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Generation of one control and four iPSCs clones from patients with Emery-Dreifuss muscular dystrophy type 1

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Lab Resource: Multiple Stem Cell Lines - template

Title: Generation of one control and four iPSCs clones from patients with Emery-Dreifuss muscular dystrophy type 1.

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Abstract:

Emery-Dreifuss muscular dystrophy type 1 (EDMD1) is a rare genetic disease caused by mutations in the *EMD* gene coding for a nuclear envelope protein emerin. We generated and characterized induced pluripotent stem cells (iPSCs) from two EDMD1 patients bearing a mutation c.del153C and from one healthy donor. That mutation leads to generation of premature STOP codon. Established iPSCs are very valuable tool for disease pathogenesis investigation and for the development of new therapeutic methods after differentiation to cardiac or muscle cells. Obtained iPSCs show the proper morphology, pluripotency markers expression, normal karyotype and potential to differentiate into three germ layers.

Resource Table:

Unique stem cell lines identifier	*UWRBTi001-A* *UWRBTi003-B* *UWRBTi003-C* *UWRBTi004-A* *UWRBTi004-B*
Alternative names of stem cell lines	*C1M35 1.9 (UWRBTi001-A)* *E1M40 1.7 (UWRBTi003-B)* *E1M40 1.9 (UWRBTi003-C)* E1M51 1.4 (*UWRBTi004-A)* E1M51 1.8 (*UWRBTi004-B)*
Institution	*University of Wroclaw, Faculty of Biotechnology, Wroclaw, Poland*
Contact information of distributor	*Magdalena Machowska, magdalena.machowska@uwr.edu.pl*
Type of cell lines	*iPSCs*
Origin	*Human*
Cell Source	*Fibroblasts*
Clonality	*Clonal*
Method of reprogramming	*Sendai virus transduction*
Multiline rationale	*Two patients with the same disease and mutation, and control of the similar age and the same gender.*
Gene modification	*Yes*
Type of modification	*Hereditary*

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Gene/locus	*EMD gene locus Xq28, mutation c.del153C*
Method of modification	*N/A*
Name of transgene or resistance	*N/A*
Inducible/constitutive system	*N/A*
Date archived/stock date	*N/A*
Cell line repository/bank	*N/A*
Ethical approval	*Patient cells were collected according to ethical and medical standards set by Polish law and based on approval of the ethical committee (DOP-GMO.431.85.2018; DOP-GMO.431.79.2018) and after obtaining written consent from the patients.*

Resource utility

iPSCs generated from EDMD1 patients' fibroblasts are an useful and valuable cellular tool for obtaining a muscle, cardiac and nerve cells after differentiation which may be used for investigation of EDMD1 pathogenesis and for development and testing new treatment methods.

Resource Details

Emery-Dreifuss muscular dystrophy type 1 (EDMD1) is a rare genetic disorder. It is X-linked disease with prevalence of 1 per 100 000 male births, caused by mutations in the *EMD* gene coding for nuclear envelope protein emerin (Viggiano et al., 2019). The main symptoms of EDMD1 are observed in the skeletal muscles and in the heart, and they are weakness and muscle wasting, tendon contractures, and cardiac dysfunction (Emery, 1989).

