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Lab Resource: Stem Cell Line

Derivation of Genea052 human embryonic stem cell line

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ABSTRACT

The Genea052 human embryonic stem cell line was derived from a donated, fully commercially consented ART blastocyst, through ICM outgrowth on inactivated human feeders. The line showed pluripotent cell morphology and genomic analysis verified a 46, XY karyotype and male allele pattern through CGH and STR analysis. Pluripotency of Genea052 was demonstrated with 85% of cells expressing Nanog, 87% Oct4, 60% Tra1-60 and 97% SSEA4, a PluriTest Pluripotency score of 27.21, Novelty score of 1.2 and tri-lineage teratoma formation. The cell line was negative for *Mycoplasma* and any visible contamination.

Resource table

Name of stem cell line	Genea052 (alternate ID: SIVF052)
Institution	Genea biocells
Person who created resource	Omar Chami
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	November, 2009
Origin	Human embryos
Type of resource	Derived human embryonic stem cell line
Sub-type	Human pluripotent cell line
Key marker expression	Nanog, Oct4, Tra1-60, and SSEA4
Authentication	Identity and purity of cell line confirmed (Figs. 1–5 below)
Link to related literature (direct URL links and full references)	
Information in public databases	National Institutes of Health (NIH) registered NIHhESC-13-0234 UK Stem Cell Bank (UKSCB) registered SCSC14-36
Ethical approval	Obtained from the Genea Ethics Committee on 21 February 2001 under the Australian National Health and Medical Research Council (NHMRC) licence 309703

Resource details

Date of derivation	October 2009
Karyotype	46, XY — no abnormalities detected
Sex	Male
Pluripotent	Yes — by Nanog, Oct4, Tra1-60, and SSEA4 staining, tri-lineage teratoma formation and PluriTest
Disease status	Unaffected
Sterility	The cell line is tested and found negative for *Mycoplasma* and any visible contamination
Sibling lines available	No

1. Materials and methods

1.1. Cell line derivation

20% KSR medium (Amit et al., 2000) was conditioned by culture with high density mitotically inactivated feeders (72,000 cells/cm^2) for 24 h @ 37° 5% CO_2 5% O2. The medium is collected and every second day for a period of 2 weeks. Medium from the 2 weeks is combined. The zona pellucida of a blastocyst-stage human embryo was manually removed using a small blade. The embryo was bisected and plated onto human collagen type I (coated at 10 ng/mL Millipore #CC050) in a 1:1 mix of 20% Knock out serum in standard hESC culture medium (Amit et al., 2000) and conditioned media, plus 50 ng/mL Fgf2 added fresh. CGH, karyotyping and STR profiling were performed at the first cryobanking step from ICM outgrowths maintained on feeders. Alkaline phosphatase staining was performed on feeders. Cells were then enzymatically passaged as single cells in M2 pluripotent cell maintenance medium (Genea Biocells) and genetic analysis repeated, immunofluorescent pluripotent marker staining, PluriTest, teratoma and sterility testing performed.

1.2. Genetic analysis

1. Comparative genomic hybridisation (CGH) based chromosomal analysis: Passage 9 (7 on feeders, 2 enzymatic); CGH was used to screen targeted regions of the genome for gains and losses associated with chromosomal imbalances such as aneuploidy, deletions and duplications. CGH was performed using SurePrint G3 microarrays (8 × 60 K format) which were scanned with the Agilent Scanner C and analysed using Genomic Workbench Standard Edition 5.0 software (Agilent Technologies).

http://dx.doi.org/10.1016/j.scr.2015.11.014

2. DNA profiling: Passage 4; DNA 'fingerprinting' was performed using the AmpFLSTR Identifiler PCR Amplification Kit (Applied Biosystems #4322288) to provide permanent genetic identification of the cell lines. https://www.thermofisher.com/order/catalog/product/4322288

1.3. Pluripotency assessment

1. Immunofluorescence: Passage 9 (7 on feeders, 2 enzymatic); cells were fixed with formalin and stained with Nanog #560,483 1:200; Oct4 #560,217 1:150: Tra1-60 #560,121 1:150; SSEA4 #560,308 1:200 (all BD Pharmingen:). Images were acquired with an IN Cell Analyser 6000 and quantified using In Cell Developer Software (GE).
2. PluriTest: Passage 9 (7 on feeders, 2 enzymatic); RNA was collected and subjected to a PluriTest, a bioinformatic assay of pluripotency in human cells based on gene expression profiles (Müller et al., 2012)
3. Teratoma formation: Passage p7; stem cells were received one day prior to injection. On the day of injection cells were harvested by manual scraping, washed and resuspended in DMEM/20% KSR for injection. In both experiments, mice were anaesthetized before intramuscular injection of cells into the left inner thigh muscle of the hind leg. Approximately 1–2 × 10^6 cells (1xT25) in 50 μL were injected per mouse at one site only. Resultant teratomas were excised, fixed, sectioned and stained for assessment of tissues from each of the embryonic germ layers.

