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Lab resource: stem cell line

Derivation of Genea057 human embryonic stem cell line

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ABSTRACT

The Genea057 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, XX karyotype and female allele pattern through traditional karyotyping, CGH and STR analysis. Pluripotency of Genea057 was demonstrated with 97% of cells expressing Nanog, 81% Oct4, 75% Tra1-60 and 97% SSEA4, a PluriTest Pluripotency score of 27.59 and Novelty score of 1.32. The cell line was negative for Mycoplasma and any visible contamination.

1. Resource table

Name of stem cell line	Genea057 (Alternate ID: SIVF057)
Institution	Genea Biocells
Person who created resource	Biljana Dumevska
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	December, 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. 1-4 below)
Link to related literature (direct URL links and full references)	
Information in public databases	National Institutes of Health (NIH) registered NIHhESC-13-0233 UK Stem Cell Bank (UKSCB) registered SCSC14-39
Ethical approval	Obtained from the Genea Ethics Committee on 21 February 2001 under the Australian National Health and Medical Research Council (NHMRC) licence 309703

2. Resource details

Date of derivation	November 2009
Karyotype	46, XX — no abnormalities detected
Sex	Female
Pluripotent	YES — by Nanog, Oct4, Tra1-60, and SSEA4 staining and PluriTest
Disease status	Unaffected
Sterility	The cell line is tested and found negative for Mycoplasma and any visible contamination
Sibling lines available	NO

3. Materials and methods

3.1. Cell line derivation

The zona pellucida of a blastocyst-stage human embryo was manually removed using a small blade. The embryo was plated whole onto mitomycin C inactivated Detroit 510 HFF human feeders (plated 90,000 cells 1 well of 4 well — 47,368 cells/cm^2) in 20% Knock out serum in standard hESC culture medium (Amit et al., 2000) with 50 ng/mL Fgf2. CGH, karyotyping and STR profiling were performed at the first cryobanking step from ICM outgrowths maintained on feeders. Cells were then enzymatically passaged as single cells in M2 pluripotent

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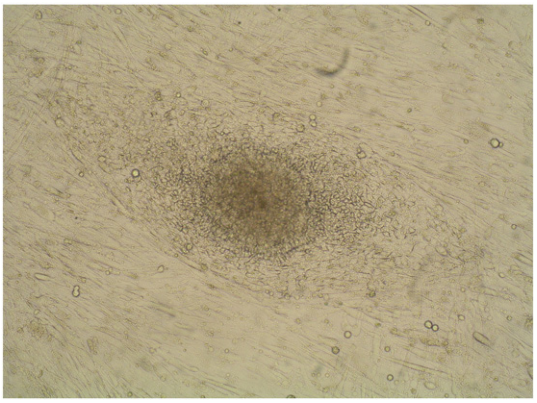

Fig. 1. Brightfield morphology of Genea057 on human inactivated feeders.

Table 1
CGH array summary.

Parameter	Result
Sample name	Genea057 p10_2
Date reported	26th May 2014
Hybridisation balance	A balanced hybridization was observed for all chromosomes, relative to reference DNA
Copy number change	No copy number changes >400 kb were detected
Interpretation	Female cell line — no abnormalities detected

cell maintenance medium (Genea Biocells) and genetic analysis repeated, immunofluorescent pluripotent marker staining, PluriTest and sterility testing performed.

3.2. Genetic analysis

1. Comparative Genomic Hybridisation (CGH) based chromosomal analysis: Passage 10 (8 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, Agilent microarrays (8 × 60 K format) at 41.5 KB overall median probe spacing. Arrays were scanned with the Agilent Scanner C and analysed using Genomic Workbench Standard Edition 5.0 software (Agilent Technologies).
2. DNA Profiling: Passage 2; 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

3.3. Pluripotency assessment

1. Immunofluorescence: Passage 10 (8 on feeders, 2 enzymatic); cells were fixed with formalin and stained with Nanog #560483 1:200; Oct4 #560217 1:150: Tra1-60 #560121 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 10 (8 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)

3.4. Sterility testing

1. Mycoplasma: Passage 10 (8 on feeders, 2 enzymatic); testing was performed as per manufacturers instructions using the MycoAlert Mycoplasma Detection Kit from LONZA
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.

4. Verification and authentication

4.1. 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. Genea057 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.

4.2. Morphology

The derived stem cell line, Genea057, morphologically displays adherent monolayer of compact cells in well-defined colonies with high nuclear to cytoplasmic ratio and prominent nucleoli (Fig. 1).

4.3. Genetic analysis

The cell line demonstrated a 46, XX karyotype and female Allele pattern, through CGH (Table 1, Supplementary Figure 1) and STR marker analysis (Fig. 2, Supplementary Figure 2), consistent with original derivation.

4.4. Disease status

Unaffected.

DNA Profile Results

CASE NO: SIVF057_p2		D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
SIVF057_p2	Identity	13 , 14	28 , 31	10	10	15 , 16	7	8 , 10	10 , 12	17 , 23	14 , 15	14 , 17	8	14 , 15	10 , 11	21 , 25

Fig. 2. STR profile.