Dermal fibroblasts were obtained from a skin biopsy from two EDMD1 patients' and healthy donor. We generated induced pluripotent stem cells (iPSCs) using non-integrating Sendal virus (SeV) vectors encoding four Yamanaka factors, Oct, Sox2, Klf4, and c-Myc. After 3-4 weeks of reprogramming, colonies were picked, transferred and expanded. Two iPSCs clones obtained from each of patient and one clone from control were established (**Table 1**) and characterized to confirm pluripotency (**Table 2**). The morphology of colonies was typical for iPSCs (**Figure 1 A**). The expression of pluripotency markers was confirmed by RT-qPCR for *Oct4, Sox2, NANOG, LIN28, hTERT* and *REX-1* using human fibroblasts as a negative control and human Episomal iPSC line (EpiPSC, A18945 Gibco) as a positive control (**Figure 1 C**). Protein expression of the pluripotency markers Oct4, Sox2, SSEA4, SSEA5 was analysed by immunofluorescence around passage 10 (**Figure 1 B**). The absence of Sendai vectors was confirmed around passage 15 (**Figure 1 F**). The karyotype of all parental cells and iPSCs was normal (**Figure 1 D**). Short tandem repeats (STRs) analysis showed the matching identity in all 16 tested loci of the parental dermal fibroblasts and generated iPSCs. The sequencing of amplified *EMD* gene confirmed the presence of c.del153C mutation in patients' iPSCs and wild type sequence in control cells (**Figure 1 H**). All iPSCs clones and fibroblasts were tested for mycoplasma with negative results (**Figure 1 G**). All iPSCs lines had the ability to differentiate into three germ layers *in vitro*, what was confirmed by immunofluorescence analysis by staining for germ layers markers: SOX17 (endoderm), Brachyury (mesoderm), Otx2 (ectoderm) (**Figure 1 E**).

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iPSC generation and expansion

Fibroblasts obtained from skin biopsies from patients affected by EDMD1 and healthy donor were cultured in DMEM with high glucose 4,5 g/L (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), Glutamax (Gibco) and Pen/Strep (Gibco). Fibroblasts were plated on 35 mm dishes and after 2 days transduced with a set of non-integrating Sendai viruses using the CytoTune™ 2.0 Sendai reprogramming kit (Invitrogen). The next day and then every other day, medium was changed to fresh fibroblast medium. At day 7, cells were plated on dishes coated with Geltrex (Gibco) in full fibroblasts' medium. From the following day, the medium was replaced with Essential 8 Medium (Gibco) every day. After 3-4 weeks individual iPSCs colonies were manually picked and transferred to new Geltrex-coated 24-well plates. iPSCs were expanded in Essential 8 Medium on Geltrex-coated vessels, they were dissociated with 0.5 mM EDTA in DPBS and split every 4-5 days at a ratio 1:8-1:12 using ROCK inhibitor. All cells were cultured at 37 °C in 5% CO_2.

Immunocytochemistry

iPSCs were plated on Geltrex-coated glass coverslips and after 3-4 days they were fixed in 4% paraformaldehyde for 15 minutes, wash with PBS, permeabilized with 0.5% Triton X-100 for 10 minutes at RT and wash again in PBS. Cells were blocked with 1% donkey serum (DS, Gibco) for 30 minutes at RT, then cells were incubated with primary unconjugated antibodies overnight at 4°C, washed with PBS and incubated with secondary antibodies or primary antibodies conjugated with fluorophore for 1 h at RT, and washed again with PBS. All antibodies were diluted in 1% DS in PBS, antibodies used are shown in **Table 3.** Coverslips were mounted on glass slides with DABCO mounting medium (Fluka) with DAPI. Staining was visualized on a Zeiss Axiovert A1 fluorescence microscope using 10 x objective using ZEN Software.

Gene expression analysis

Total RNA was extracted using small RNA miRNeasy Mini Kit (Qiagen). 1 µg of total RNA was reverse transcribed to cDNA using SuperScript VILO cDNA synthesis kit (Life Technologies). qRT-PCR were performed using SYBR Green Master Mix (Applied Biosystems). Each sample was analyzed in triplicate using QuantStudio 6 Flex Real-Time PCR System Software (Applied Biosystems). The relative gene expression for pluripotency markers was expressed relatively to a certified Episomal iPSC lineage (EpiPSC) and normalized to Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (**Table 3**).

The episomal vectors presence

Total RNA was isolated using Universal RNA Purification Kit (EurX). cDNA was synthesized using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific). PCR analysis of episomal vectors presence was performed using primers shown in **Table 3**. PCR was performed with Taq polymerase (EURx), with 35 cycles of amplification using thermocycler TOne 96G (Biometra). Normal human dermal fibroblasts (Lonza) were used as a negative control, cells short after Sendai transduction were used as a positive control. *GAPDH* was used as a control.

