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Lab Resource: Stem Cell Line

Derivation of human embryonic stem cell line Genea019

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ABSTRACT

The Genea019 human embryonic stem cell line was derived from a donated, fully commercially consented ART blastocyst, through ICM outgrowth on inactivated feeders. The line showed pluripotent cell morphology and genomic analysis verified a 46, XX karyotype, female Allele pattern and unaffected Htt CAG repeat length, compared to HD affected sibling Genea020. Pluripotency of Genea019 was demonstrated with 75% of cells expressing Nanog, 89% Oct4, 48% Tra1-60 and 85% SSEA4, a Pluritest Pluripotency score of 22.97, Novelty score of 1.42, trilineage teratoma formation and Alkaline Phosphatase activity. The cell line was negative for Mycoplasma and any visible contamination.

Resource table

Name of stem cell line	Genea019 (Alternate ID: SIVF019)
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	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: GSM638426, GEO: GSM638427, GEO: GSM638428
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	October 2004
Karyotype	46, XX — no abnormalities detected
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	Unaffected
Sterility	The cell line is tested and found negative for Mycoplasma and any visible contamination
Sibling lines available	YES — GENEA020 (HD affected, XX — UKSCB: SCSC14-62)

Materials and methods

Cell line derivation

The zona pellucida of a blastocyst-stage human embryo was removed using pronase. The embryo was plated whole onto mitomycin C inactivated human feeders (Detroit 551 HFF, plated 200,000 cells per organ culture dish — 69,204 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 was performed at the first cryobanking step from ICM outgrowths maintained on feeders. Alkaline Phosphatase staining was 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.

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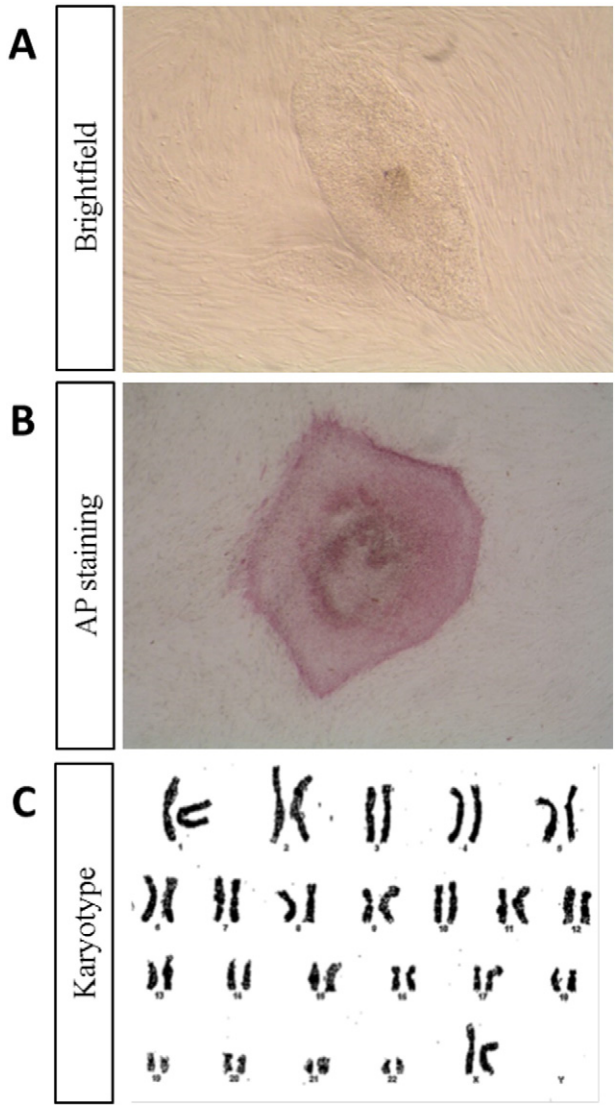

Fig. 1. Morphology and karyotype of Genea019. A) Brightfield (passage 55) growing on human inactivated feeders. B) Alkaline phosphatase activity (passage 46) on human inactivated feeders. C) Karyotypic analysis (passage 3) showing 46, XX normal, female karyotype.

