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Lab Resource: Stem Cell Line

Derivation of Huntington Disease affected Genea017 human embryonic stem cell line

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ABSTRACT

The Genea017 human embryonic stem cell line was derived from a donated, fully commercially consented ART blastocyst, carrying Htt gene CAG expansion of 40 repeats, indicative of Huntington Disease. Following ICM outgrowth on inactivated human feeders, genetic analysis confirmed a 46, XY karyotype and male allele pattern through CGH and STR analysis. The hESC line had pluripotent cell morphology, 87% of cells expressed Nanog, 95% Oct4, 88% Tra1-60 and 99% SSEA4, gave a PluriTest pluripotency score of 34.74, novelty of 1.27, 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	Genea017 — HD affected (alternate ID: SIVF017)
Institution	Genea Biocells
Person who created resource	Teija Peura
Contact person and email	Biljana Dumevska (biljana.dumevska@geneabiocells.com)
Date archived/stock date	July 2007
Origin	Human embryos
Type of resource	Derived human embryonic stem cell line
Sub-type	HD 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)	Tay et al. (2009) http://www.ncbi.nlm.nih.gov/pubmed/?term=20058197 Laurent et al. (2010) http://www.ncbi.nlm.nih.gov/pubmed/?term=20038950 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 Bradley et al. (2011) http://www.ncbi.nlm.nih.gov/pubmed/?term=20649476 McQuade and Balachandran (2014) http://www.ncbi.nlm.nih.gov/pubmed/25316320
Information in public databases	National Institutes of Health registered, NIH: NIHhESC-12-0166 UK Stem Cell Bank registered, UKSCB: SCSC14-29 SNP Data GEO accession number, GEO: GSM638422
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	June 2007
Karyotype	46, XY
Sex	Male
Pluripotent	Yes — by Nanog, Oct4, Tra1-60, and SSEA4 staining, PluriTest, alkaline phosphatase and tri-lineage teratoma formation
Disease status	Expansion of CAG repeats (12 and 40 CAG repeats) — Huntington Disease (HD) affected, OMIM: 143100
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 embryo was plated whole onto mitomycin C inactivated human feeders (Detroit 551 HFF – 200,000 cells per organ culture dish – 69,204 cells/cm^2) in 20% KSR medium with 50 ng/mL FGF added fresh (Amit et al., 2000). 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) for freezing of single cell stocks and karyotyping/DNA profiling was repeated as well as immunofluorescent pluripotent marker staining, PluriTest and sterility testing were performed.

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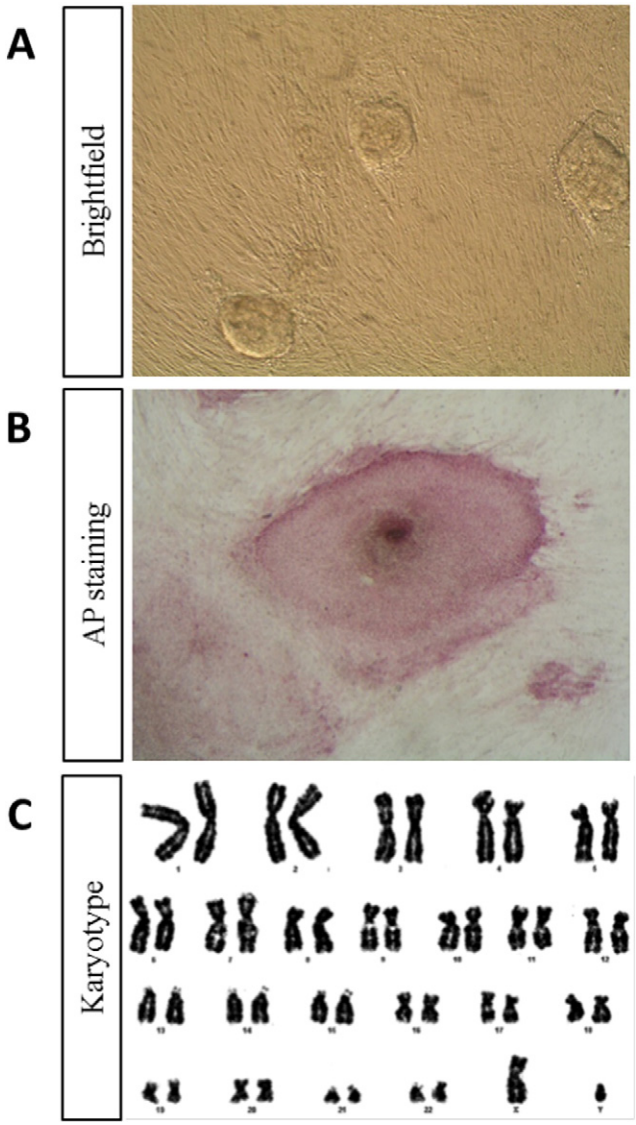

Fig. 1. Morphology and karyotype of Genea017. A) Brightfield (passage 2) growing on human inactivated feeders. B) Alkaline phosphatase activity (passage 35) on human inactivated feeders. C) Karyotypic analysis (passage 5) showing 46, XY normal, male karyotype.

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 12 (10 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).

Pluripotency assessment

1. Alkaline phosphatase: Passage 35; Genea017 grown on feeders were stained as per manufacturer's protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004).
2. Immunofluorescence: Passage 12 (10 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 12 (10 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).
4. Teratoma formation: Passage 43; two CB17-SCID mice sourced from the Animal Resource Centre (ARC) in Western Australia. Stem cells were received one day prior to injection and harvested using Collagenase IV treatment, washed in DMEM/F12 and resuspended in injection buffer supplemented with 10% FBS and 30% BD Matrigel™. In both experiments, mice were anaesthetised before intramuscular injection of the cells into the hind leg of the mouse. Approximately 1×10^6 cells (1 × T25) 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.

Sterility testing

1. Mycoplasma: Passage 10; 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 a lack of bacterial, fungal or yeast contamination. Clarity of the solution was assessed by the 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. Genea017 has been

Table 1
CGH analysis summary; Genea017 (passage 12, 2 enzymatic) reporting a male cell line and no abnormalities detected.

	CGH summary
Sample name	Genea017 p12_2
Date reported	22nd July 2013
Hybridisation balance	Balanced hybridisation was observed for all chromosomes, relative to reference DNA
Copy number change	No copy number changes above 400 kb were detected
Interpretation	Male cell line — no abnormalities detected