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Lab resource: Stem Cell Line

Generation of the human induced pluripotent stem cell (hiPSC) line PSMi005-A from a patient carrying the KCNQ1-R190W mutation

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A B S T R A C T

We generated human induced pluripotent stem cells (hiPSCs) from dermal fibroblasts of a woman carrier of the heterozygous mutation c.568C > T p.R190W on the *KCNQ1* gene. hiPSCs, obtained using four retroviruses enconding the reprogramming factors OCT4, SOX2, cMYC and KLF4, display pluripotent stem cell characteristics, and can be differentiated into spontaneously beating cardiomyocytes (hiPSC-CMs).

Resource table

Unique stem cell line identifier	PSMi005-A
Alternative name of stem cell line	HDF29-LQT1-iPS
Institution	Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
Contact information of distributor	Massimiliano Gnecchi, m.gnecchi@unipv.it
Type of cell line	hiPSC
Origin	human
Additional origin info	Age: 37 Gender: female Ethnicity: Caucasian
Cell source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Retroviruses encoding for the human cDNA of OCT4, SOX2, cMYC, KLF4
Genetic modification	No
Type of modification	N/A
Associated disease	Long QT Syndrome type 1 (OMIM #192500)
Gene/locus	568C > T mutation on *KCNQ1,* 11p15.5-p15.4 (NM_000218.2)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/Constitutive system	N/A
Date archived/stock date	Jan 7, 2013
Cell line repository/bank	No
Ethical approval	The study has been approved by the Ethics Committee of our Institution, Fondazione IRCCS Policlinico San Matteo, on the 29th of October 2010, protocol number 20100004354, proceeding P-20100003369. We obtained patient written informed consent for both skin biopsy procedure and conservation of biological samples.

Resource utility

It has been proven that iPSCs and iPSC-CMs can be efficiently used to model LQTS and test targeted therapies (Mehta et al., 2018; Gnecchi

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et al., 2017; Mura et al., 2017; Rocchetti et al., 2017). PSMi005-A cell line will be of help to: (1) modelling of LQTS type 1; (2) targeted drug testing.

Resource details

The PSMi005-A cell line was generated by reprogramming of dermal fibroblasts derived from a skin biopsy of 37 years old woman diagnosed with Long QT Syndrome type 1 (LQT1). LQTS is an autosomal dominant inherited disease characterized by the prolongation of cardiac repolarization, reflected by a prolongation of the QT interval on the surface electrocardiogram (ECG) (Schwartz et al., 2012). The repolarization defect predisposes to "Torsades de Pointes" (TdP), a type of ventricular tachycardia that can cause syncope or even sudden cardiac death (SCD). LQT1 is the most common LQTS sub-type, accounting for ~40–50% of all LQTS cases. It is caused by mutations in the *KCNQ1* gene, encoding for the α-subunit of the voltage-dependent potassium channel responsible for the delayed rectifier potassium current (I_{Ks}), one of the repolarization currents in the heart (Schwartz et al., 2012).

The patient carries the heterozygous mutation c.568C/T on the *KCNQ1* gene, that causes the substitution of the arginine in position 190 with tryptophan. Despite the fact that her QTc (QT corrected for heart rate) in basal condition is normal and she never experienced cardiac symptoms, she was placed in preventive therapy with propranolol 2.0 mg/kg/die.

Fibroblasts were reprogrammed by retroviral infection of OCT4, SOX2, KLF4 and c-MYC. The hiPSCs were maintained on feeders. The disease causing mutation on the *KCNQ1* gene is present on both patient's fibroblasts and the derived hiPSCs, as shown by DNA sequencing (Fig. 1A. *The KCNQ1 coding sequence -CDS- used as a reference is the NCBI sequence NM_000218.2*). They also present an identical DNA profile at 7 polymorphic loci, as shown by Short tandem Repeat (STR) analysis (available with authors). Moreover the DNA karyotyping revealed normal female features (46, XX) (Fig. 1B). PSMi005-A retains embryonic stem cell (ES)-like morphology and pluripotent features up to passage 50 (Fig. 1C). It uniformly expresses the human ES surface antigens Tumor Related Antigen-1-60 and − 1-81 (TRA-1-60, TRA-1-81), Stage Specific Embryonic Antigen-4 (SSEA-4), and shows alkaline phosphatase (AP) activity (Fig. 1C). Likewise, it expresses the pluripotent markers NANOG, OCT4, SOX2, REX1, GDF3, ESG1, DPPA2, DPPA4 and NODAL (Fig. 1C and D), and shows OCT4 promoter demethylation (Fig. 1E*, open circles indicate unmethylated CpG dinucleotides, while closed circles indicate methylated CpGs*). The percentage of $NANOG^+$ cells determined by immunocytochemistry counting was 99.4%, (Fig. 1F). RT-PCR analysis in Fig. 1G shows no expression of the four viral transgenes (Tg) in naïve fibroblasts (HDF), clear expression of Tg OCT4, SOX2, KLF4 and cMYC in fibroblasts 5 days after transduction (OSKM) and silencing of the four Tg in PSMi005-A at passage 3.

