Derivation of the human embryonic stem cell line RCe010-A (RC-6)

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Lab Resource: Stem Cell Line

Derivation of the human embryonic stem cell line RCe010-A (RC-6)

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ABSTRACT

The human embryonic stem cell line RCe010-A (RC-6) was derived from a frozen and thawed blastocyst voluntarily donated as unsuitable and surplus to fertility requirements following ethics committee approved informed consent under licence from the UK Human Fertilisation and Embryology Authority. The cell line shows normal pluripotency marker expression and differentiation to the three germ layers in vitro. It has a normal 46XY male karyotype and microsatellite PCR identity, HLA and blood group typing data are available.

Resource table.

Name of stem cell construct	RCe010-A
Alternative name	RC-6, RC6
Institution	Roslin Cells Ltd.
Person who created resource	B.J. Tye, K. Bruce, P. Dand, G. Russell, D.M. Collins, H. Bradburn, J. Gardner
Contact person and email	Paul.desousa@roslincells.com; Paul.desousa@ed.ac.uk Janet.downie@roslincells.com Aidan.courtney@roslincells.com Malcolm.bateman@roslinfoundation.com
Date archived/stock date	30 January 2009 (pre bank at passage 8 on feeders) 06 December 2010 (seed bank at passage 24)
Type of resource	Biological reagent: Cell line
Sub-type	hESC, research grade
Origin	Blastocyst with ICM and Trophoblast
Key transcription factors	Oct4 (confirmed by flow cytometry and immunocytochemistry) Nanog (confirmed by immunocytochemistry)
Authentication	See Quality Control Test Summary, Table 1
Link to related literature (direct URL links and full references)	N/A
Information in public databases	http://hpscreg.eu/cell-line/RCe010-A
Ethics	Informed consent obtained. Scotland A Research Ethics committee approval obtained (07/MRE00/56). Conducted under the UK Human Fertilisation and Embryology Authority licence no R0136 to centre 0202.

1. Resource details

RCe010-A (RC-6) was derived from a frozen and thawed blastocyst that was surplus to requirement or unsuitable for clinical use. The cell line was derived by whole embryo outgrowth on mitotically inactivated human fibroblast (HDF) feeder cells using HDF conditioned medium and expanded under feeder free conditions.

RCe010-A (RC-6) was shown to be pluripotent by expression of Oct-4, Nanog, Tra-1-60 and SSEA-4, but low levels of SSEA-1, using immunocytochemistry (Table 1, Fig. 1). By flow cytometric analysis, the expression of pluripotency makers Oct 4 and SSEA-4 was 86.2% and 96.8%, respectively, whereas low expression of the differentiation marker SSEA-1 (5.9%) was observed (Fig. 2). Differentiation to the three germ layers, endoderm, ectoderm and mesoderm, was demonstrated using embryoid body formation and expression of the germ layer markers α-fetoprotein, β-tubulin and muscle actin (Fig. 3).

A microsatellite PCR profile has been obtained for the cell line, and HLA Class I and II typing is available (Table 2). Blood group genotyping gave the blood group O_1O_1 (Table 2).

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Table 1
Summary of quality control testing and results for RC-6 (RCe010-A).

Classification	Test	Purpose	Result
Donor screening	HIV 1 + 2 Hepatitis B Hepatitis C	Donor screening for adventitious agents	Negative
Identity	Microsatellite PCR (mPCR)	DNA Profiling to give cell line its signature, gender/species	Performed
Phenotype	Immunocytochemisty Flow cytometry	To assess levels of staining for the pluripotency markers Assess antigen levels & cell surface markers commonly associated with hESC	Expression of Oct, 4, Nanog, SSEA-4, Tra-1-60 Oct 3/4: 84.0% SSEA-4: 99.2% SSEA-1: 3.8%
Genotype (Details provided in Table 2)	Blood Group Genotyping (DNA Analysis) Karyology (G-Banding) HLA tissue Typing	To establish blood group of the line Confirmation of normal ploidy by G-banding To establish full HLA Type I and II genotype of the line	O_1O_1 46XY HLA typed Class I and Class II
Microbiology and virology	Mycoplasma Endotoxin	Mycoplasma testing by RT-qPCR Screening for Endotoxin levels	Negative 1.21 EU/mL
Morphology	Photography	To capture a visual record of the line	Normal
Differentiation potential	Embryoid body formation	To show differentiation to three germ layers	Expression of muscle actin, β-tubulin and α-feto protein

2. Verification and authentication

The cell line was analysed for genome stability by G-banding (Fig. 4) and showed a normal 46XY male genotype. The cell line is free from mycoplasma contamination as determined by RC-qPCR. Microsatellite PCR DNA profiling for cell identity is shown in Table 2.

