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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.

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

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.

Materials and methods

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.

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 precoated 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).

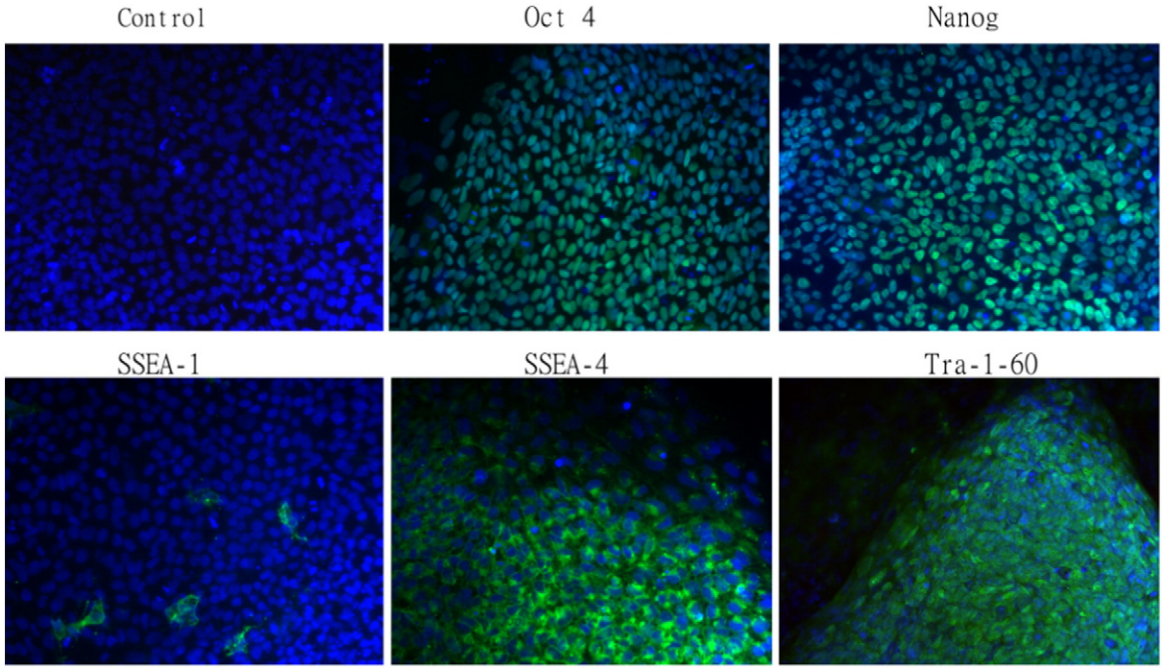

Fig. 1. RCe010-A (RC-6) expresses pluripotency markers Oct-4, Nanog, SSEA-4 and Tra-1-60, but no significant expression of the differentiation maker SSEA-1. Specific staining shown in green, cell nuclei are counterstained with DAPI (blue).