Karyotyping

Obtained iPSC lines and fibroblasts were treated with 0.67 µg/mL colcemid (BioWest) for 2 hours, dissociated by Trypsin-EDTA (Gibco) and centrifuged 1400 rpm, 7 min. After incubation in hypotonic solution (Ohnuki's solution) and fixation in methanol:glacial acetic acid (3:1) mixture (Chempur) microscopic slides were prepared. G-banded metaphase analysis was performed according to International System for Human Cytogenomic Nomenclature 2016 (ISCN 2016; AGT manual) employing the Imager.M1 (Zeiss) microscope and the Ikaros

from fibroblasts (resolution 350-400) were karyotyped.

STR Analysis

Profiling of the human cell lines was done by Microsynth company using highly polymorphic short tandem repeat loci (STRs). STR loci were amplified using the PowerPlex® 16 HS System (Promega). Fragment analysis was done on an ABI3730xl (Life Technologies) and the resulting data were analyzed with GeneMarker HID software (Softgenetics).

Sequencing

Genomic DNA was isolated using Basic DNA Purification Kit, EurX. The *EMD* gene was amplified with Phusion hot start II DNA Polymerase (Thermo Scientific), with 1x Phusion GC buffer and 3% DMSO; primers are listed in **Table 3**. PCR product was purified with PCR/DNA Clean-Up Purification Kit (EuX). The DNA sequencing was performed by Microsynth company with sequencing primer listed in **Table 3**.

Mycoplasma test

Mycoplasma test was performed using PCR Venor®GeM Classic Mycoplasma Detection Kit (Minerva Biolabs) according to manufacturer's instructions.

In vitro differentiation to three germ layers

Trilineage differentiation *in vitro* was carried out using Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems), that contains specifically supplemented media and growth factors that can be used to differentiate iPSCs into endoderm, ectoderm, and mesoderm. For characterization of each of the three cell types, antibodies listed in **Table 3** were used for immunofluorescence staining.

Additional files:

Figure 1

Table 1, 2 and 3

STR analysis

Analysis Reports for Cell Line Typing, Table with STR profiles summary

Supplementary files

Supplementary figure 1

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iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
C1M35 1.9 (UWRBTi001-A)	*C1M35 1.9*	*Male*	*35*	*Caucasian*	*EMD gene locus Xq28, CCCCCCAG*	*N/A*
E1M40 1.7 (UWRBTi003-B)	*E1M40 1.7*	*Male*	*40*	*Caucasian*	*EMD gene locus Xq28, mutation c.153delC CCCCC_AG*	*Emery-Dreifuss Muscular Dystrophy type 1 (EDMD1)*
E1M40 1.9 (UWRBTi003-C)	*E1M40 1.9*	*Male*	*40*	*Caucasian*	*EMD gene locus Xq28, mutation c.153delC CCCCC_AG*	*Emery-Dreifuss Muscular Dystrophy type 1 (EDMD1)*
E1M51 1.4 (*UWRBTi004-A)*	E1M51 1.4	*Male*	*51*	*Caucasian*	*EMD gene locus Xq28, mutation c.153delC CCCCC_AG*	*Emery-Dreifuss Muscular Dystrophy type 1 (EDMD1)*
E1M51 1.8 (*UWRBTi004-B)*	E1M51 1.8	*Male*	*51*	*Caucasian*	*EMD gene locus Xq28, mutation c.153delC CCCCC_AG*	*Emery-Dreifuss Muscular Dystrophy type 1 (EDMD1)*