3. Materials and methods

3.1. Ethics

Derivation of hESC from surplus to requirement and failed to fertilise/develop embryos was approved by The Scotland A Research Ethics Committee and local ethics board at participating fertility clinics and conducted under licence no R0136 from the UK HFEA with informed donor consent.

3.2. Cell culture

Frozen embryos were thawed using Embryo Thawing Pack (Origio (Medicult), Denmark) using standard techniques and cultured in BlastAssist (Origio) after Day 3 of development. Embryos were cultured at 36.5–37.5 °C, 5 ± 0.5% CO_2, 5 ± 0.5% O_2 in drops under paraffin oil (Origio) and transferred to fresh medium at least every 2–3 days.

By Day 8 of development, or when spontaneous hatching occurred, embryos were placed in derivation conditions consisting of mitotically inactivated neonatal human dermal fibroblasts (HDFs) (ThermoFisher Scientific (Cascade Biologics), Paisley, UK) on tissue culture plastic pre-coated with 2 μg/cm^2 human laminin (Sigma Aldrich, Dorset, UK) as per manufacturer's recommendation. If required, assisted hatching was performed by removing the zona pellucidae mechanically using Swemed Cutting tools (Vitrolife, Göteborg, Sweden).

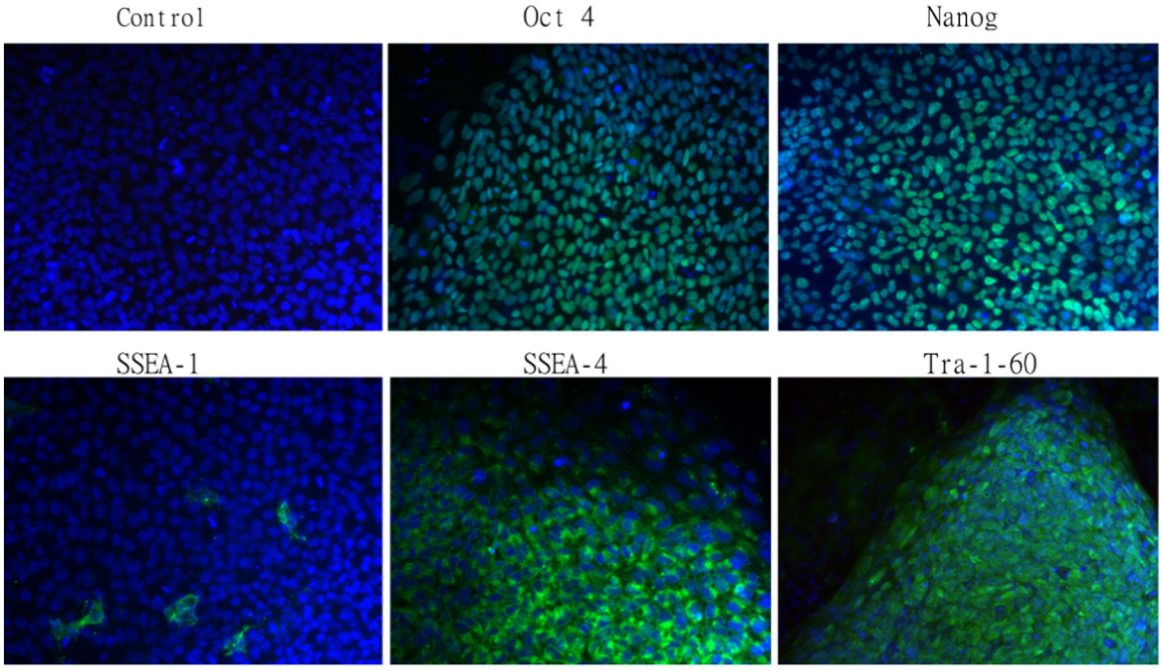

Fig. 1. RCe010-A (RC-6) expresses pluripotency markers Oct-4, Nanog, SSEA-4 and Tra-1-60, but no significant expression of the differentiation maker SSEA-1. Specific staining shown in green, cell nuclei are counterstained with DAPI (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