As expected, PSMi005-A spontaneously forms embryoid bodies (EBs) able to differentiate into cells belonging to the three germ layers: endoderm (alpha-fetoprotein-AFP), mesoderm (alpha smooth muscle actin-αSMA, troponin I- TnI) and ectoderm (tubulin beta III-Tuj, microtubule-associated protein 2-MAP2) (Fig. 1H). Moreover, PSMi005-A differentiated also into spontaneously beating cardiomyocytes, expressing the sarcomeric proteins alpha-actinin (α-SA) and troponin T (TnT) (Fig. 1I*, the insets show areas of cross-striation*). We also verified the absence fo mycoplasma contamination in our PSMi005-A line (Fig. 1J).

Materials and methods

Detailed protocols are provided as Supplemental Methods.

hiPSC generation

Skin fibroblasts were reprogrammed using four retroviruses encoding OCT4, SOX2, KLF4 and c-MYC. Emerging iPSC clones were manually picked, individually placed into a separate cell culture well and expanded on a feeder-layer of mitotically-inactivated mouse embryonic fibroblasts (iMEF), in DMEM/F12, 20% Knockout Serum Replacement (KO-SR), 2 mM L-glutamine, 50 U/ml penicillin, 50 U/ml streptomycin, 1% Non-Essential Amino Acids (NEAA), 0.1 mM beta-mercaptoethanol, 10 ng/ml basic Fibroblast Growth Factor (bFGF) (Table 1).

Mutation analysis

KCNQ1 exon 3 was amplified with primers in Table 2 and the GoTaq G2 DNA polymerase (Promega). The resulting amplicon (size in Table 2) was purified and sequenced (Lightrun service - GATC Biotech AG – Germany).

STR analysis

STR analysis was carried out using PowerPlex® CS7 System (Promega) kit, following the manufacturer's protocol. Fragments were run on a 3130xl capillary sequencer (Applied Biosystems). Genotypes were assigned using GeneMarker software (SoftGenetics).

Karyotyping

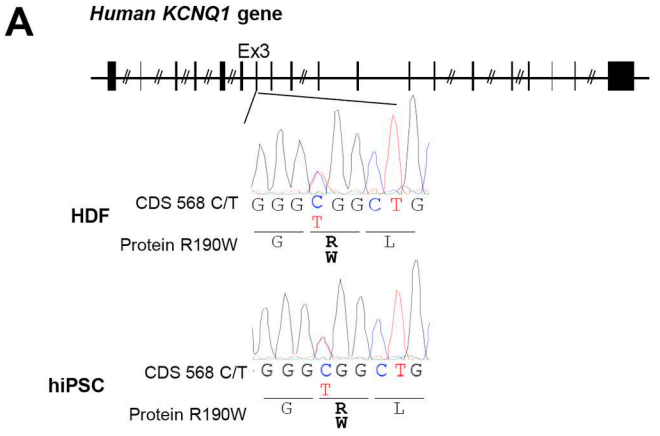
hiPSCs were blocked at metaphase by exposition to 10 μg/ml demecolcine solution (Sigma Aldrich) for 3 h. Karyotyping was performed using 300 G-banding chromosome analysis.

