GmbH.

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Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.dib.2021.107140.
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