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Classification	Test	Result	Data
Morphology	Photography	*Visual record of the line: normal*	*Figure 1 panel A*
Phenotype	Qualitative analysis Immunocytochemistry	Positive staining for pluripotency markers: Oct4, Sox2, SSEA4, SSEA5	Figure 1 panel B
	Quantitative analysis RT-qPCR	Expression of the pluripotency markers: Oct4, Sox2, NANOG, LIN28	Figure 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XY Resolution 350-450	Figure 1 panel D, Supplementary Figure 1 A
Identity	Microsatellite PCR (mPCR)	DNA Profiling not performed	N/A
	STR analysis	16 loci tested, all matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	DNA sequencing	X-linked mutation c.153delC	Figure 1 panel H, Supplementary Figure 1 B
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: negative	Figure 1 panel G
Differentiation potential	Directed differentiation	Positive staining for three germ layers: SOX17 (endoderm), Brachyury (mesoderm), Otx2 (ectoderm)	Figure 1 panel E
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

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Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	Rabbit anti-OCT4 Mouse anti-SSEA4 DyLight550 Mouse anti-SOX2 DyLight550 Mouse anti-SSEA5 DyLight488	1:100 1:100 1:50 1:100	Cell Signaling Cat# 2750, RRID# AB_823583 Invitrogen Cat# MA1-021-D550, RRID# AB_2536689 Invitrogen Cat# MA1-014-D550, RRID# AB_2536669 Invitrogen Cat# MA1-144-D488, RRID# AB_2536849
Differentiation Markers	Goat anti-SOX17 Goat anti-Otx2 Goat anti-Brachyury	1:10 1:10 1:10	R&D Systems Cat# AF1924, RRID# AB_355060 R&D Systems Cat# AF1979, RRID# AB_2157172 R&D Systems Cat# AF2085, RRID# AB_2200235
Secondary antibodies	Donkey anti-Goat AF568 Donkey anti-Rabbit TRITC Donkey anti-Rabbit AF488	1:200 1:50 1:200	Invitrogen Cat# A11057, RRID# AB_142581 Jackson Immunoresearch Cat# 711-025-152, RRID# AB_2340588 Jackson Immunoresearch Cat# 711-545-152, RRID# AB_2313584
Primers			
	Target	**Forward/Reverse primer (5’-3’)**	
Episomal Plasmids (PCR)	SeV (genomic sequence of Sendai virus) KOS (Human Klf4, Oct3/4, Sox2) Klf4 c-Myc	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG	
Pluripotency Markers (qPCR)	NANOG Oct4 Sox2 LIN28 hTERT REX-1	CCCAAAGGCAAACAACCCACTTCT/AGCTGGGTGGAAGAGAACACAGTT AGTTTGTGCCAGGGTTTTTG/ACTTCACCTTCCCTCCAACC AGCTACAGCATGATGCAGGA/GGTCATGGAGTTGTACTGCA AGTAAGCTGCACATGGAAGG/ATTGTGGCTCAATTCTGTGC GGAGCAAGTTGCAAAGCATTG/TCCCACGACGTAGTCCATGTT TCGCTGAGCTGAAACAAATG/CCCTTCTTGAAGGTTTACAC	
House-Keeping Genes (qPCR)	GAPDH for qPCR	GTGAAGGTCGGAGTCAACG/GGTGGAATCATATTGGAACATG	

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		GGTC
Targeted mutation sequencing	*EMD* gene amplification Amplicon sequencing	CTCCCGCGGTTAGGTCCCG/TTCCCCAAAGACCTAGCTCTG CTCCCGCGGTTAGGTCC

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contribution

MM, CB and RR designed the study. CB and MM generated and expanded iPSCs. MM performed immunofluorescence analysis, *in vitro* differentiation to three germ layers, Mycoplasma test and prepared cells or cell pellets for karyotyping, STR analysis, sequencing and analysis of episomal vector presence. CB carried out gene expression analysis. IŁ conducted karyotyping. KP performed the analysis of episomal vectors presence and prepare samples for sequencing. MM wrote the original draft of the manuscript. CB, IŁ, KP and RR reviewed and edited the manuscript.

