

Lab Resource: Stem Cell Line

Derivation of the human embryonic stem cell line RCe008-A (RC-4)

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ABSTRACT

The human embryonic stem cell line RCe008-A (RC-4) was derived from a 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 ectoderm and mesoderm *in vitro*. It has a mixed 46XX/45X female karyotype and microsatellite PCR identity and blood group typing data is available.

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Resource table.

Name of stem cell construct	RCe008-A
Alternative name	RC-4, RC4
Institution	Roslin Cells Ltd.
Person who created resource	B. Tye, K. Bruce, P. Dand, 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	11 June 2008
Type of resource	Biological reagent: Cell line
Sub-type	hESC, research grade
Origin	Blastocyst with ICM and Trophoblast
Key transcription factors	Oct4 and 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://hpscereg.eu/cell-line/RCe008-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

RCe008-A (RC-4) was derived from a fresh blastocyst that was surplus to requirement or unsuitable for clinical use. The cell line was

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derived by whole embryo outgrowth on a chemically defined matrix consisting of laminin, fibronectin, Collagen IV and vitronectin (Ludwig et al., 2006) using human fibroblast (HDF) conditioned medium and expanded under feeder free conditions.

RCe008-A (RC-4) was shown to be pluripotent by expression of Oct-4 and Nanog using immunocytochemistry (Table 1, Fig. 1). By flow cytometric analysis, the expression of pluripotency markers Tra-1-60, Tra-1-81 and SSEA-4 was 74%, 58% and 75%, respectively, whereas low expression of SSEA-1 (5%) was observed (Fig. 2). Germinal lineage marker expression is not available due to poor EB formation.

A microsatellite PCR profile has been obtained for the cell line and blood group genotyping gave the blood group AO₁ (Table 2).

Verification and authentication

The cell line was analysed for genome stability by G-banding (Fig. 3) and showed a mixture of a normal female karyotype (46XX in 16 cells) and monosomy X (45X in 3 cells). The cell line is free from mycoplasma contamination as determined by RT-qPCR.

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.

Table 1
Summary of quality control testing and results for RCe008-A (RC-4).

Classification	Test	Purpose	Result
Donor screening	HIV 1 + 2 Hepatitis B Hepatitis C	Donor screening for adventitious agents	Negative
Identity	Microsatellite PCR (mPCR) Immunocytochemistry	DNA profiling to give cell line its signature, gender/species To assess levels of staining for the pluripotency markers.	Performed Expression of Oct-4 and Nanog
Phenotype	Flow cytometry	Assess antigen levels & cell surface markers commonly associated with hESC	CD9: 84% Tra 1-60: 74% Tra 1-81: 58% SSEA-4: 75% SSEA-1: 5%
Genotype (details provided in Table 2)	Blood group genotyping (DNA analysis) Karyology (G-banding)	To establish blood group of the line Confirmation of normal ploidy by G-banding	AO ₁ 46XX (16)/45X (3)
Microbiology and virology	Mycoplasma Endotoxin	Mycoplasma testing by RT-qPCR Screening for endotoxin levels	Negative 1.87 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	Poor EB formation – germinal lineage marker expression not available

Cell culture

Fresh embryos were cultured EmbryoAssist (Origio (Medicult), Denmark) until Day 3 or BlastAssist (Origio) after Day 3 of development. Embryos were cultured at 36.5–37.5 °C, 5 ± 0.5% CO₂, 5 ± 0.5% O₂ 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 a mixture of laminin (5 µg/cm²; Sigma Aldrich, Dorset, UK), fibronectin (5 µg/cm²; BD, Oxford, UK), vitronectin (0.2 µg/cm²; BD), and collagen IV (10 µg/cm²; Sigma) (Ludwig et al., 2006) in human dermal fibroblast

(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, Paisley, UK) over 24 h intervals over 7 days) supplemented with an additional 24 ng/ml human bFGF. If required, assisted hatching was performed by removing the zona pellucida mechanically using Swemed cutting tools (Vitrolife, Göteborg, Sweden). Cells were cultured at 36.5–37.5 °C, 5 ± 0.5% CO₂, 5 ± 0.5% O₂ 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

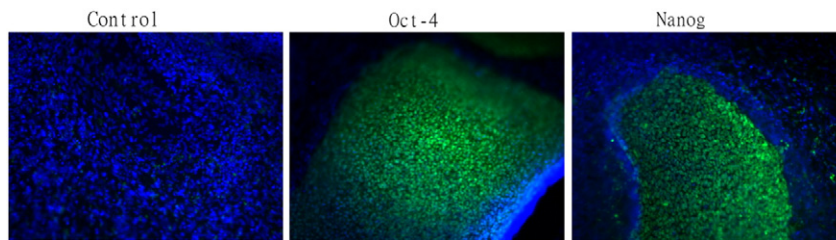


Fig. 1. RCe008-A (RC-4) expresses pluripotency markers Oct-4 and Nanog. Cell nuclei are counterstained with DAPI (blue).

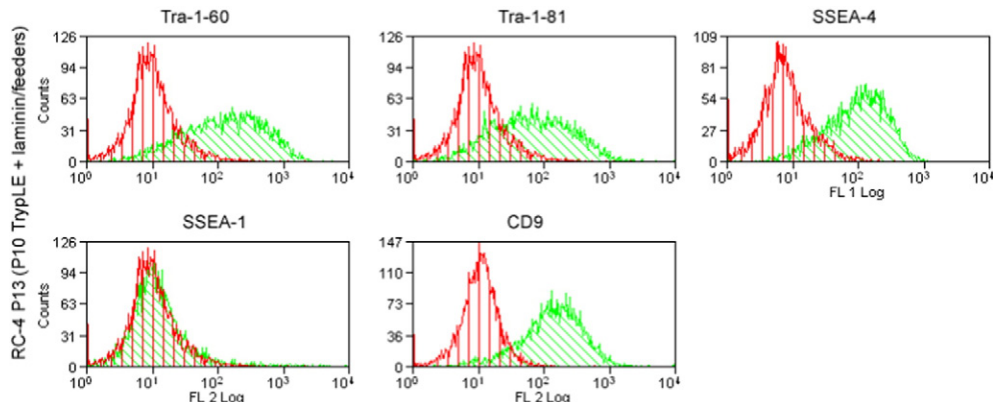


Fig. 2. Flow cytometry staining of RCe008-A (RC-4) at Passage 13. Isotype/negative control is shown in red, specific staining in green. Percentage staining is indicated in Table 1.

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