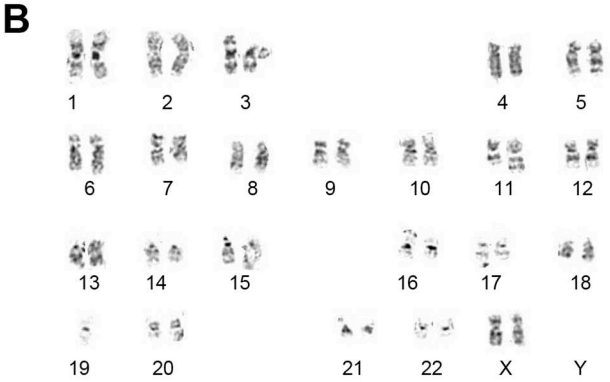
Immunocytochemistry

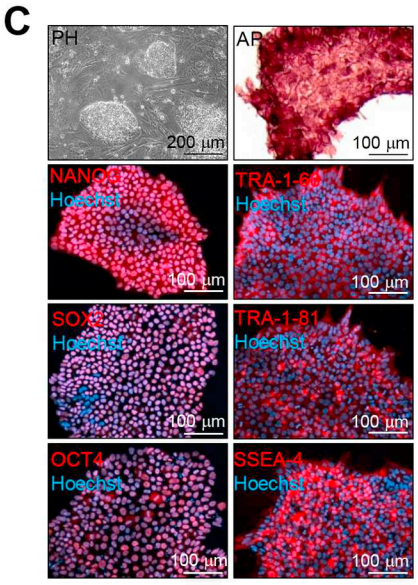
hiPSCs and their derivatives were grown on glass coverslips, and then fixed for 15 min in 4% paraformaldehyde (Affymetrix USB), permeabilized with 0.1% Triton X-100 (Sigma Aldrich) for 5 min, and blocked in 1% bovine serum albumin (BSA, Sigma Aldrich) for 1 h at room temperature (RT). Then they were incubated for 1 h at RT with the primary antibody (Table 2) diluted in blocking solution, washed three times, and incubated for 1 h at RT with an appropriate secondary antibody (Table 2). Finally, the cells were stained with 1 μg/ml of Hoechst 33258 (Sigma Aldrich). Images were acquired using the Carl Zeiss fluorescence microscope Observer.Z1 equipped with the Apotome system and AxioVision 6.0 software (Zeiss GmbH, Gottingen, Germany).

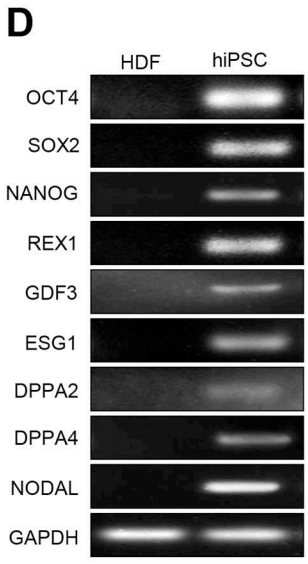
Immunocytochemistry counting

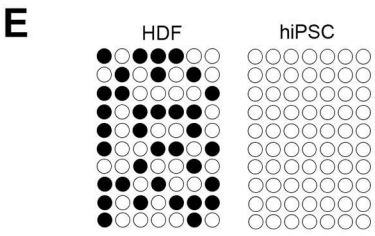
$Nanog^+$ cells and the nuclei stained with Hoechst 33258 were counted in five fields using the AxioVision 6.0 software (Zeiss GmbH, Gottingen, Germany).





