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Lab Resource: Stem Cell Line

Derivation of Genea047 human embryonic stem cell line

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ABSTRACT

The Genea047 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 Genea047 was demonstrated with 88% of cells expressing Nanog, 95% Oct4, 59% Tra1-60 and 99% SSEA4, a PluriTest Pluripotency score of 30.86, Novelty score of 1.23 and tri-lineage teratoma formation. The cell line was negative for *Mycoplasma* and any visible contamination.

Resource table

Name of stem cell line	Genea047 (alternate ID: SIVF047)
Institution	Genea Biocells
Person who created resource	Biljana Dumevska
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	May, 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. 1–6 below)
Link to related literature (direct URL links and full references)	(Laurent et al., 2011) http://www.ncbi.nlm.nih.gov/pubmed/?term=21211785
Information in public databases	National Institutes of Health (NIH) registered NIHhESC-13-0230 UK Stem Cell Bank (UKSCB) registered SCSC14-41 SNP Data GEO accession numbers GSM638449
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	April 2009
Karyotype	46, XX — no abnormalities detected
Sex	Female
Pluripotent	YES — by Nanog, Oct4, Tra1-60, and SSEA4 staining, PluriTest and tri-lineage teratoma formation
Disease status	Unaffected
Sterility	The cell line is tested and found negative for *Mycoplasma* and any visible contamination
Sibling lines available	No

Materials and methods

Cell line derivation

The zona pellucida of a blastocyst-stage human embryo was manually removed using a small blade. The embryo was bisected and both ICM and trophectoderm were plated onto mitomycin C inactivated Detroit 551 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 20 ng/mL Fgf2. 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.

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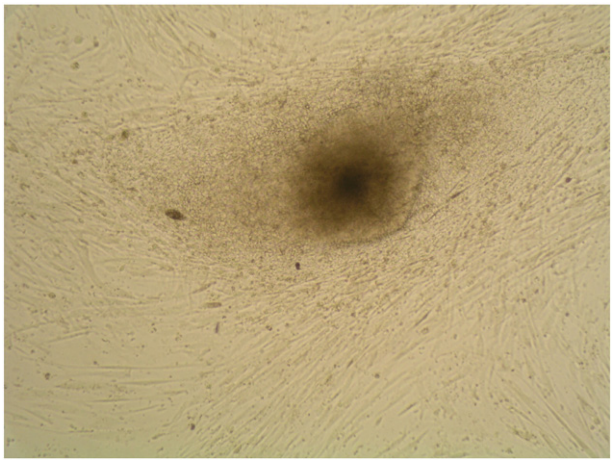

Fig. 1. Brightfield morphology of Genea047 on human inactivated feeders.

Table 1
CGH array summary.

Parameter	Result
Sample name	Genea047p8_2
Date reported	14th August 2013
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

Genetic analysis

1. Karyotyping: Passage 5; For enrichment of metaphase cells, cellular outgrowths were incubated with either 0.22 ng/ml colcemid (Invitrogen) and 37.5 g/mL BrdU (Sigma) for 17–19 h or 5 ng/mL colcemid for 2.5 h. Single cells were subsequently obtained using Non-Enzymatic Cell Dissociation Solution (Sigma) and transferred to Sydney IVF's (now Genea) diagnostic cytogenetics laboratory for preparation of metaphase spreads and cytogenetic analysis by G-banding. A minimum of 15 metaphase cells were examined, of which a full karyotype or chromosome counts was performed for 5 and 10 of the metaphases respectively.
2. Comparative genomic hybridisation (CGH) based chromosomal analysis: Passage 8 (6 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).
3. 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

Pluripotency assessment

1. Immunofluorescence: Passage 8 (6 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 #560308 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 (6 on feeders, 3 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 8; 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 $1–2 \times 10^6$ cells (1xT25) in 50 μL were injected per mouse at one site only. Resultant teratomas were

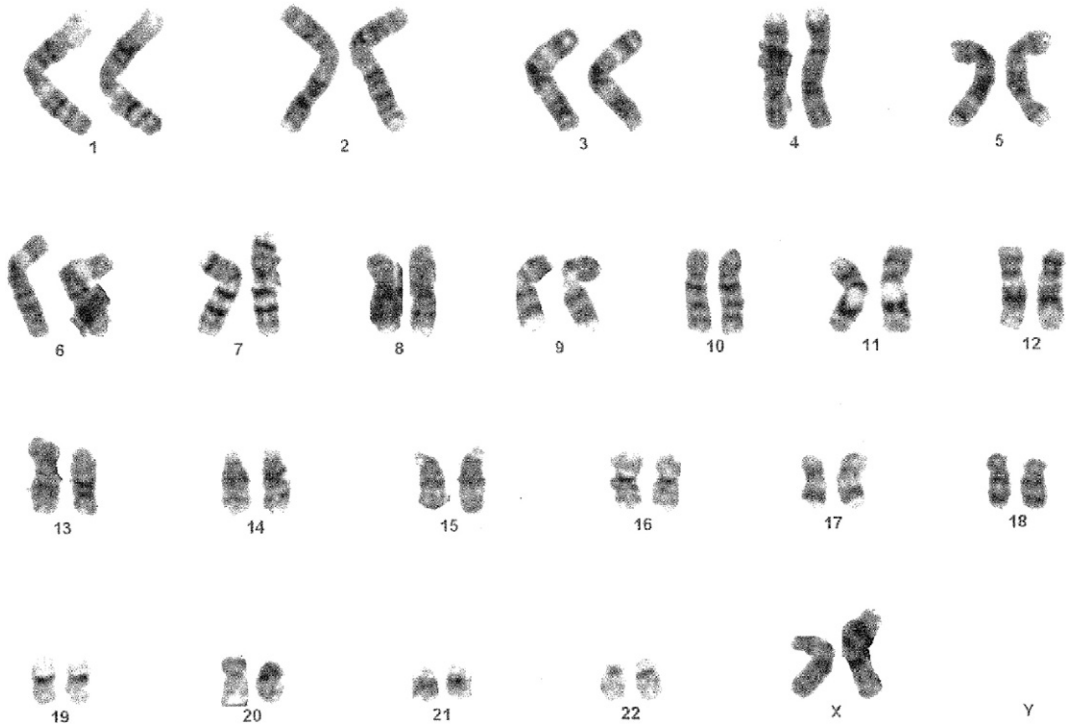

Fig. 2. Karyotype.