1.4. Sterility testing

1. *Mycoplasma*: Passage 2; testing was performed at the Victorian Infectious Diseases Reference Laboratory using *Mycoplasma* Genus PCR
2. Microbial contamination: testing was performed in conjunction with our QC measures. Cells were thawed and cultured in 7 mL antibiotic free medium (Genea Biocells M2 medium) for 2–3 days at 37 °C. A clear solution at ~48–72 h indicated lack of bacterial, fungal or yeast contamination. Clarity of the solution was assessed by Cell Production Team.

2. Verification and authentication

2.1. Ethics/consents

Ethics approval for the project ('development of human embryonic stem cells from excess ART embryos') was obtained from the Genea Ethics Committee on 21 February 2001. Excess ART embryos were fully consented for stem cell derivation by all responsible people through an informed consent process (signed de-identified consent form can be provided upon request). Donors have received no payment or financial benefits for their donation. Genea052 has been derived from a donated, fully commercially consented human embryo, originally created by assisted reproduction technology (ART) for the purpose of procreation, under Australian National Health and Medical Research Council (NHMRC) licence 309703. This licence was issued to GENEA on 16 April 2004. More information about the licence can be obtained from the NHMRC webpage at http://www.nhmrc.gov.au/health-ethics/human-embryos-and-cloning/database-licences-authorising-use-excess-art-embryos.

2.2. Morphology

The derived stem cell line, Genea052, morphologically displays adherent monolayer of compact cells in well-defined colonies with high nuclear to cytoplasmic ratio and prominent nucleoli (Fig. 1).

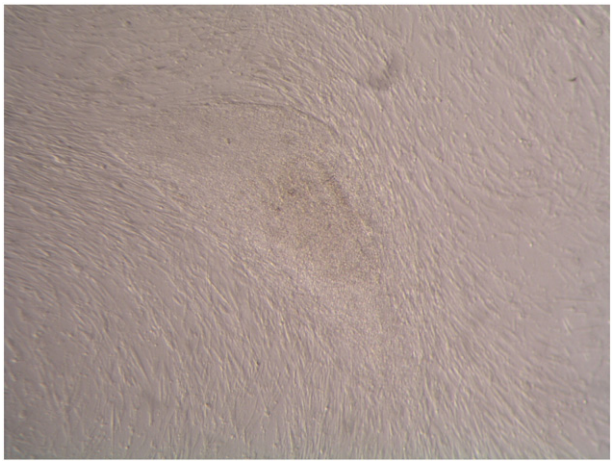

Fig. 1. Brightfield morphology of Genea052 on human inactivated feeders.

2.3. Genetic analysis

The cell line has been tested by CGH (Table 1, Supplementary Fig. 1), which demonstrated 46, XY karyotype, consistent with original derivation. Analysis of STR markers showed allele pattern consistent with male genotype (Fig. 2, Supplementary Fig. 2).

2.4. Disease status

Unaffected.

2.5. Pluripotency

GENEA052 is pluripotent by;

1. Immunofluorescence with 85% Nanog positive, 87% Oct4 positive, 60% Tra1-60 positive, and 97% SSEA4 positive (Fig. 3A, quantified in 3B).
2. PluriTest with a 27.21 Pluripotency score and 1.2 Novelty score (Fig. 4).
3. Teratoma formation which contained tissues derived from each of the embryonic germ layers: endoderm, mesoderm, and ectoderm (Fig. 5).

2.6. Sterility

The cell line is tested and found negative for *Mycoplasma* and any visible contamination (Supplementary Fig. 3).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2015.11.014.

Table 1
CGH array summary.

Parameter	Result
Sample name	Genea052p9_2
Date reported	30th May 2014
Hybridisation balance	A balanced hybridisation was observed for all chromosomes, relative to reference DNA
Copy number change	Loss of 875 kb at chromosome region 4p12 — not clinically relevant
Interpretation	Male cell line — no abnormalities detected