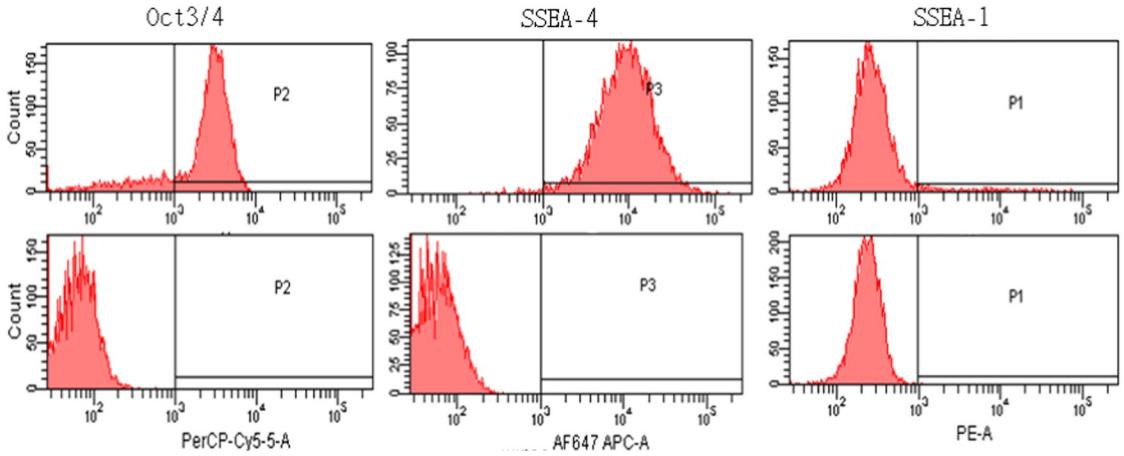

Fig. 2. RCe010-A (RC-6) was subjected to flow cytometry analysis for markers of pluripotency with specific antibody (top row) or isotype control (bottom row) as indicated above the histograms. Percentage staining is indicated in Table 1.

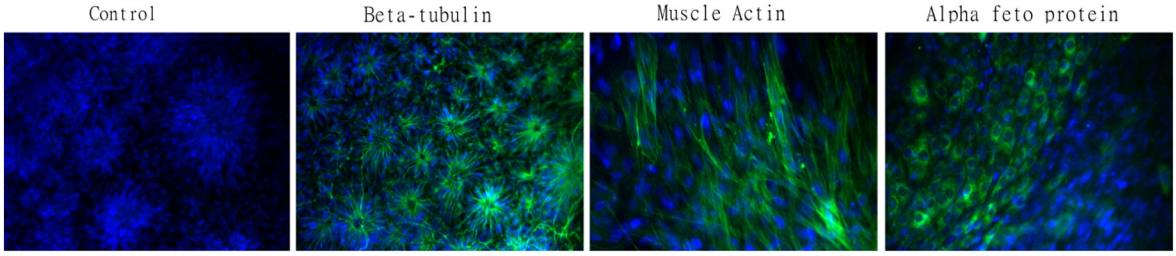

Fig. 3. In vitro differentiation of RCe010-A (RC-6) to ectoderm (β-tubulin III), mesoderm (muscle Actin), and endoderm (α-fetoprotein). Specific staining shown in green, cell nuclei are counterstained with DAPI (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

HDF cells were cultured in DMEM (Lonza, Slough, UK), 10% FCS (GE Healthcare (PAA), Buckinghamshire, UK) and 2 mM L-glutamine (ThermoFisher Scientific). HDF were mitotically inactivated using gamma irradiation at 50 GY using a Gammacell Elite 1000 machine. For use as a feeder layer, irradiated HDFs were plated at 2–50,000 cells/cm^2 in HDF conditioned medium (80% Knockout-DMEM, 20% Knockout serum replacement (KOSR), 1 mM glutamine, 0.1 mM β-mercaptoethanol, 1% nonessential amino acids, and 4 ng/ml human bFGF (all ThermoFisher Scientific) over 24 h intervals over 7 days) supplemented with an additional 2 ng/ml human bFGF Cells were cultured at 36.5–37.5 °C, 5 ± 0.5% CO_2, 5 ± 0.5% O_2 and 50% medium exchanged 6 days a week.

