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Lab Resource

Derivation of the clinical grade human embryonic stem cell line RCe021-A (RC-17)

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ABSTRACT

The human embryonic stem cell line RCe021-A (RC-17) was derived under quality assured compliance with UK regulation, European Union Directives and International guidance for tissue procurement, processing and storage according to Good Manufacturing Practice (GMP) standards. The cell line was derived from a day 3 embryo voluntarily donated as unsuitable or surplus to fertility requirements following informed consent. RCe021-A (RC-17) shows normal pluripotency marker expression and differentiation to the three germ layers in vitro. It has a normal 46XX female karyotype and microsatellite PCR identity, HLA and blood group typing data are available.

Resource table

Name of stem cell construct	RCe021-A
Alternative name	RC-17, RC17
Institution	Roslin Cells Ltd.
Person who created resource	B.J. Tye, K. Bruce, P. Dand, G. Russell, D.M. Collins, A. Greenshields, K. McDonald, H. Bradburn, D. Allan, A. Laurie, M. A. Canham
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 Tilo.kunath@ed.ac.uk
Date archived/stock date	20 May 2011 (seed bank)
Type of resource	Biological reagent: cell line
Sub-type	hESC, clinical grade
Origin	Cleavage stage embryo (Mitosis)
Key transcription factors	Oct4 (confirmed by flow cytometry),
Authentication	See Quality Control Certificate of Analysis (Fig. 1)
Link to related literature (direct URL links and full references)	N/A
Information in public databases	http://hpscreg.eu/cell-line/RCe021-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 and UK Human Tissue Authority (HTA) licensing number 22631.

Resource details

RCe021-A (RC-17) was received as day 3 embryo that was surplus to requirement or unsuitable for clinical use and was cultivated to the blastocyst stage in medium containing GMP grade granulocyte-macrophage colony-stimulating factor (GM-CSF) to improve survival of the inner cell mass (Sjöblom et al. 1999). Human embryonic stem cell (hESC) isolation, expansion and qualification were performed in a facilities whose specification, operation and monitoring complied with GMP standards enabling; i) a fully traceable procurement procedure with informed ethical consent which includes provision for commercial use, ii) detailed medical history and blood borne virus (BBV) screening of donors, and iii) compilation of a cell line history providing details on hESC manufacturing process and quality control testing regime.

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URL's: http://www.crm.ed.ac.uk/research/associate/pluripotent-cell-translation, http://www.roslincells.com (P.A. De Sousa).

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Human ESC culture and processing were performed in a grade A tissue culture cabinet in a grade B clean room environment monitored for particulate and microbiological contamination during cell processing in accordance with Rules and Guidance for Pharmaceutical Manufacturers and Distributors — The Orange Guide, compiled by the UK Medicines Healthcare Products Regulatory Authority (go to: https://www.gov.uk/guidance/good-manufacturing-practice-and-good-distribution-practice). Accordingly, the facility was operating under a mature Quality Management System, compliant with ISO9001:2008 standards. HESC derivation was performed under licensure from the UK HFEA (R0136 to centre 0202) and HTA (Licensing Number 22631).

HESC derivation involved whole embryo outgrowth on mitotically inactivated human dermal fibroblast (HDF) feeder cells. HDFs were derived and manufactured according to GMP and had been approved for clinical use by the Food and Drug Administration, USA. During derivation on HDFs, hESCs were grown in a xeno-free cell therapy grade media (XF KODMEM) supplemented with xeno-free human recombinant bFGF. The cell line was subsequently expanded in a GMP grade serum-free medium (StemPro hESC Serum Free Medium) on a xeno-free matrix (CellStart). The former contained bovine serum albumin (BSA) from a Transmissible Spongiform Encephalopathy (TSE)-free country of origin. The cell line was cryopreserved in a GMP compliant cryopreservation solution (CryoStor CS10).

By flow cytometry, RCe021-A (RC-17) expressed the pluripotency makers Oct-4, Tra-1-60 and SSEA-4 (89.8%, 70.8% and 94.7%, respectively), whereas lower expression of the differentiation marker SSEA-1 (10.2%) was observed (Figs. 1, 2). Differentiation to the three germ layers, endoderm, ectoderm and mesoderm, was demonstrated using embryoid body formation in vitro, and expression of the germ layer markers α-fetoprotein, β-tubulin and muscle actin was observed (Fig. 3).

