Stem Cell Research 16 (2016) 452–455

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Derivation of the human embryonic stem cell line RCe006-A (RC-2)

P.A. De Sousa [a,b,c,*], B. Tye [a], K. Bruce [a], P. Dand [a], G. Russell [a], J. Gardner [a], J.M. Downie [a], M. Bateman [a], A. Courtney [a]

[a] Roslin Cells Limited, Nine Edinburgh Bio-Quarter, 9 Little France Road, Edinburgh EH16 4UX, UK
[b] Centre for Clinical Brain Sciences, University of Edinburgh, UK
[c] MRC Centre for Regenerative Medicine, University of Edinburgh, UK

ARTICLE INFO

Article history:
Received 9 February 2016
Accepted 11 February 2016
Available online 16 February 2016

ABSTRACT

The human embryonic stem cell line RCe006-A (RC-2) was derived from a frozen and thawed blastocyst voluntarily donated as surplus to fertility requirements following ethics committee approved informed consent under licence from the UK Human Fertilisation and Embryology Authority. The cell line exhibits expression of expected pluripotency markers and *in vitro* differentiation potential to three germinal lineage representative cell populations. It has a male trisomy 12 karyotype (47XY, +12). Microsatellite DNA marker identity and HLA and blood group typing data are available.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Resource table

Name of stem cell construct	RCe006-A
Alternative name	RC-2, RC2
Institution	Roslin Cells Ltd.
Person who created resource	B. Tye, K. Bruce, P. Dand, G. Russell, 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	21 December 2007 (pre-bank at passage 12 on feeders) 29 November 2010 (banked at passage 31)
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)
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/RCe006-A http://www.nibsc.org/science_and_research/advanced_therapies/uk_stem_cell_bank/cell_lines.aspx
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

RCe006-A (RC-2) was derived from a frozen and thawed, surplus to requirement, blastocyst. The cell line was derived by whole embryo outgrowth on mitotically inactivated human dermal fibroblast (HDF) feeder cells using HDF conditioned medium and expanded under feeder free conditions.

RCe006-A (RC-2) was shown to be pluripotent by expression of the pluripotency markers Oct4, Nanog Tra-1-60 and Tra-1-81, but not the differentiation marker SSEA-1 using immunocytochemistry (Table 1, Fig. 1). By flow cytometric analysis, expression of the pluripotency makers SSEA-4, Tra-1-60 and Tra-1-81 was 81.8%, 55.0% and 43.6%, respectively, whereas low expression of the differentiation marker SSEA-1 (2.3%), was observed at passage 6 (Fig. 2). Differentiation to the three germ layers, endoderm, ectoderm and mesoderm, was demonstrated using embryoid body formation with all three germ layers present as shown by expression of 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 BO_1 (Table 2).

Verification and authentication

The cell line was analysed for genome stability by G-banding (Fig. 4) and showed an abnormal 47XY, +12 male genotype in all 20 cells analysed. The cell line is free from mycoplasma contamination as determined by RC-qPCR. Microsatellite PCR DNA profiling for cell identity is available.

* Corresponding author at: University of Edinburgh, Roslin Cells Limited, Nine Edinburgh Bio-Quarter, 9 Little France Road, Edinburgh EH16 4UX, UK.
E-mail addresses: paul.desousa@ed.ac.uk, Paul.desousa@roslincells.com (P.A. De Sousa).

http://dx.doi.org/10.1016/j.scr.2016.02.029
1873-5061/© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Table 1
Summary of quality control testing and results for RCe006-A (RC-2).

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	To assess levels of staining for pluripotency markers	Expression of Oct4, Nanog, Tra-1-60 and Tra-1-81
	Flow cytometry	Assess antigen levels & cell surface markers commonly associated with hESC	Tra 1-60: 55.0% Tra 1-81: 43.6% SSEA-4: 81.8% SSEA-1: 2.3%
Genotype (details provided in Table 2)	Blood group genotyping (DNA analysis)	To establish blood group of the line	BO_1
	Karyology (G-Banding)	Confirmation of normal ploidy by G-banding	47XY, +12
	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	4.19 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

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 were cultured EmbryoAssist (Origio) until Day 3 or 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).

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 50 Gy using a Gammacell Elite 1000 machine. For use as a feeder layer, irradiated HDFs were plated at 2–50,000 cells/cm^2 in 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) over 24 h intervals over 7 days) supplemented with an additional 24 ng/ml human bFGF. Cells

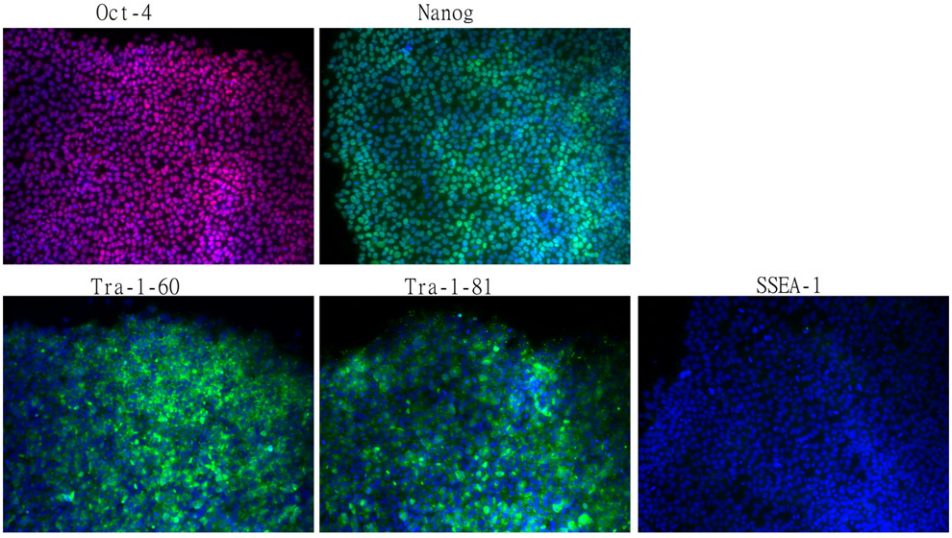

Fig. 1. Immunostaining of RCe006-A (RC-2) show expression of the pluripotency markers Oct-4 (red), Nanog, Tra-1-60, Tra-1-81 (green), but not the differentiation marker SSEA-1 (green). Cell nuclei are counterstained with DAPI (blue).

Download English Version:

https://daneshyari.com/en/article/2093995

Download Persian Version:

https://daneshyari.com/article/2093995

Daneshyari.com