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Viggiano, E., Madej-Pilarczyk, A., Carboni, N., Picillo, E., Ergoli, M., Gaudio, S.D., Marchel, M., Nigro, G., Palladino, A., Politano, L., 2019. X-Linked Emery-Dreifuss Muscular Dystrophy: Study Of X-Chromosome Inactivation and Its Relation with Clinical Phenotypes in Female Carriers. Genes (Basel) 10.

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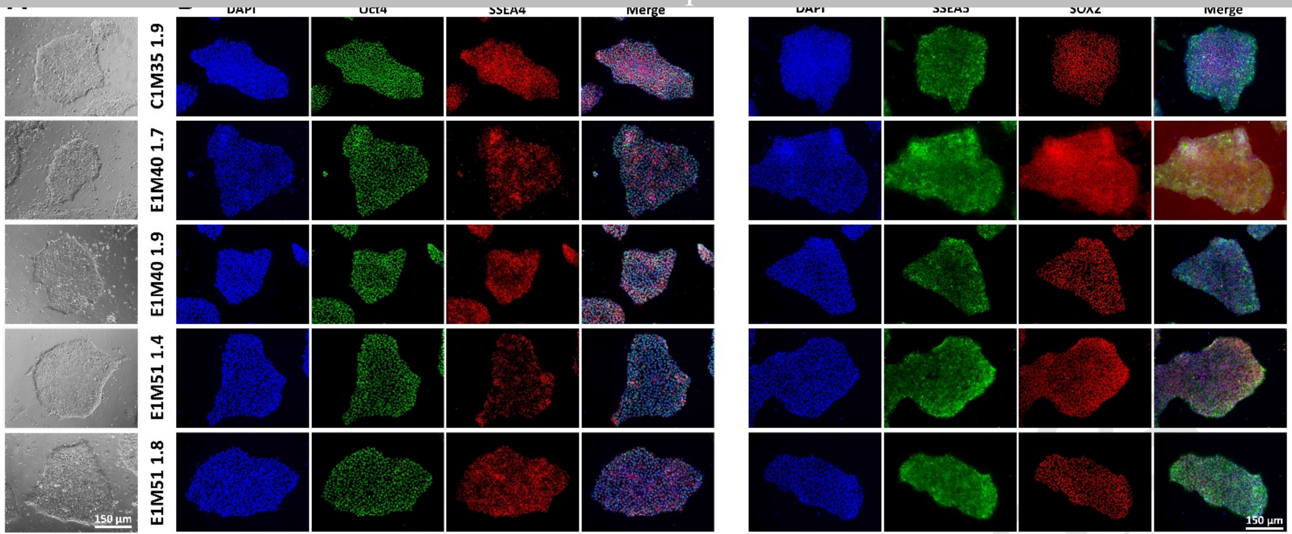
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SSEA4
Merge
SSEA3
SOX2
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E1M40 1.7
E1M40 1.9
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E1M51 1.8
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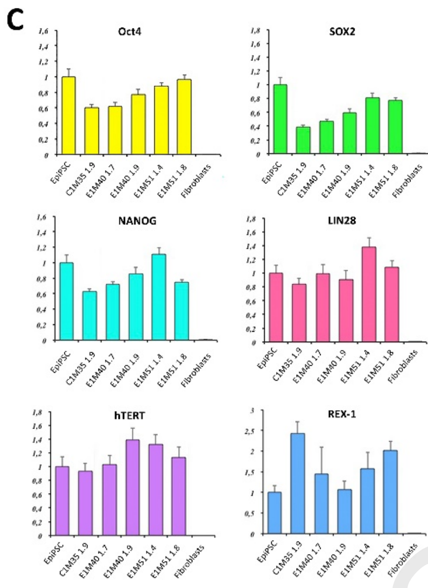
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NANOG
