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Lab Resource: Stem Cell Line

Derivation of FSHD1 affected human embryonic stem cell line Genea050

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ABSTRACT

The Genea050 human embryonic stem cell line was derived from a donated, fully commercially consented ART blastocyst, carrying a deletion in 4q35 with only 5 D4Z4 repeats by PGD linkage analysis, indicative of FSHD1. Following ICM outgrowth on inactivated human feeders, karyotype was confirmed as 46, XY and STR analysis demonstrated a male Allele pattern. The hESC line had pluripotent cell morphology, 92% of cells expressed Nanog, 97% Oct4, 79% Tra1-60 and 99% SSEA4, gave a Pluritest Pluripotency score of 25.45, Novelty of 1.45 demonstrated Alkaline Phosphatase activity and tri-lineage teratoma formation. The cell line was negative for *Mycoplasma* and visible contamination.

Resource table

Name of stem cell line	Genea050 — FSHD1 affected (Alternate ID: SIVF050)
Institution	Genea Biocells
Person who created resource	Omar Chami
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	August, 2009
Origin	Human embryos
Type of resource	Derived human embryonic stem cell line
Sub-type	FSHD1 affected human pluripotent cell line
Key marker expression	Nanog, Oct4, Tra1-60, and SSEA4
Authentication	Identity and purity of cell line confirmed (Figs. 1–2 and Tables 1–2 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 registered, NIH: NIHhESC-12-0184 UK Stem Cell Bank registered, UKSCB: SCSC14-54 SNP Data Gene Expression Omnibus accession number, GEO: GSM638452
Ethical Approval	Obtained from the Genea Ethics Committee on 13 September 2005 under the Australian National Health and Medical Research Council (NHMRC) licence 309710

Resource details

Date of derivation	July 2009
Karyotype	46, XY
Sex	Male
Pluripotent	YES — by Nanog, Oct4, Tra1-60, and SSEA4 staining, Pluritest, Alkaline Phosphatase and teratoma
Disease status	deletion in 4q35, 5 D4Z4 repeats — Facioscapulohumeral muscular dystrophy 1 (FSHD1) affected, OMIM: 158900
Sterility	The cell line is tested and found negative for *Mycoplasma* and any visible contamination
Sibling lines available	YES — GENEA049 (FSHD1 affected — XX, NIH: NIHhESC-12-0183, UKSCB: SCSC14-54)

Materials and methods

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 @ 37degrees 5% CO_2 5% O_2. The medium is collected every second day for a period of 2 weeks. Medium from the 2 weeks is combined. The fresh embryo was plated whole onto mitomycin C inactivated human feeders (Detroit 551 HFF — 90,000/well in 4-well) in hESC conditioned 20% KSR medium with 20 ng/ml FGF added fresh. CGH karyotyping and STR profiling were performed at the first cryobanking step from ICM outgrowths maintained on feeders. Alkaline Phosphatase staining and teratoma formation were performed from feeders. Cells were then enzymatically passaged as single cells in M2 pluripotent cell maintenance medium (Genea Biocells) and CGH/karyotyping repeated,

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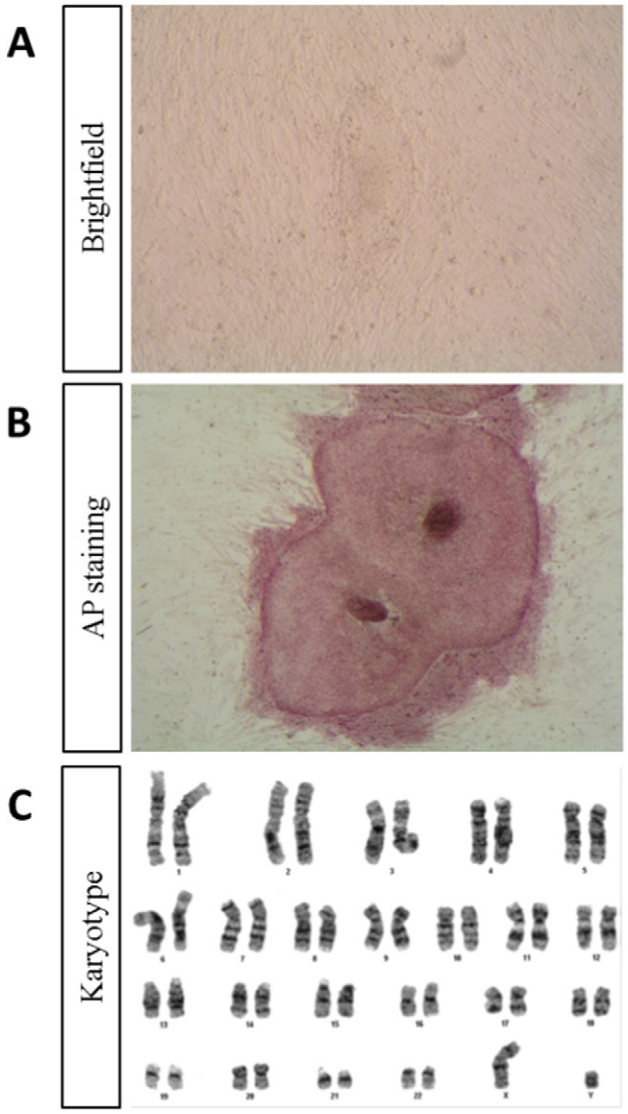

Fig. 1. Morphology and karyotype of Genea050. A) Brightfield (passage 1) growing on human inactivated feeders. B) Alkaline Phosphatase activity (passage 6) on human inactivated feeders. C) Karyotypic analysis (passage 5, 1 enzymatic) showing 46, XY normal, male karyotype.

immunofluorescent pluripotent marker staining, Pluritest and sterility testing performed.

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 were performed for 5 and 10 of the metaphases respectively.
2. *Comparative Genomic Hybridisation (CGH) based chromosomal analysis:* Passage 8; 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 (8x 60K 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 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

Pluripotency assessment

1. *Immunofluorescence:* Passage 7 (5 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; and 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 8 (5 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. *Alkaline Phosphatase:* Passage 6; Genea050 grown on feeders was stained as per manufacturer's protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004).
4. *Teratoma formation:* Passage 5; Genea050 was injected intramuscularly to immuno-deficient mice and resultant teratomas excised, fixed, sectioned and stained for assessment of tissues from each of the embryonic germ layers.

Sterility testing

1. *Mycoplasma:* Passage 12; 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.

Verification and authentication

Ethics/consents

Ethics approval for the project ('Derivation of human embryonic stem cells from embryos identified through pre-implantation genetic diagnosis to be affected by known genetic conditions') was obtained from the Genea Ethics Committee on 13 September 2005. 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. Genea050 has been derived from a donated, fully commercially consented embryo, originally created by assisted reproduction technology (ART) for the purpose of procreation. The embryo was identified through pre-implantation genetic diagnosis to be affected by a genetic mutation and was declared

Table 1
CGH analysis summary; Genea050 (passage 8, 3 enzymatic) reporting a male cell line and no abnormalities detected.

	CGH summary
Sample name	Genea 50_p8_TE3
Date reported	1st May 2013
Hybridisation balance	Balanced hybridization was observed for all chromosomes, relative to reference DNA
Copy number change	No copy number changes >400 kb were detected
Interpretation	Male cell line — no abnormalities detected