The established cell line was expanded and banked using CellStart matrix and Stempro hESC Serum Free Medium (ThermoFisher Scientific). Passaging was performed mechanically using an EZ passage tool (ThermoFisher Scientific). hESC lines were expanded to 25–30 wells of a 6-well plate and cryopreserved in 0.5–1 ml KOSR based

Table 2
Microsatellite PCR, blood group and HLA tissue typing results for RCe010-A (RC-6).

Microsatellite PCR results							
D3S1358 1	D3S1358 2	vWA 1	vWA 2	D16S539 1	D16S539 2	D2S1338 1	D2S1338 2
15	15	18	19	9	10	18	19
Amelogenin 1	Amelogenin 2	D8S1179 1	D8S1179 2	D21S11 1	D21S11 2	D18S51 1	D18S51 2
X	Y	lw*	lw*	29	32.2	15	16
D19S433 1	D19S433 2	THO1 1	THO1 2	FGA 1	FGA 2	CSF1PO 1	CSF1PO 2
14	15	7	7	20	24	12	12
D5S818 1	D5S818 2	D7S820 1	D7S820 2	D13S317 1	D13S317 2	TPOX 1	TPOX 2
10	11	lw*	lw*	11	12	8	9

*Peak falls below threshold to confidently score.

Blood group genotyping							
RhD	RhC	Rhc	RhE	Rhe	Fy a	Fy b	Fy GATA
pos	pos	pos	neg	pos	pos	pos	neg
Jka	Jkb	K	k	M	N	S	S
pos	neg	neg	pos	pos	pos	neg	pos
Kp a	Kp b	Do a	Do b	ABO			
neg	pos	pos	pos	O1O1			
HLA tissue typing							
HLA Class I Type		HLA-A*01, A*03; B*08, B*44; Cw*05, Cw*07					
HLA Class II Type		HLA-DRB1*03, DRB1*04; DRB3*01; DRB4*01; DQB1*02, DQB1*03					
Comment		DRB1*03 is expressed serologically as DR17, DQB1*03 is expressed serologically as DQ7.					

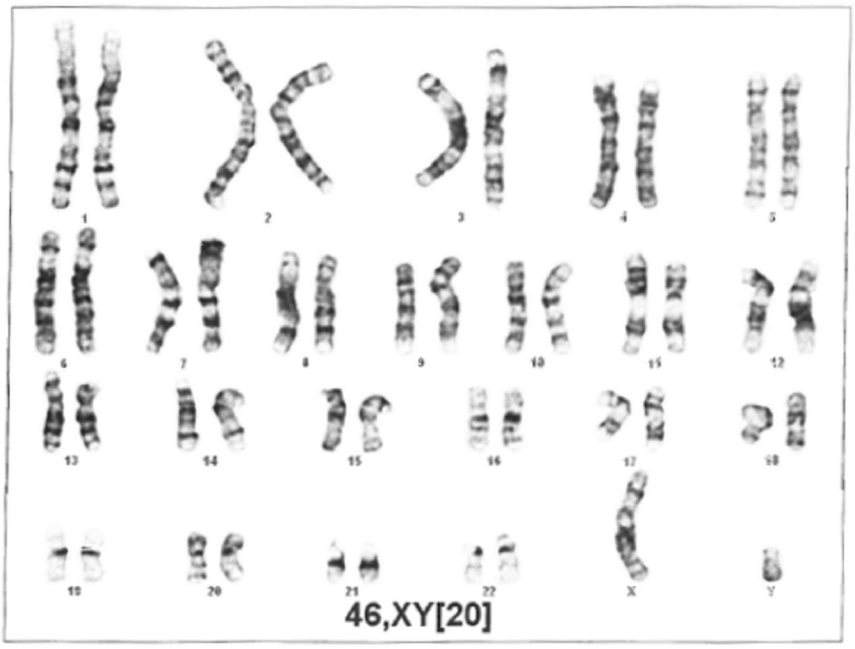

Fig. 4. RCe010-A (RC-6) was analysed by Giesma staining of 20 metaphase spreads and showed a normal 46XY male karyotype.

cryopreservation solution (75% KO-DMEM, 15% Xeno-free KOSR (ThermoFisher Scientific) and 10% DMSO (Origen Biomedical, Texas, USA)) or Cryostor CS10 (Biolife Solution, Washington, USA).