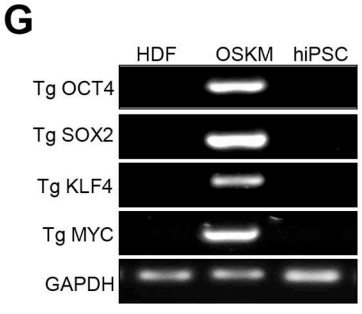


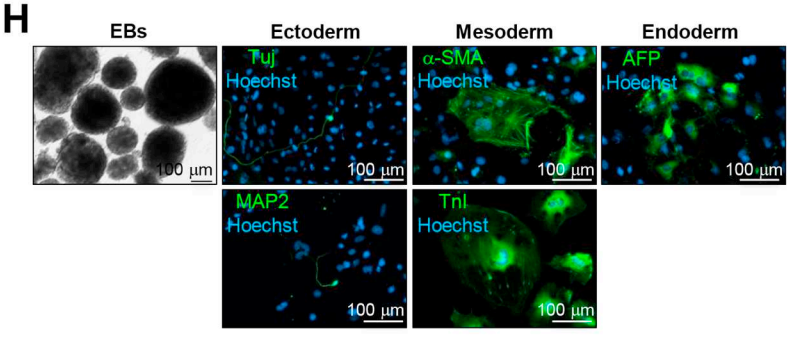


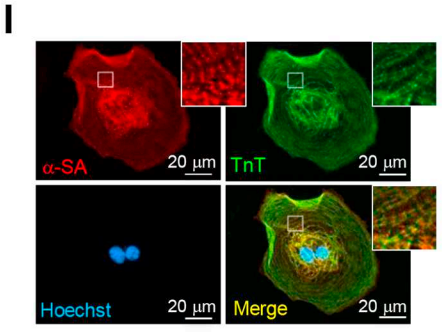


F

	N° nuclei	N° NANOG+ cells	%
Field 1	371	361	97,3
Field 2	161	161	100
Field 3	444	444	100
Field 4	398	398	100
Field 5	383	383	100
TOT	1757	1747	**99,4**







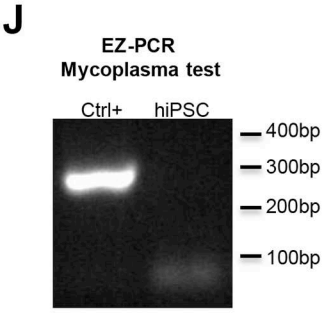


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Fig. 1. Characterization of the PSMi005-A cell line. **A.** Top: schematic representation of *KCNQ1* gene (exons are vertical lines/boxes). The *KCNQ1* coding sequence (CDS) used as a reference is the NCBI sequence NM_000218.2. Bottom: DNA sequencing results showing the mutation 568 C/T in the *KCNQ1* gene in heterozygosis in both patient-derived dermal fibroblasts (HDF) and PSMi005-A cell line (hiPSC). **B**. Karyotype analysis of PSMi005-A (300 G-bandings) showing normal female karyotype (46, XX). **C**. Top left: phase contrast image showing PSMi005-A morphology (PH). Top right: alkaline phosphatase colorimetric staining (AP). Bottom panels: immunofluorescence stainings showing uniform expression of pluripotency markers in the PSMi005-A. Nuclei were counterstained with Hoechst 33258 (Hoechst, blue). **D**. RT-PCR analysis showing expression of the indicated markers of pluripotency in PSMi005-A (hiPSC) compared with its parental fibroblasts (HDF). **E**. *OCT4* promoter methylation analysis with bisulfite sequencing in patient's dermal fibroblasts (HDF) and in the derived hiPSCs. Open circles indicate unmethylated CpG dinucleotides, while closed circles indicate methylated CpGs. **F.** Immunocytochemistry counting of anti Nanog-positive ($Nanog^+$) cells. The total number of cells in each of the five fields analyzed was quantified by counting the nuclei stained with Hoechst 33258. **G.** RT-PCR analysis showing no expression of the four viral transgenes (Tg) in naïve fibroblasts (HDF), expression of Tg OCT4, SOX2, KLF4 and cMYC five days after transduction (OSKM) and silencing of the four Tg in PSMi005-A at passage 6. **H.** Far left panel: floating embryoid bodies (EBs) formed after 7 days of PSMi005-A culture in suspension. Panels on the right: immunofluorescence staining for markers of the 3 germ layers in iPSC-derived EBs: neuronal class tubulin beta III (Tuj) and microtubule-associated protein 2 (MAP2) for ectoderm, smooth muscle actin (α-SMA) and troponin I (TnI) for mesoderm, and alpha Fetoprotein (AFP) for endoderm. **I.** Co-immunofluorescence staining for the alpha-sarcomeric actinin (α-SA, red) and troponin T (TnT, green) in cardiomyocytes differentiated from the PSMi005-A. Nuclei were counterstained with Hoechst 33258 (Hoechst, blue). Magnifications show areas of cross-striations. **J.** EZ-PCR test showing the absence of mycoplasma contamination in PSMi005-A. Ctrl + is the positive PCR control provided by the kit.

