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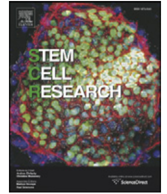

Lab Resource: Stem Cell Line

Derivation of Huntington Disease affected Genea018 human embryonic stem cell line

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ABSTRACT

The Genea018 human embryonic stem cell line was derived from a donated, fully commercially consented ART blastocyst, carrying Htt gene CAG expansion of 46 repeats, indicative of Huntington Disease. Following ICM outgrowth on inactivated human feeders, karyotype was confirmed as 46, XX by CGH and STR analysis demonstrated a female Allele pattern. The hESC line had pluripotent cell morphology, 75% of cells expressed Nanog, 91% Oct4, 73% Tra1-60 and 96% SSEA4, gave a Pluritest pluripotency score of 31.12, Novelty of 1.45, demonstrated Alkaline Phosphatase activity and tri-lineage teratoma formation. The cell line was negative for Mycoplasma and visible contamination.

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Resource Table

Name of stem cell line	Genea018 — Huntington Disease affected (Alternate ID: SIVF018)
Institution	Genea Biocells
Person who created resource	Teija Peura
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	September 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 and 2 and Tables 1 and 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-12-0169 UK Stem Cell Bank registered, UKSCB: SCSC14-45 SNP Data Gene Expression Omnibus accession number, GEO: GSM638425
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	August 2007
Karyotype	46, XX
Sex	Female
Pluripotent	YES — by Nanog, Oct4, Tra1-60, and SSEA4 staining, Pluritest, Alkaline Phosphatase and tri-lineage teratoma
Disease status	Expansion of CAG repeats (17 and 46 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 zona pellucida of a blastocyst-stage human embryo was manually removed using a small blade. The embryo was plated whole onto irradiated human feeders (Detroit 551 HFF – 200,000 cells per organ culture dish – 69,204 cells/cm^2) in 20% KSR medium (Amit et al., 2000) with 50 ng/mL FGF added fresh. Karyotyping and STR profiling was 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/DNA profiling repeated as well as immunofluorescent pluripotent marker staining, Pluritest and sterility testing performed.

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Genetic analysis

1. Karyotyping: Passage 6; 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. DNA Profiling: Passage 8; 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

3. Immunofluorescence: Passage 15 (12 on feeders, 3 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).
4. Pluritest: Passage 16 (12 on feeders, 4 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)
5. Alkaline Phosphatase: Passage 9; Genea018 grown on feeders were stained as per manufacturers protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004)
6. Teratoma formation: Passage 43; 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 (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

7. Mycoplasma: Passage 13; testing was performed at the Victorian Infectious Diseases Reference Laboratory using Mycoplasma Genus PCR
8. 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. Genea018 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 excess to reproductive needs. Derivation was performed under Australian National Health and Medical Research Council (NHMRC) licence 309710. This licence was issued to GENEA on 7 May 2007. More information about the licence can be obtained from the NHMRC

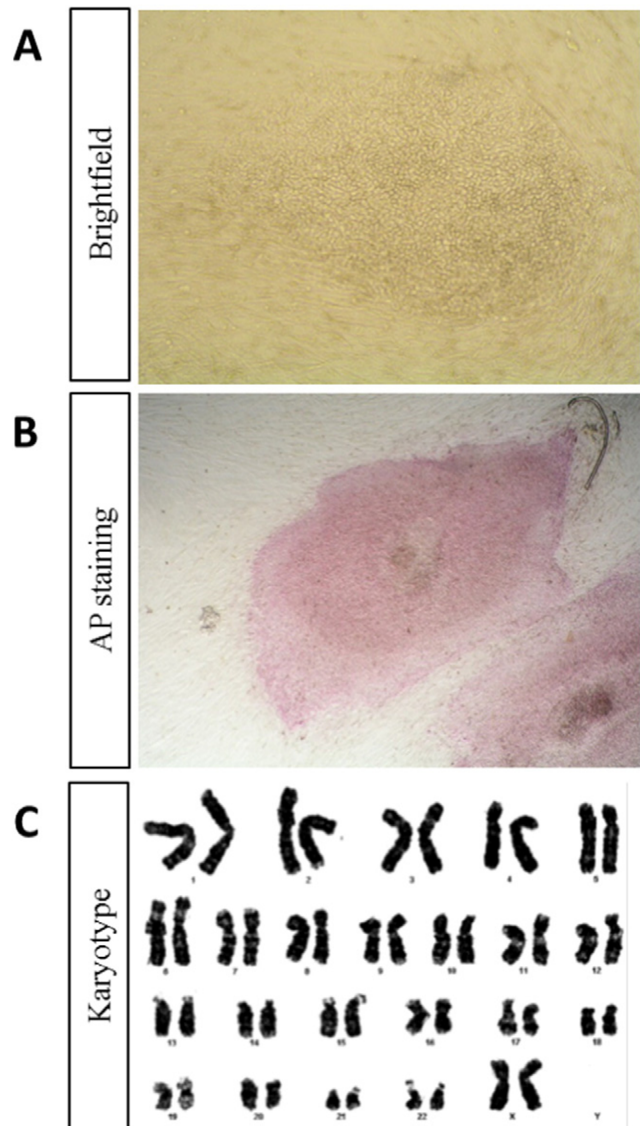

Fig. 1. Morphology and karyotype of Genea018. A) Brightfield (passage 52) growing on human inactivated feeders. **B)** Alkaline Phosphatase activity (passage 9) on human inactivated feeders. **C)** Karyotypic analysis (passage 6) showing 46, XX normal, female karyotype.

Table 1
CGH analysis summary; Genea018 (passage 16, 4 enzymatic) reporting a female cell line and no abnormalities detected.

	CGH summary
Sample name	Genea018 p16_TE4
Date reported	12th February 2013
Hybridisation balance	Balanced hybridization was observed for all chromosomes, relative to reference DNA
Copy number change	No copy number changes above 400 kb were detected
Interpretation	Female cell line — no abnormalities detected

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