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Lab Resource: Stem Cell Line

Derivation of Huntington Disease affected Genea091 human embryonic stem cell line

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ABSTRACT

The Genea091 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, karyotype was confirmed as 46, XX by CGH and STR analysis demonstrated a female Allele pattern. The hESC line had pluripotent cell morphology, 92% of cells expressed Nanog, 97% Oct4, 79% Tra1-60 and 98% SSEA4 and gave a Pluritest pluripotency score of 38.36, Novelty of 1.35. The cell line was negative for Mycoplasma and visible contamination.

Resource Table

Name of Stem Cell line	Genea091 — Huntington Disease affected (Alternate ID: SIVF091)
Institution	Genea Biocells
Person who created resource	Biljana Dumevska
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	June 2012
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 (Fig. 1 and Tables 1 and 2 below)
Link to related literature (direct URL links and full references)	(McQuade & Balachandran, 2014) http://www.ncbi.nlm.nih.gov/pubmed/25316320
Information in public databases	National Institutes of Health registered, NIH: NIHhESC-14-0246 UK Stem Cell Bank registered, UKSCB: SCSC14-49
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	May 2012
Karyotype	46, XX
Sex	Female
Pluripotent	YES — by Nanog, Oct4, Tra1-60, and SSEA4 staining and Pluritest
Disease status	Expansion of CAG repeats (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	YES — Genea089 (XX — HD affected, NIH: NIHhESC-14-0247), Genea090 (XX — HD affected, NIH: NIHhESC-14-0245)

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 bisected and plated onto irradiated human feeders (Detroit 551 HFF — 90,000/well in 4-well) in 20% KSR medium with 20 ng/mL FGF added fresh (Amit et al., 2000). CGH karyotyping and STR profiling were performed at the first cryobanking step from ICM outgrowths maintained on feeders. Cells were then enzymatically passaged as single cells in M2 pluripotent cell maintenance medium (Genea Biocells) and CGH/karyotyping

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Table 1
CGH analysis summary; Genea091 (passage 3) reporting a female cell line and no abnormalities detected.

	CGH summary
Sample name	Genea091p3
Date reported	16th August 2012
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

repeated, immunofluorescent pluripotent marker staining, Pluritest and sterility testing performed.

Genetic analysis

1. Comparative Genomic Hybridisation (CGH) based chromosomal analysis: Passage 3; 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).
2. DNA profiling: Passage 3; 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 9 (4 on feeders, 5 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).
2. Pluritest: Passage 8 (4 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).

Sterility testing

1. Mycoplasma: Passage 8 (4 on feeders, 4 enzymatic); testing was performed as per manufacturer's instructions using the MycoAlert Mycoplasma Detection Kit from LONZA
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

Table 2
STR profile; Genea091 (passage 3) demonstrating female allele pattern.

	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
GENEA091p3	13,15	29,31	10,11	12,14	17	6	9,11	11,12	17	12,13	18,19	9,11	14	10,12	22,23

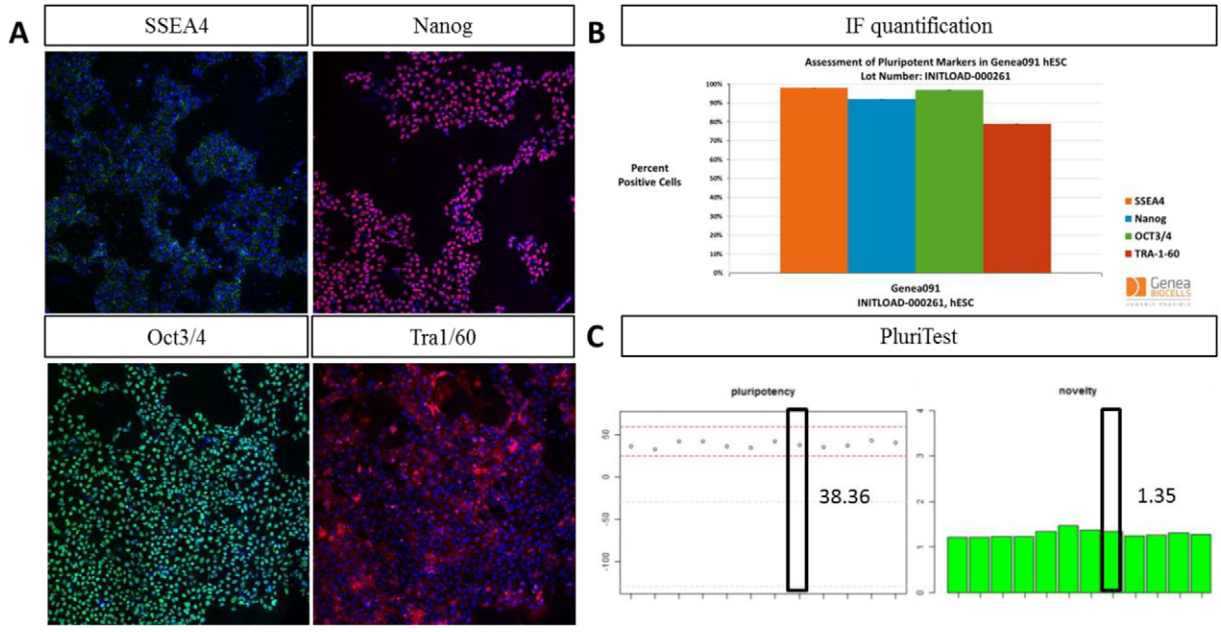

Fig. 1. Pluripotency validation of Genea090. A) Immunofluorescent staining (10×) of pluripotent cell markers SSEA4 (green), Nanog (red), Oct3/4 (green) and Tra1/60 (red), (passage 9, 5 enzymatic). B) Quantitation of expression of pluripotent markers. C) Pluritest pluripotency (left) and novelty (right) scores with Genea091 (passage 8, 4 enzymatic) outlined in black.

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. Genea091 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 309,710. This licence was issued to GENEA on 7 May 2007. More information about the licence can be obtained from the NHMRC webpage at http://www.nhmrc.gov.au/health-ethics/human-embryos-and-cloning/database-licences-authorising-use-excess-art-embryos.

PGD analysis conclusion

Mutation; expansion of CAG (40 CAG repeats) in HTT gene. Family tree; father has 41 repeats; inherited from his father. Huntington Disease (HD) affected.

Morphology

The derived stem cell line, Genea091, morphologically displays adherent monolayer of compact cells in well-defined colonies with high nuclear to cytoplasmic ratio and prominent nucleoli.

Genetic analysis

The cell line has been karyotyped and tested by CGH, which demonstrated 46, XX karyotype (Table 1, Supplementary Fig. 1) consistent with original derivation and pre-implantation genetic diagnosis (PGD). Analysis of STR markers showed Allele pattern consistent with female genotype (Table 2, Supplementary Fig. 2).

Pluripotency

GENEA091 is pluripotent by;

1. Immunofluorescence with 92% Nanog positive, 97% Oct4, 79% Tra1-60 and 98% SSEA4 positive (Fig. 1A, quantified in 1B).
2. Pluritest with a pluripotency score of 38.36 and Novelty score of 1.35 (Fig. 1C).

Sterility

The cell line is tested and found negative for Mycoplasma and any visible contamination (Supplementary Fig. 3).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2016.02.022.

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