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Lab resource: Stem cell line

Derivation of Genea016 human embryonic stem cell line

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ABSTRACT

The Genea016 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 Genea016 was demonstrated with 77% of cells expressing Nanog, 95% Oct4, 53% Tra1-60 and 98% SSEA4, a PluriTest Pluripotency score of 28.4, Novelty score of 1.37 and Alkaline Phosphatase activity. The cell line was negative for Mycoplasma and any visible contamination.

Resource table

Name of stem cell line	Genea016 (Alternate ID: SIVF016)
Institution	Genea Biocells
Person who created resource	Teija Peura
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	June, 2007
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)	(Bradley et al. 2010) http://www.ncbi.nlm.nih.gov/pubmed/?term=20198447 (Laurent et al. 2011) http://www.ncbi.nlm.nih.gov/pubmed/?term=21211785
Information in public databases	National Institutes of Health (NIH) registered NIHhESC-13-0229 UK Stem Cell Bank (UKSCB) registered SCSC14-37 SNP Data GEO accession numbers GSM638421
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	May 2007
Karyotype	46, XX — no abnormalities detected
Sex	Female
Pluripotent	YES — by Alkaline Phosphatase stain positivity, 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	YES — GENEA015 (XY NIHhESC-13-0228)

Materials and methods

Cell line derivation

The zona pellucida of a blastocyst-stage human embryo was removed with pronase. The embryo was bisected and plated onto mitomycin C inactivated Detroit 551 HFF (plated 200,000 cells per organ culture dish — 69,204 cells/cm2) in 20% Knock out serum in standard hESC culture medium (Amit et al. 2000) with 50 ng/ml Fgf2. Karyotyping, CGH and DNA profiling was 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 and genetic analysis repeated as well as immunofluorescent pluripotent marker staining, PluriTest and sterility testing performed.

Genetic analysis

1. Karyotyping: Passage 5; for the 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

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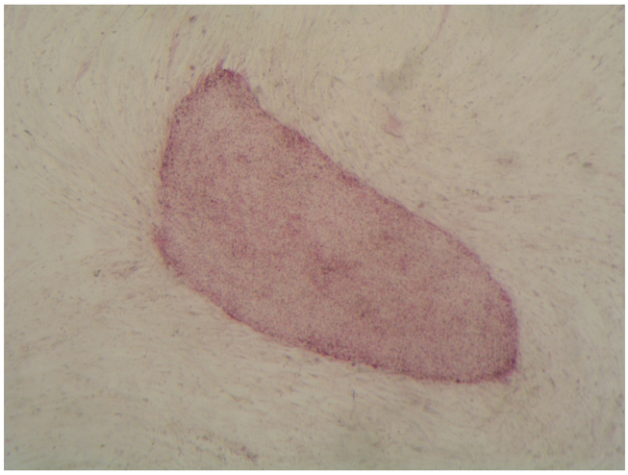

Fig. 1. Alkaline Phosphatase staining.

by G-banding. A minimum of 15 metaphase cells were examined, of which full karyotype or chromosome counts were performed for 5 and 10 of the metaphases respectively.

2. Comparative Genomic Hybridisation (CGH) based chromosomal analysis: Passage 15 (13 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 5; 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

Table 1
CGH analysis summary.

Parameter	Result
Sample name	Genea016p15_2
Date reported	20th 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

Pluripotency assessment

1. Alkaline Phosphatase: Passage 21; Genea016 was stained as per manufacturers protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004).
2. Immunofluorescence: Passage 15 (13 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).
3. PluriTest: Passage 15 (13 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).

Sterility testing

1. Mycoplasma: Passage 27; testing was performed at the Victorian Infectious Diseases Reference Laboratory using Mycoplasma Genus PCR.

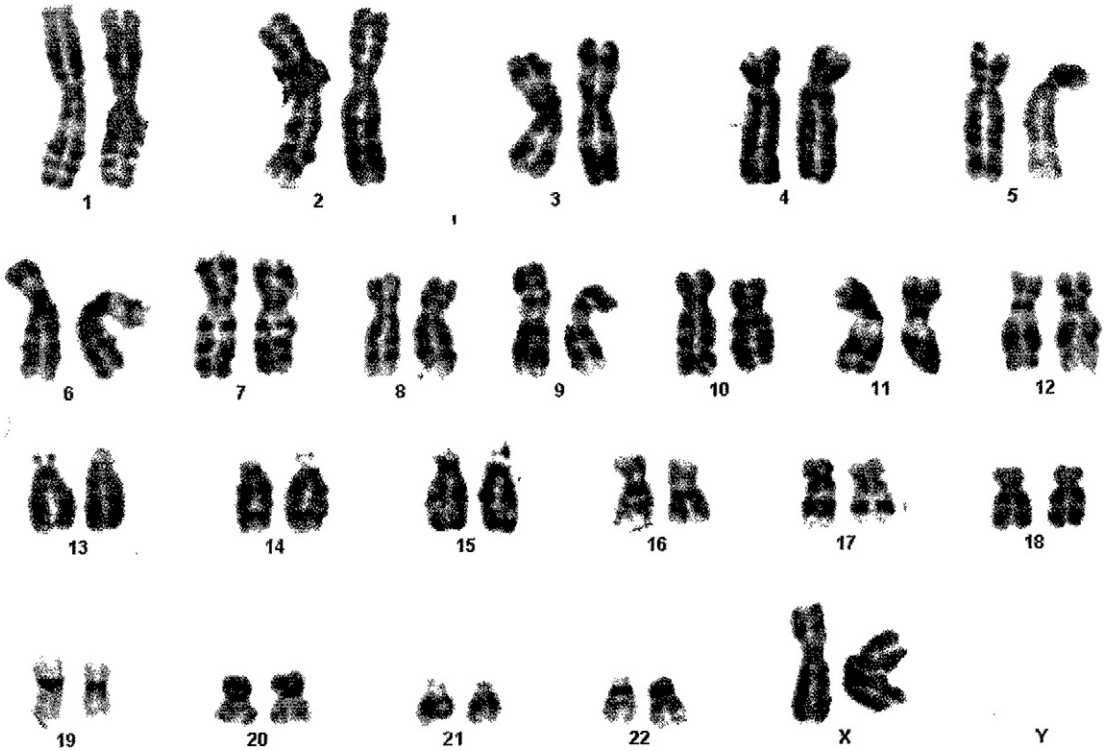

Fig. 2. Karyotype.