Genetic analysis

1. Karyotyping: Passage 3; 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. Comparative Genomic Hybridisation (CGH) based chromosomal analysis: Passage 16 (12 on feeders, 4 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 analyzed using Genomic Workbench Standard Edition 5.0 software (Agilent Technologies).
3. DNA Profiling: Passage 30; 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 46; Genea019 grown on feeders were stained as per manufacturers protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004).
2. Immunofluorescence: Passage 16 (14 on feeders, 2 enzymatic); cells were fixed with formalin and stained with Nanog #560483 1:200; Oct4 #560217 1:150: Tra1–60 #560,121 1:150; SSEA4 #560308 1:200 (all BD Pharmingen:). Images were acquired with an IN Cell Analyzer 6000 and quantified using In Cell Developer Software (GE).
3. Pluritest: Passage 14 (12 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 72; 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 intramuscular injection of cells into the left inner thigh muscle of the hind leg. Approximately $1–2 \times 10^6$ cells (1xT25) in 50 uL 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 20; 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 ('the possibility that karyotyped embryonic cells derived from non-viable "investigation" embryos could be developed into stem cell lines') was 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*. Karyotyped embryo cells were fully consented for development of stem cells by all responsible people through an informed consent process (signed de-identified consent form can be provided upon request). Donors have received no payment of other benefits for their donation. Donated embryos originally created by

Table 1
CGH analysis summary; Genea019 (passage 16, 4 enzymatic) reporting a female cell line and no abnormalities detected.

	CGH summary
Sample name	Genea019 p16-TE4
Date reported	20th September 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

Table 2
STR profile; Genea019 (passage 30) demonstrating female allele pattern.

	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
SIVF19	12,13	29,30	7,8	10,11	15	9.3,10	11,12	11	18,23	13,14	17	11	12,18	11,12	22,23

assisted reproduction technology (ART) for the purpose of procreation. Embryos were identified as unsuitable for implantation, biopsy or freezing due to abnormal development. Embryonic outgrowths were developed for consented clinical investigation studies. Results of the studies have been reported back to the patient.

PGD analysis conclusion

No mutation; 15 and 18 CAG repeats in HTT gene. No family tree; embryo was de-identified. See (Bradley et al., 2011) for siblingship and sequencing analysis.

Morphology

The derived stem cell line, Genea019, morphologically displays adherent monolayer of compact cells in well-defined colonies with high nuclear to cytoplasmic ratio and prominent nucleoli (Fig. 1A/B).

Genetic analysis

The cell line has been karyotyped (Fig. 1C, Supplementary Fig. 1) and also tested by CGH (Table 1, Supplementary Fig. 2), demonstrated 46, XX karyotype, consistent with original derivation. Analysis of STR markers showed Allele pattern consistent with female genotype (Table 2, Supplementary Fig. 3).

Pluripotency

GENEA019 is pluripotent by;

1. Alkaline Phosphatase stain positivity (Fig. 1B).
2. Immunofluorescence with 75% Nanog positive, 89% Oct4 positive, 48% Tra1-60 positive, and 85% SSEA4 positive (Fig. 2A, quantified in 2B).
3. Pluritest with a 22.97 Pluripotency score and 1.42 Novelty score (Fig. 2C).
4. Teratoma formation which contained tissues derived from each of the embryonic germ layers: endoderm, mesoderm, and ectoderm (Fig. 2D).

Sterility

The cell line is tested and found negative for Mycoplasma and any visible contamination (Supplementary Fig. 4).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2016.02.008.

References

Amit, M., Carpenter, M.K., Inokuma, M.S., Chiu, C.P., Harris, C.P., Waknitz, M.A., ... Thomson, J.A., 2000. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Dev. Biol. 227 (2), 271–278. http://dx.doi.org/10.1006/dbio.2000.9912.

