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Lab Resource: Stem Cell Line

Derivation of the human embryonic stem cell line RCE012-A (RC-8)

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c MRC Centre for Regenerative Medicine, University of Edinburgh, UK

ABSTRACT

The human embryonic stem cell line RCE012-A (RC-8) was derived from a frozen and thawed day 5 embryo cultured to the blastocyst stage. The embryo was 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 46XX female karyotype and microsatellite PCR identity, HLA and blood group typing data is available.

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Resource table

Name of stem cell construct: RCE012-A
Alternative name: RC-8, RC8
Institution: Roslin Cells Ltd.
Person who created resource: B. J. Tye, K. Bruce, P. Dand, G. Russell, D.M. Collins, A. Greenshields, H. Bradburn
Contact person and email: Paul.desousa@roslincells.com; Janet.downie@roslincells.com
Aidan.courtney@roslincells.com; Malcolm.bateman@roslinfoundation.com

Date archived/stock date: 06 September 2010 (seed bank)
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)
Authentication: See Quality Control test summary, Table 1
Link to related literature (direct URL links and full references): http://hpscreg.eu/cell-line/RCE012-A
http://dx.doi.org/10.1016/j.scr.2016.02.033
Information in public databases: http://hpscreg.eu/cell-line/RCE012-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

RCE012-A (RC-8) was derived from a thawed day 5 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 xeno-free medium (XF KODMEM) and expanded under xeno free and feeder free conditions.

By flow cytometry, RCE012-A (RC-8) expressed the pluripotency makers Oct-4, Tra-1-60 and SSEA-4 (94.7%, 89.4% and 99.8%, respectively), whereas low expression of the differentiation marker SSEA-1 (2.2%) 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 AO1 (Table 2).

Verification and authentication

The cell line was analysed for genome stability by G-banding (Fig. 3) and showed a normal 46XX female genotype. The cell line is free from mycoplasma contamination as determined by RC-qPCR. Microsatellite PCR DNA profiling for cell identity is available (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

Vitrified embryos were thawed using Vitriﬁed Embryo Safety Thawing Pack (Kitazato/Dibimed, Valencia, Spain) according to manufacturers’ instruction and were cultured in system SAGE Quinn’s Advanced Blastocyst medium (Rochford Medical, Coventry) 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 (Rochford Medical) and transferred to fresh medium at least every 2–3 days.

By day 8 of development, embryos were placed in derivation conditions consisting of mitotically inactivated neonatal human dermal ﬁbro-blasts (HDFs) (ThermoFisher Scientiﬁc (Cascade Biologics), Paisley, UK) on tissue culture plastic in XF KODMEM medium (Knockout-DMEM, 15% KOSR-XF, 2 mM l-glutamine, 1% MEM Non-essential amino acids, 2% XF Growth Factor Cocktail, 0.1 mM β-mercaptoethanol, all ThermoFisher Scientiﬁc, Paisley, UK) supplemented with 80 ng/ml human bFGF (ThermoFisher Scientiﬁc). Assisted hatching was performed by removing the zona pellucidae mechanically using Swemed Cutting tools (Vitrolife, Göteborg, Sweden).

HDF cells were cultured in DMEM (Lonza, Slough, UK), 10% FCS (GE Healthcare (PAA), Buckinghamshire, UK) and 2 mM l-glutamine (ThermoFisher Scientiﬁc). HDFs were mitotically inactivated using gamma irradiation at 50GY using a Gammacell Elite 1000 machine. For use as a feeder layer, irradiated HDFs were plated at 50,000 cells/cm2 in XF KODMEM medium supplemented with 80 ng/ml human bFGF (ThermoFisher Scientiﬁc). 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 (ThermoFisher Scientiﬁc). hESC lines were expanded to 25–30 wells of a 6-well plate and cryopreserved in 0.5-1 ml Cryostor CS10 (Biolife Solution, Washington, USA).

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.

