Stem Cell Research 16 (2016) 397–400

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

# Derivation of human embryonic stem cell line Genea019

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ARTICLE INFO

*Article history:*
Received 26 January 2016
Accepted 1 February 2016
Available online 3 February 2016

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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http://dx.doi.org/10.1016/j.scr.2016.02.008

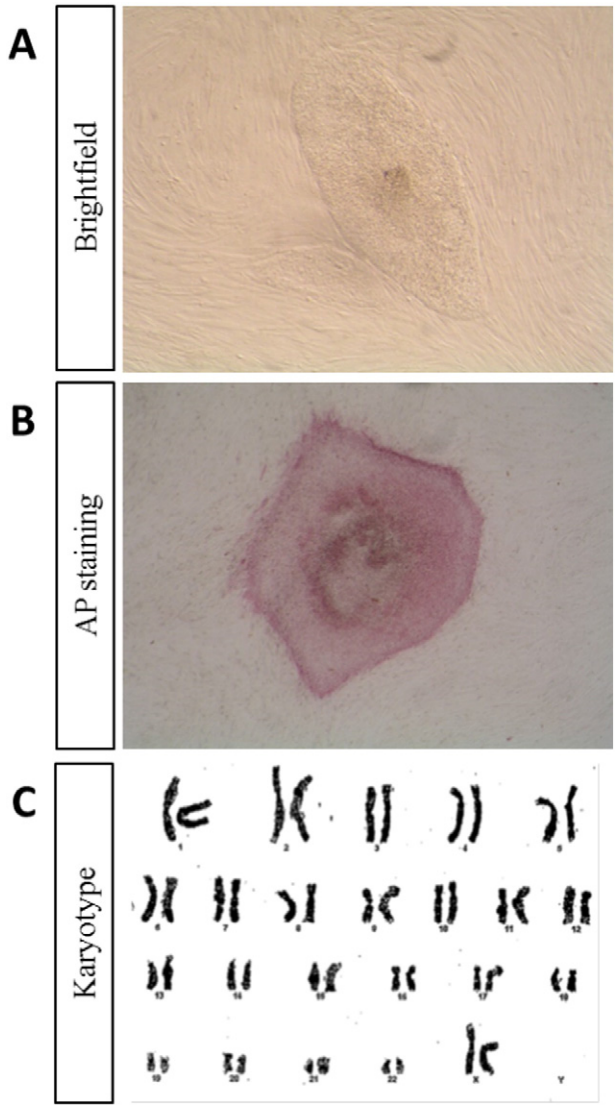


**Fig. 1.** Morphology and karyotype of Genea019. A) Brightfield (passage 55) growing on human inactivated feeders. B) Alkaline phosphatase activity (passage 46) on human inactivated feeders. C) Karyotypic analysis (passage 3) showing 46, XX normal, female karyotype.

*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 ($8 \times 60$ 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 |