Table 1
Characterization and validation of PSMi005-A cell line.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel C
Phenotype	Qualitative analysis	Positive immunostaining for the pluripotency markers OCT4, NANOG, SOX2, TRA-1-60, TRA-1-81, SSEA-4	Fig. 1 panel C
		Positive staining for the alkaline phosphatase	Fig. 1 panel C
		Expression of the pluripotency markers OCT3/4, SOX2, NANOG, Rex1, GDF3, ESG1, DPPA2, DPPA4, NODAL measured by RT-PCR	Fig. 1 panel D
	Quantitative analysis	99,4% $NANOG^+$ cells quantified by immunocytochemistry counting	Fig. 1 panel F
Genotype	Karyotype (300 G-banding) and resolution	46XX, Resolution 450–500	Fig. 1 panel B
Identit	Microsatellite PCR (mPCR)	Not performed	Not available
	STR analysis	7 sites tested for iPSC, all sites matched with donor HDF STR profile	Available with authors
Mutation analysi	Sequencing	Heterozygous for the mutation c. 568C > T p.R190W on the KCNQ1 gene	Fig. 1 panel A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR. Negative	Fig. 1 panel J
Differentiation potential	Embryoid body formation	The EBs expressed neuronal class tubulin beta III (Tuj) and microtubule-associated protein (MAP) (ectoderm); smooth muscle actin (SMA) and troponin I (mesoderm); alpha Fetoprotein (AFP) (endoderm).	Fig. 1 panel H
	Differentiation into cardiomyocytes	The iPSC-derived cardiomyocytes expressed the cardiac sarcomeric proteins alpha-sarcomeric actinin (α-SA) and troponin T (TnT)	Fig. 1 panel I
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	Not available
Genotype additional info	Blood group genotyping	Not performed	Not available
	HLA tissue typing	Not performed	Not available

AP assay

AP was detected by using the Alkaline Phosphatase Staining kit (00–0009 Stemgent).

RT-PCR

Total RNA was purified using TRIzol (ThermoFisher). cDNA was synthesized using the Superscript II Reverse Transcriptase (ThermoFisher). RT-PCR was performed with the GoTaq G2 DNA polymerase (Promega) and primers in Table 2.

OCT4 promoter demethylation analysis

Genomic DNA were treated with the EZ DNA methylation kit (Zymo Research, Orange, CA, USA). The promoter region of the human OCT4 gene was amplified with biotinylated primers (Table 2) using Amplitaq gold 360 (Applied Biosystems). PCR products were sequenced (sequencing primer sequence in Table 2) using Pyrosequencing PSQ96 HS System (Biotage, Uppsala, Sweden). The methylation status of each locus was analyzed using PyroQ-CpG software (Qiagen).

EB formation

hiPSCs were grown for 7 days in suspension in a modified iPS medium deprived of bFGF and containing 20% FBS instead of KO-SR. Forming EBs were then transferred to gelatin-coated dishes to allow differentiation in adhesion for additional 7 days, and finally immunostained for the three germ layers.

Cardiac differentiation

Cardiac differentiation was induced using the PSC Cardiomyocyte Differentiation Kit (ThermoFisher).

Table 2
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti NANOG	1:200	Stemgent *Cat#* 09–0020, *RRID:* AB_2298294
	Mouse anti OCT3/4 (C-10)	1:500	SCBT *Cat#* sc-5279, *RRID:* AB_628051
	Mouse anti SOX2	1:500	R&D Systems *Cat#* MAB2018, *RRID:* AB_358009
	Mouse anti TRA-1-60	1:100	Stemgent *Cat#* 09–0010, *RRID:* AB_1512170
	Mouse anti TRA-1-81	1:100	Stemgent *Cat#* 09–0011, *RRID:* AB_1512171
	Mouse anti SSEA-4	1:100	Stemgent *Cat#* 09–0006, *RRID:* AB_1512169
Differentiation Markers (EBs)	Mouse anti neuronal class tubulin beta III (Tuj)	1:500	Covance *Cat#* MMS-435P, *RRID:* AB_2313773
	Anti microtubule-associated protein 2 (MAP2)	1:200	Millipore *Cat#* MAB3418, *RRID:* AB_94856
	Mouse anti smooth muscle actin	1:1000	Millipore *Cat#* CBL171, *RRID:* AB_2223166
	Mouse anti Troponin I (TnI)	1:200	Millipore *Cat#* MAB1691, *RRID:* AB_2256304
	Mouse anti alpha-fetoprotein	1:500	Millipore *Cat#* SCR030, *RRID:* AB_597591
Cardiac Markers	Mouse anti Troponin T[a]	1:250	ThermoFisher *Cat#* MA5–12960, *RRID:* AB_11000742
	Mouse anti alpha actinin[a]	1:800	Sigma Aldrich *Cat#* A7811, *RRID:* AB_476766
Secondary antibodies	Alexa-Fluor® 546 Goat anti-rabbit IgG	1:500	ThermoFisher Cat# A11010, *RRID*: AB_ AB_143156
	Alexa-Fluor® 488 Goat anti-mouse IgG	1:500	ThermoFisher Cat# A11001, *RRID*: AB_2534069
	Alexa-Fluor® 546 Goat anti-mouse IgG	1:500	ThermoFisher Cat# A11003, *RRID*: AB_141370