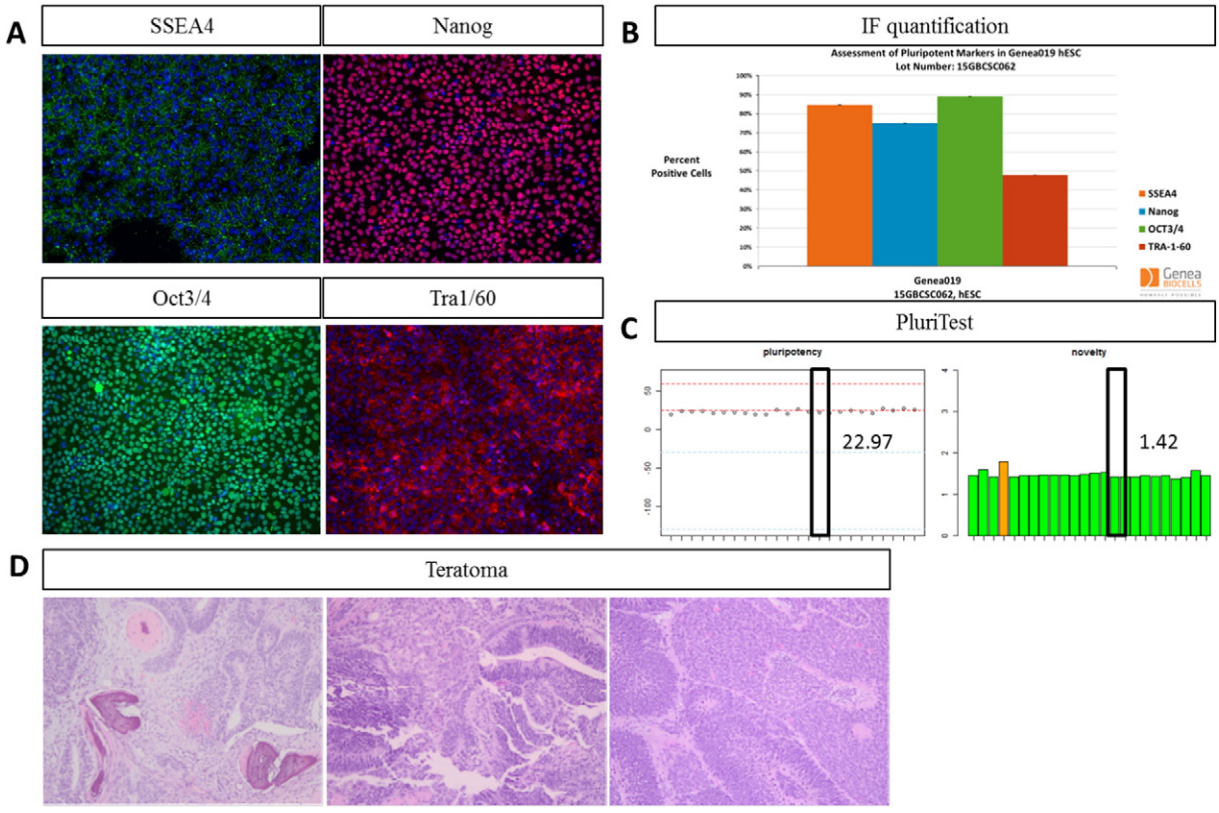

Fig. 2. Pluripotency validation of Genea019. A) Immunofluorescent staining (10×) of pluripotent cell markers SSEA4 (green), Nanog (red), Oct3/4 (green) and Tra1/60 (red), (passage 16, 2 enzymatic). B) Quantitation of expression of pluripotent markers. C) PluriTest pluripotency (left) and novelty (right) scores with Genea019 (passage 14, 2 enzymatic) outlined in black. D) Tri-lineage teratoma formation (passage 72) with (left) bone, (middle) primitive mesenchyme, gut, (right) neuroglia, neuroepithelium and primitive neural anlage.

Bradley, C.K., Chami, O., Peura, T.T., Bosman, A., Dumevska, B., Schmidt, U., Stojanov, T., 2010. Derivation of three new human embryonic stem cell lines. In Vitro Cell. Dev. Biol. Anim. 46 (3–4), 294–299. http://dx.doi.org/10.1007/s11626-010-9298-y.

Bradley, C.K., Scott, H.A., Chami, O., Peura, T.T., Dumevska, B., Schmidt, U., ... Al, B.E.T., 2011. Derivation of Huntington disease-affected human embryonic stem cell lines. Stem Cells Dev. 20 (3).

Laurent, L.C., Nievergelt, C.M., Lynch, C., Fakunle, E., Harness, J.V., Schmidt, U., ... Loring, J.F., 2010. Restricted ethnic diversity in human embryonic stem cell lines. Nat. Methods 7 (1), 5–6. http://dx.doi.org/10.1038/nmeth0110-6.

Laurent, L.C., Ulitsky, I., Slavin, I., Tran, H., Schork, A., Morey, R., ... Loring, J.F., 2011. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Cell Stem Cell 8 (1), 106–118. http://dx.doi.org/10.1016/j.stem.2010.12.003.

McQuade, L., Balachandran, A., 2014. Proteomics of Huntington disease-affected human embryonic stem cells reveals an evolving pathology involving mitochondrial dysfunction and metabolic. J. Proteome (Retrieved from http://pubs.acs.org/doi/abs/10.1021/pr500649m).

Müller, F.-J., Schuldt, B.M., Williams, R., Mason, D., Altun, G., Papapetrou, E., ... Loring, J.F., 2012. A bioinformatic assay for pluripotency in human cells. Nat. Methods 8 (4), 315–317. http://dx.doi.org/10.1038/nmeth.1580.A.

Tay, Y., Peter, S., Rigoutsos, I., Barahona, P., Ahmed, S., Dröge, P., 2009. Insights into the regulation of a common variant of HMGA2 associated with human height during embryonic development. Stem Cell Rev. Rep. 5 (4), 328–333. http://dx.doi.org/10.1007/s12015-009-9095-8.