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Lab Resource: Stem Cell Line

Derivation of Huntington disease affected Genea020 human embryonic stem cell line

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ABSTRACT

The Genea020 human embryonic stem cell line was derived from a donated, fully commercially consented ART blastocyst, carrying Htt gene CAG expansion of 48 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, 89% of cells expressed Nanog, 95% Oct4, 29% Tra1-60 and 99% SSEA4, gave a Pluritest pluripotency score of 27.51, novelty of 1.43 and demonstrated alkaline phosphatase activity. The cell line was negative for *Mycoplasma* and visible contamination.

Resource table

Name of stem cell line	Genea020 — HD affected (Alternate ID: SIVF020)
Institution	Genea Biocells
Person who created resource	Teija Peura
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	December 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	UK Stem Cell Bank registered, UKSCB: SCSC14-62 SNP Data Gene Expression Omnibus accession numbers, GEO: GSM638429, GEO: GSM638430
Ethical approval	Obtained from the Genea Ethics Committee on 13 September 2005 under the *Ethical Guidelines on the Use of Assisted Reproductive Technology in Clinical Practice and Research* (ART guidelines, 2004) and the *National Statement on Ethical Conduct in Human Research*.

Resource details

Date of plating	November 2004
Karyotype	46, XX
Sex	Female
Pluripotent	YES — by Nanog, Oct4, Tra1-60, and SSEA4 staining as well as tri-lineage teratoma formation, Pluritest and alkaline phosphatase stain positivity
Disease status	Expansion of CAG repeats (17 and 48 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	YES — GENEA019 (XX — UKSCB: SCSC14-62)

Materials and methods

Cell line derivation

The embryo was plated whole onto mitomycin C inactivated human feeders (Detroit 551 HFF, plated 130,000 cells 1 well of 4 wells — 68,421 cells/cm^2) in 20% knock out serum in standard hESC culture medium with 50 ng/ml Fgf2 (Amit et al., 2000). CGH karyotyping and STR profiling were performed at the first cryobanking step from ICM outgrowths maintained on feeders. Alkaline phosphatase staining was

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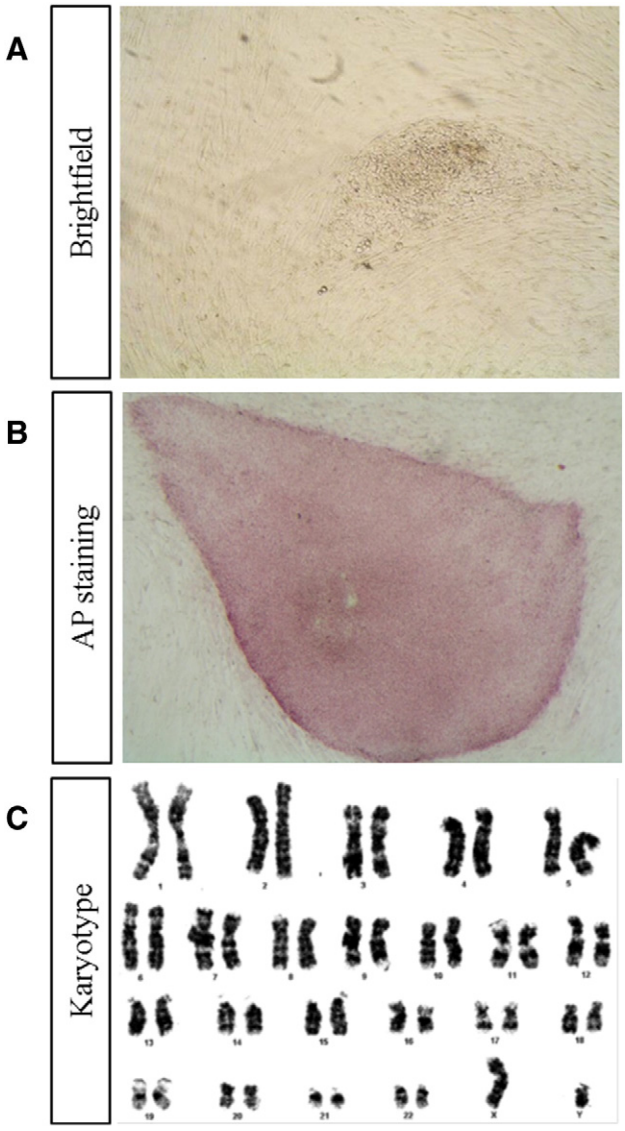

Fig. 1. Morphology and karyotype of Genea020. A) Brightfield (passage 1) growing on human inactivated feeders. B) Alkaline phosphatase activity (passage 21) on human inactivated feeders. C) Karyotypic analysis (passage 5) showing 46, XY normal, male karyotype.

Table 1
CGH analysis summary; Genea020 (passage 12, 3 enzymatic), reporting a female cell line and no abnormalities detected.

	CGH summary
Sample name	Genea020 p12-TE3
Date reported	12th February 2013
Hybridisation balance	Balanced hybridization was observed for all chromosomes
Copy number change	No copy number changes above 400 kb were detected
Interpretation	Female cell line

performed on feeders. Cells were then enzymatically passaged as single cells in M2 pluripotent cell maintenance medium (Genea Biocells) and CGH/karyotyping repeated, immunofluorescent pluripotent marker staining, Pluritest, teratoma and sterility testing performed.

Genetic analysis

1. *Karyotyping*: Passage 2; 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 (9 on feeders, 3 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 29; 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 45; Genea020 grown on feeders were stained as per manufacturer's protocol using the Merck Millipore alkaline phosphatase detection kit (SCR004)
2. *Immunofluorescence*: Passage 22 (19 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).
3. *Teratoma formation*: Passage 42 (18 on feeders, 24 enzymatic); 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 excised, fixed, sectioned and stained for the assessment of tissues from each of the embryonic germ layers.
4. *Pluritest*: Passage 12 (9 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)

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

Table 2
STR profile; Genea020 (passage 29) demonstrating female allele pattern.

	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
SIVF-20	12,13	30,33.2	8	10	15	7,9	10,12	11	24	13,16.2	14,17	8,11	14,15	11,12	19,20