Primers

	Target	Forward/Reverse primer (5′-3′)
Targeted mutation analysis/sequencing	*KCNQ1* Exon 3	Fw: 5′- gttcaaacaggttgcagggtctga −3’
	256 bp	Rev.: 5′- ccaggtttccagaccaggaag −3’
Pluripotency Markers (RT-PCR)	OCT4	Fw: 5’-gtactcctcggtccctttcc-3’
	168 bp	Rev.: 5′-caaaaaccctggcacaaact-3’
	SOX2	Fw: 5’-acaccaatcccatccacact-3’
	273 bp	Rev.: 5′-tttttcgtcgcttggagact-3’
	NANOG	Fw: 5’-ttccttcctccatggatctg-3’
	213 bp	Rev.: 5′-tctgctggaggctgaggtat-3’
	REX1	Fw: 5’-cagatcctaaacagctcgcagaat-3’
	306 bp	Rev.: 5′-gcgtacgcaaattaaagtccaga-3’
	GDF3	Fw: 5’-cttatgctacgtaaaggagctggg-3’
	631 bp	Rev.: 5′-gtgccaacccaggtcccggaagtt-3’
	ESG1	Fw: 5’-atatcccgccgtgggtgaaagttc-3’
	243 bp	Rev.: 5′-actcagccatggactggagcatcc-3’
	DPPA4	Fw: 5’-ggagccgcctgccctggaaaattc-3’
	408 bp	Rev.: 5′-tttttcctgatattctattcccat-3’
	DPPA2	Fw: 5’-ccgtccccgcaatctccttccatc-3’
	606 bp	Rev.: 5′-atgatgccaacatggctcccggtg-3’
	NODAL	Fw: 5’-gggcaagaggcaccgtcgacatca-3’
	234 bp	Rev.:5′-gggactcggtggggctggtaacgtttc-3’
House-Keeping Genes (RT-PCR)	GAPDH	Fw 5’-catgttccaatatgattccaccc-3’
	112 bp	Rev. 5′-gggatctcgctcctggaagat-3’
Retroviral transgenes	OCT4 cDNA on pMXs-hOCT3/4	Fw: 5’-ccccagggccccattttggtacc-3’
	339 bp	
	SOX2 cDNA on pMXs-hSOX-2	Fw: 5’-ggcacccctggcatggctcttggctc-3’
	496 bp	
	cMYC cDNA on pMXs-hcMYC	Fw: 5’-caacaaccgaaaatgcaccagccccag-3’
	542 bp	
	KLF4 cDNA on pMXs-hKLF4	Fw: 5’-acgatcgtggccccggaaaaggacc-3’
	518 bp	
	pMX viral vector	Rev: 5’-ccctttttctggagactaaataaa-3’
OCT4 promoter demethylation analysis/ Bisulfite sequencing	OCT4 promoter	Fw: 5’-gaggttggagtagaaggattgttttggttt-3’
	467 bp	Rev.: 5′-ccccctaacccatcacctccaccacctaa-3′
	Sequencing primer	Fw: 5′-agagaggggttgagtagtttt-3’

[a] To perform the co-staining with these two antibodies, we used the Zenon Tricolor Mouse IgG labeling Kit (Molecular Probes)

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101437.

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