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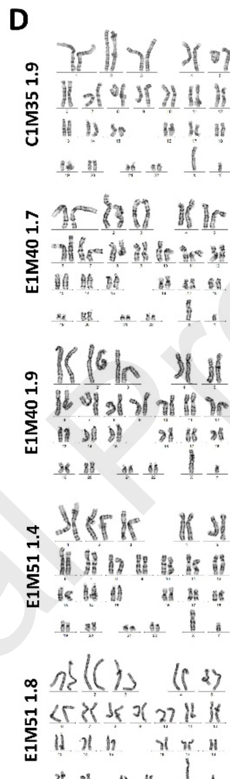
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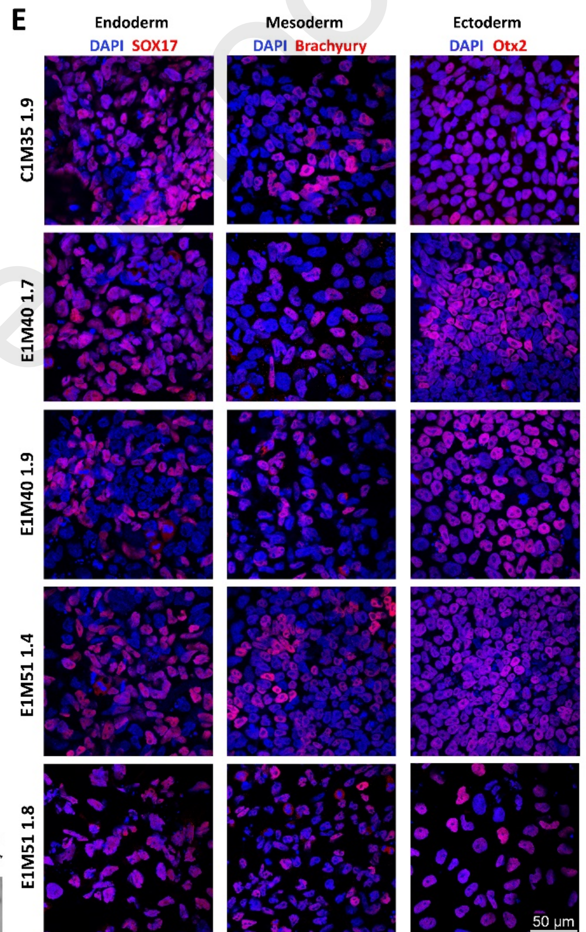
E
Endoderm
DAPI SOX17
Mesoderm
DAPI Brachyury
Ectoderm
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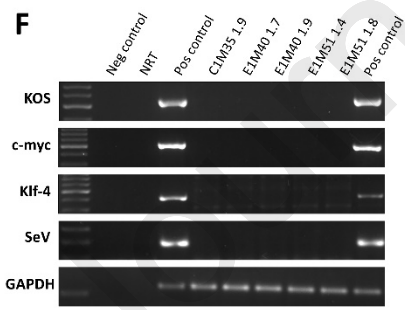
C1M35 1.9
E1M40 1.7
E1M40 1.9
E1M51 1.4
E1M51 1.8
50 µm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NRT
Pos control
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E1M40 1.7
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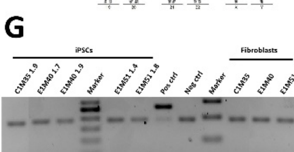
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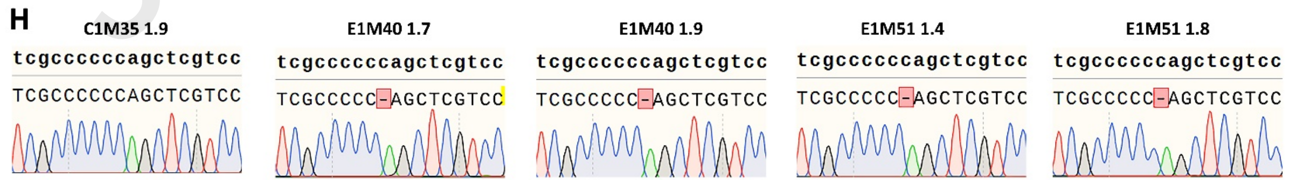
iPSCs
Fibroblasts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E1M40 1.7
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