A microsatellite PCR profile has been obtained for the cell line, and HLA Class I and II typing is available (Table 1). Blood group genotyping gave the blood group O_1O_1, expected to give rise to blood group O^+ (Table 1). The cell line is free from mycoplasma contamination as determined by RT-qPCR.

Verification and authentication

The cell line was analysed for genome stability by G-banding and showed a normal 46XX female genotype (Fig. 4). SNP genotyping was carried out using the Illumina HumanCytoSNP-12 v2.1 BeadChip and revealed a 144 kb gain on chromosome12p13.31 as described in Canham et al. 2015. This region contains the genes, *SLC2A14*; *SLC2A3*. Duplications and deletions of this region are found commonly in healthy individuals (1 n 25) as documented by the Database of Genomic Variants (MacDonald et al. 2014).

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. The processing and storage of hESC cells for human application were conducted under licence number 22631 from the UK Human Tissue Authority.

Roslin Cells

Quality Control Test Certificate

Sample Point 2 Test Results

Certificate Number:	QCC-11-056	**Version:**	1
Grade:	CLINICAL		
Sample ID:	RC-17 P12A		

Assay	Test Method	Roslin Cells Assay Code	Date of Assay	Result	
Mycoplasma Detection	RT-qPCR (SOP/QCP/22)	MYCO-11-007	19 May 11	**Not Detected**	
Endotoxin Detection	Kinetic Chromogenic LAL (SOP/QCP/12)	ENDO-11-011	20 May 11	**2.27 EU/ml**	
Viral Screening*	PCR (CMV,HTLV1,HIV1,HCV, HBV,EBV) (SOP/QCP/60)	N/A	21 May 11	**Not Detected**	
Karyotype*	G-banding (SOP/QCP/59)	N/A	09 Jun 11	**46,XX**	
Pluripotency / Differentiation	Flow Cytometry (SOP/QCP/25)	FLOW-11-010	20 May 11	**Antibody**	**% Positive**
				SSEA-4	**94.7**
				Oct 3/4	**89.8**
				Tra-1-60	**70.8**
				SSEA-1	**10.2**
Microsatellite Genotyping*	PCR (SOP/QCP/6)	MPCR-11-001	02 Aug 11	**ID Obtained**	

*Subcontracted to a Third Party

Certificate Prepared by (QC): E. Clarke Date: 30 NOV 11.

Certificate Reviewed by (QC): [signature] Date: 30 NOV 11.

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Roslin Cells

Quality Control Test Certificate

Sample Point 2 Test Results

Certificate Number:	QCC-11-056	**Version:**	1
Grade:	CLINICAL		
Sample ID:	RC-17 P12A		

Assay	Test Method	Roslin Cells Assay Code	Date of Assay	Result
HLA Typing*	PCR-SSO (SOP/QCP/62)	HLA-11-001	28 Jul 11	**HLA Typed Class I and Class II**
Blood Group Genotyping*	PCR (SOP/QCP/63)	N/A	08 Jun 11	**ABO Genotype: $0^1 0^1$**
Differentiation	Embryoid Body Formation (Endoderm, Ectoderm, Mesoderm) (SOP/QCP/7 & SOP/QCP/58)	EB-CUL-11-006 EB-STAIN-11-003	20 May 11 15 Jun 11	**Endoderm - Detected**
				Ectoderm - Detected
				Mesoderm - Detected
Viability	Flow Cytometry (SOP/QCP/40)	VIAB-11-004	20 May 11	**% Non-Viable: 5 ± 2.6**

*Subcontracted to a Third Party

Certificate Prepared by (QC): E. Clarke Date: 30 NOV 11.

Certificate Reviewed by (QC): [signature] Date: 30 NOV 11.

Certificate Approved by (QA): Julie Uren Date: 30 NOV 2011.

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Fig. 1. Quality Control Certificate of Analysis for RC-17 (RCe021-A) P12A seed lot.