3.3. Mycoplasma

Mycoplasma detection was performed using Applied Biosystems PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit and MicroSEQ™ Mycoplasma Real-Time PCR Detection Kit (ThermoFisher Scientific (Applied Biosystems)) according to manufacturer's instruction.

3.4. Endotoxin

Endotoxin levels were determined using the Kinetic-QCL assay (Lonza) and an incubating plate reader (BioTek ELx808) according to manufacturer's instructions. Briefly, an unknown sample was compared with a standard curve of known levels of control endotoxin. An assay was deemed valid if the coefficient of correlation, $r \geq 0.980$ and the CV (%) for the standard curve was ≤10%.

3.5. Flow cytometry

Pluripotency was determined using the Human and Mouse Pluripotent Stem Cell Analysis kit (BD, Oxford, UK). Oct 3/4 and SSEA-4 were included as pluripotency markers, and SSEA-1 as a differentiation marker. Fixed and permeabilised cells were analysed using a FACS Aria flow cytometer (BD).

3.6. Immunocytochemistry

hESC were fixed in 4% paraformaldehyde (ThermoFisher Scientific (Alfa Aesar)), permeabilised using 100% ethanol (ThermoFisher Scientific) and stained with AFP (1:500; Sigma), β-tubulin III (1:1000; Sigma), muscle-specific actin (1:50; DAKO, Glostrup, Denmark), Oct-4 (1:200; Santa Cruz Biotechnlogy, Texas, USA), Nanog (1:20; R&D Systems, Abingdon, UK), Tra-1-60, SSEA-1 and SSEA-4 (all 1:50; BD) and secondary antibodies anti-mouse IgG-FITC (1:200; Sigma), anti mouse IgG- AlexaFluor 488, anti-goat IgG-AlexaFluor 488, anti-goat IgG-AlexaFluor-594 and anti-donkey polyclonal AlexaFluor-594 (all 1:200; ThermoFisher Scientific). Images were acquired using a Zeiss S100 Axiovert fluorescence microscope or Nikon eC1 confocal microscope.

3.7. In vitro differentiation

hESC cells were pre-treated for 1 h with 10 μM ROCK inhibitor in Stempro hESC SFM (ThermoFisher Scientific) and embryoid bodies EBs generated in ultra low attachment plates (Corning) for 7 days before being transferred into EB medium (20% FBS (GE Healthcare (PAA)), 80% KO-DMEM 1 mM L-glutamine, 0.1 mM β-mercaptoethanol, 1% nonessential amino acids (all ThermoFisher Scientific)), on glass slide tissue culture chambers (Nunc, ThermoFisher Scientific) coated with 0.5% gelatin (Sigma) at 0.1 ml/cm^2 for 14 days.

3.8. Genomic analysis

All outsourced assays were carried out under a Quality and Technical Agreement. DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Manchester, UK) according to manufacturer's recommendations and provided in recommended quantities to the service providers.

Microsatellite PCR, or Short Tandem Repeat analysis, was used to determine cell line identity and was carried out by Public Health England. A profile was obtained for the following core alleles: vWA, D16S539, Amelogenin, THO1, CSF1PO, D5S818, D75820, D135317 and TPOX.

Human Leukocyte Antigen (HLA) tissue typing was carried out by the Scottish National Blood Transfusion Service.

Blood group genotyping was carried out by the Molecular Diagnostics laboratory at NHSBT.

Karyotype analysis was carried out by The Doctors Laboratory (London, UK) or the Western General Cytogenetics Laboratory (Edinburgh, UK) Live cells at 60–70% confluency were shipped overnight in warm containers, fixed and analysed by standard G-banding analysis. For research grade lines, 20 spreads were analysed.

Acknowledgements

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