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Lab Resource: Stem Cell Line

Derivation of the human embryonic stem cell line RCe011-A (RC-7)

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ABSTRACT

The human embryonic stem cell line RCe011-A (RC-7) was derived from a failed to fertilise oocyte 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	RCe011-A
Alternative name	RC-7, RC7
Institution	Roslin Cells Ltd.
Person who created resource	B.J. Tye, D.M. Collins, K. Bruce, P. Dand, G. Russell, H. Bradburn
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	02 July 2009 (pre seed bank at passage 4 on feeders) 15 July 2009 (seed bank at passage 7)
Type of resource	Biological reagent: cell line
Sub-type	hESC, research grade
Origin	Zygote (activated oocyte)
Key transcription factors	Oct4 (confirmed by flow cytometry)
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/RCe011-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

RCe011-A (RC-7) was derived from a failed to fertilise oocyte/late developing embryo that had undergone activation using strontium chloride ($SrCl_2$)-containing media (Bos-Mikich et al., 1997). As the oocyte resulting in RCe011-A (RC-7) divided shortly after activation, it is likely to be a late developing embryo rather than oocyte activation, although this has not been verified. The cell line was derived by whole embryo outgrowth on mitotically inactivated human fibroblast (HDF) feeder cells using xeno free medium and expanded under xeno free and feeder free conditions.

By flow cytometry, RCe011-A (RC-7) expressed the pluripotency makers Oct 4 and SSEA-4 (87.9% and 99.6%, respectively), whereas low expression of the differentiation marker SSEA-1 (2.5%) was observed (Fig. 1, Table 1). 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. 2).

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).

Verification and authentication

The cell line was analysed for genome stability by G-banding (Fig. 3) 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.

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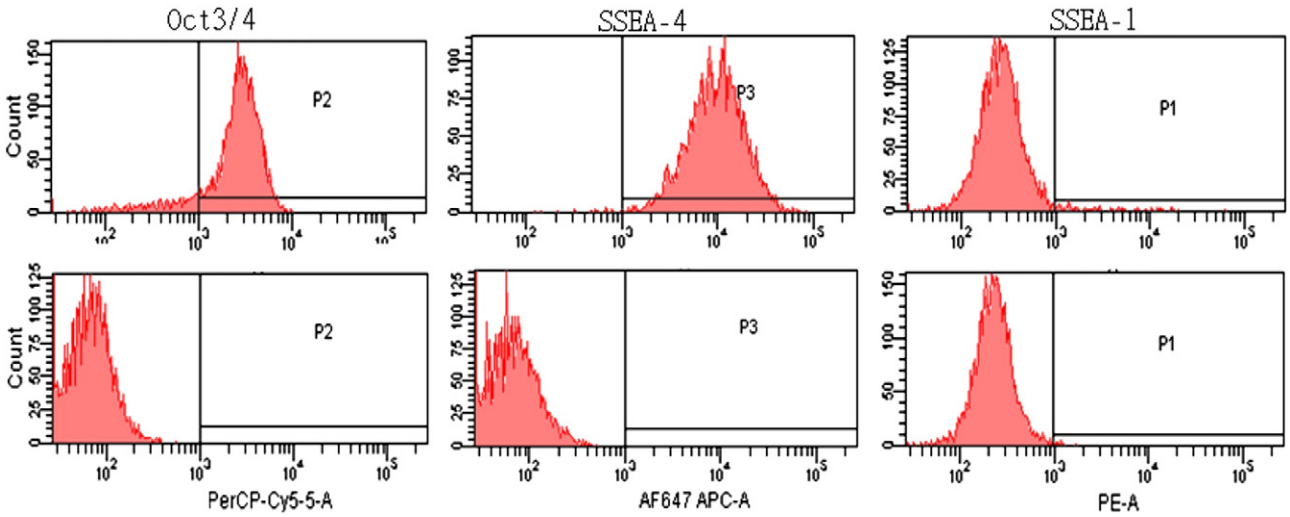

Fig. 1. RCe011-A (RC-7) was subjected to flow cytometry analysis for markers of pluripotency with specific antibody (top row) or isotype control (bottom row) as indicated above the histograms. Percentage staining is indicated in Table 1.

Materials and methods

Ethics

Derivation of hESC from surplus to requirement and failed to fertilise/develop embryos and oocytes 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

Failed to fertilise oocytes were appraised as clinically unusable at the treatment centre and transferred to Roslin Cells using a portable 37 °C incubator. They were subjected to an activation regimen consisting of 10 mM $SrCl_2$ (Sigma-Aldrich, Dorset, UK) in DMEM (ThermoFisher Scientific, Paisley, UK) containing 10% v/v HSA (Sigma) for 1 h. All oocytes were transferred to pre-warmed 30 μl microdrops of Embryo Assist under paraffin oil (both Origio, CooperSurgical, Trumbull, CT,

Table 1
Summary of quality control testing and results for RCe011-A (RC-7).

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	Flow cytometry	Assess antigen levels & cell surface markers commonly associated with hESC	Oct 3/4: 87.9% SSEA-4: 99.6% SSEA-1: 2.5%
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	BO_1 46XY HLA typed Class I and Class II
Microbiology and virology	Mycoplasma Endotoxin	Mycoplasma testing by RT-qPCR Screening for endotoxin levels	Negative 1.73 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

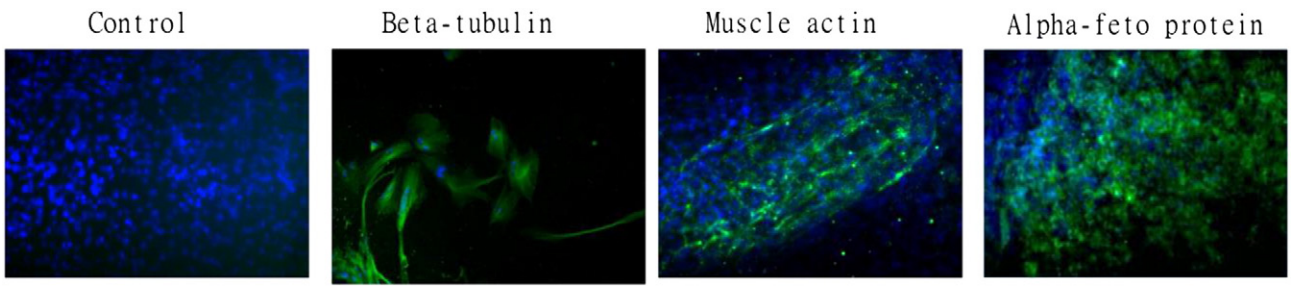

Fig. 2. In vitro differentiation of RCe011-A (RC-7) to ectoderm (β-tubulin III), mesoderm (muscle actin), and endoderm (α-fetoprotein). Specific staining shown in green, cell nuclei are counterstained with DAPI (blue).