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 ≥ 0.980 and the CV (%) for the standard curve was ≤ 10%.

| Table 1 Summary of quality control testing and results for RCe012-A (RC-8). |
|----------------|----------------|----------------|
| **Classification** | **Test** | **Purpose** | **Result** |
| Donor screening | HIV 1 + 2 | Donor screening for adventitious agents | Negative |
| | Hepatitis B | | |
| | Hepatitis C | | |
| 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: 94.7%
| | | | Tra 1-60: 89.4%
| | | | SSEA-4: 99.8%
| | | | SSEA-1: 2.2% |
| Genotype | Blood group genotyping (DNA analysis) | To establish blood group of the line | O1O1 |
| | Karyology (G-banding) | Confirmation of normal ploidy by G-banding | 46XX |
| | HLA tissue typing | To establish full HLA type I and II genotype of the line | HLA typed class I and class II |
| Microbiology and virology | Mycoplasma | Mycoplasma testing by RT-qPCR | Negative |
| | Endotoxin | Screening for endotoxin levels | 0.54 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 |
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. FITC conjugated Tra-1-60 (BD) was used as an additional pluripotency marker. Fixed and permeabilised cells were analysed using a FACS Aria flow cytometer (BD). Percentage expression of each marker was compared to isotype control or unstained cells.

Immunocytochemistry

hESC were fixed in methanol (ThermoFisher Scientific), blocked using 10% goat serum (Sigma-Aldrich, Dorset, UK) in PBS (Lonza) containing 0.01% Tween-20 (Sigma), and stained with AFP (1:500; Sigma), β-tubulin III (1:1000; Sigma), muscle-specific actin (1:50; DAKO, Glostrup, Denmark), and secondary antibody anti-goat IgG-AlexaFluor 488 (1:200; ThermoFisher Scientific). Images were acquired using a Zeiss S100 Axiovert fluorescence microscope or Nikon eC1 confocal microscope.

In vitro differentiation

Confluent hESC cells lifted using a cell scraper (Corning) and embryoid bodies EBs generated in ultra low attachment plates (Corning) in EB medium (20% FBS (GE Healthcare (PAA)), 80% KO-DMEM 1 mM l-glutamine, 1% nonessential amino acids (all ThermoFisher Scientific)). After 9 days in suspension culture, EBs were being transferred onto glass slide tissue culture chambers (Nunc, ThermoFisher Scientific) coated with 0.1% gelatin (Sigma) at 0.1 ml/cm² and cultured for 14 days.

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, D7S820, D13S317 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

Research culminating in the derivation of this line was funded by a grant from Scottish Enterprise Economic Development Agency (PM07321) to PDS, MB and AC.

Images of embroy body staining were kindly provided by S. Greenhough and J. Gardner, Roslin Cellab Ltd.

Table 2

<table>
<thead>
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<th>Microsatellite PCR results</th>
<th>D3S1358 1</th>
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<td>X</td>
<td>D8S1179 1</td>
<td>D8S1179 2</td>
<td>D21S11 1</td>
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Blood group genotyping

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<th>HLA tissue typing</th>
<th>HLA class I type</th>
<th>HLA class II type</th>
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<tbody>
<tr>
<td>HLA-A<em>02, A</em>32, B<em>15, B</em>38, C<em>03, C</em>12</td>
<td>HLA-DRB1<em>04, DRB1</em>15, DRB4<em>01, DRB5</em>01, DQB1<em>06, DQB1</em>03</td>
<td>C<em>03 is expressed serologically as C10, B</em>15 is expressed serologically as B62, DQB1*03 is expressed serologically as a DQ8.</td>
</tr>
</tbody>
</table>
Fig. 3. RCe012-A (RC-8) was analysed by Giesma staining of 20 metaphase spreads at passage 23 and showed a normal 46XX female karyotype in 17 of 20 spreads. Abnormalities in the three remaining cells are believed to be results of harvesting artefacts. An independent assessment of karyology on the same cell line at passage 54 indicated normal 46XX karyology all 20 cells examined.