

Lab Resource: Stem Cell Line

Derivation of human embryonic stem cell line Genea022

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ABSTRACT

The Genea022 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, XY karyotype and male allele pattern through CGH and STR analysis. Pluripotency of Genea022 was demonstrated with 84% of cells expressed Nanog, 98% Oct4, 55% Tra1-60 and 97% SSEA4, gave a Pluritest Pluripotency score of 42.95, Novelty of 1.23, 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	Genea022 (Alternate ID: SIVF022)
Institution	Genea Biocells
Person who created resource	Alexis Bosman
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	December, 2005
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 1–3 below) (Laurent et al., 2010)
Link to related literature (direct URL links and full references)	http://www.ncbi.nlm.nih.gov/pubmed/?term=20038950 (Laurent et al., 2011) http://www.ncbi.nlm.nih.gov/pubmed/?term=21211785 (McQuade & Balachandran, 2014) http://www.ncbi.nlm.nih.gov/pubmed/25316320 (Bosman et al., 2015) http://www.ncbi.nlm.nih.gov/pubmed/25645121
Information in public databases	UK Stem Cell Bank registered, UKSCB: SCS14-60 SNP Data Gene Expression Omnibus accession numbers, GEO: GSM638433 , GEO: GSM638434

Resource details

Date of derivation	December 2004
Karyotype	46, XY – no abnormalities
Sex	Male
Pluripotent	YES – by Nanog, Oct4, Tra1-60, and SSEA4 staining, Pluritest and Alkaline Phosphatase
Disease status	Unaffected
Sterility	The cell line is tested and found negative for Mycoplasma and any visible contamination
Sibling lines available	YES – Genea021 (XY, TS21 affected – UKSCB: SCSC14-60), Genea023 (XY, UKSCB: SCSC14-60)

Materials and methods

Cell line derivation

The zona pellucida of a fresh, blastocyst stage embryo was removed with a small blade. The embryo was bisected and plated onto Mitomycin C inactivated human feeders (Detroit 551 HFF - 130,000/well in 4-well) in 20% KSR medium with (Amit et al., 2000) with 50 ng/mL FGF added fresh. CGH karyotyping and STR profiling were performed at the first cryobanking step from ICM outgrowths maintained on feeders. Alkaline Phosphatase staining and teratoma formation was performed from 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 and sterility testing performed.

¹ Equal contributions.

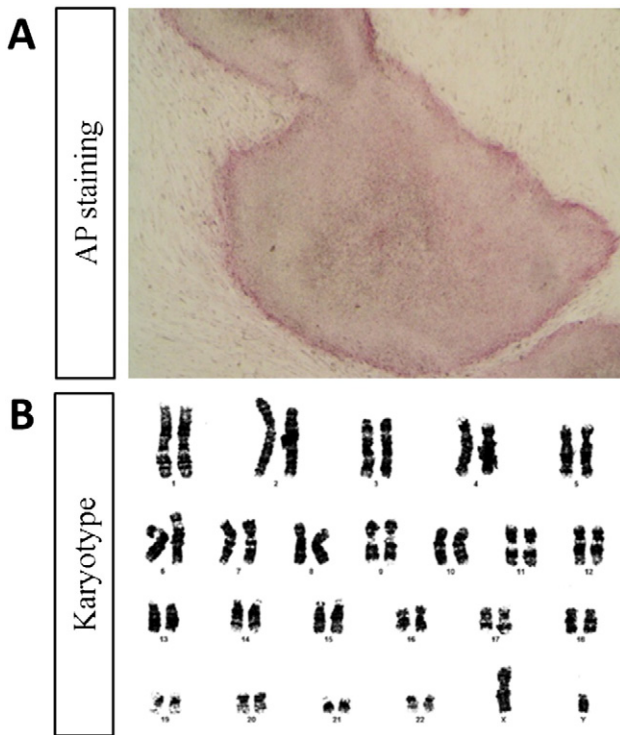


Fig. 1. Morphology and karyotype of Genea022. A) Alkaline Phosphatase activity (passage 14) on human inactivated feeders. B) Karyotypic analysis (passage 38) showing 46, XY normal, male karyotype.

Genetic analysis

1. Karyotyping: Passage 38; 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 count was performed for 5 and 10 of the metaphases respectively.
2. Comparative Genomic Hybridisation (CGH) based chromosomal analysis: Passage 10; 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

Table 1

CGH analysis summary; Genea022 (passage 10, 4 enzymatic) reporting a male cell line and no abnormalities detected.

	CGH summary
Sample name	Genea022p10_TE4
Date reported	8th October 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	Male cell line — no abnormalities detected

Table 2

STR profile; Genea022 (passage 8) demonstrating male allele pattern.

	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
SIVF022p8	12, 13	28, 29	10, 13	11, 13	15	6	12	10, 13	17, 18	13, 14	15, 19	8, 11	14, 16	11	20, 22

using Genomic Workbench Standard Edition 5.0 software (Agilent Technologies).

3. DNA Profiling: Passage 8; DNA 'fingerprinting' was performed using the AmpFLSTR Identifier PCR Amplification Kit (Applied Biosystems #4322288) to provide permanent genetic identification of the cell lines. <https://www.thermofisher.com/order/catalog/product/4322288>
4. Siblingship testing: Due to the de-identifying of embryos the siblingship of Genea021, Genea022 and Genea023 was tested at DNA Labs (a subsidiary of Sydney IVF; Sydney, Australia) via analysis 15 highly variable loci, 15 variable Y chromosome loci and mitochondrial DNA sequences in the D-loop HVRI and HVRII regions.

Pluripotency assessment

1. Immunofluorescence: Passage 8 (6 on feeders, 2 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 9 (6 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)
3. Alkaline Phosphatase: Passage 14; Genea022 grown on feeders were stained as per manufacturer's protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004)
4. Teratoma formation: Passage 64; 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 36; 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

Karyotyped embryo cells were fully consented for the 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 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

Table 3

Summary of siblingship testing: de-identified Genea021, Genea022 and Genea023 share identical maternal and paternal lines and with variable loci analysis are likely related as full siblings.

	Result
Variable locus analysis	SIVF021 and SIVF022 – full sibs inconclusive SIVF021 and SIVF023 – full sibs practically proven SIVF022 and SIVF023 – full sibs inconclusive
Y chromosome comparison	No mismatches between the lines thus common paternal lineage
Mitochondrial DNA analysis	Identical mitochondrial sequences thus common maternal line
Interpretation	It is likely that all cell lines are related

investigation studies. Results of the studies have been reported back to the patient.

Morphology

The derived stem cell line, Genea022, morphologically displays adherent monolayer of compact cells in well-defined colonies with high nuclear to cytoplasmic ratio and prominent nucleoli (Fig. 1A).

Genetic analysis

The cell line has been karyotyped (Fig. 1B, Supplementary Fig. 1) and tested by CGH (Table 1, Supplementary Fig. 2), which demonstrated 46, XY karyotype, consistent with original derivation. Analysis of STR markers showed allele pattern consistent with male genotype (Table 2, Supplementary Fig. 3). Siblingship analysis showed

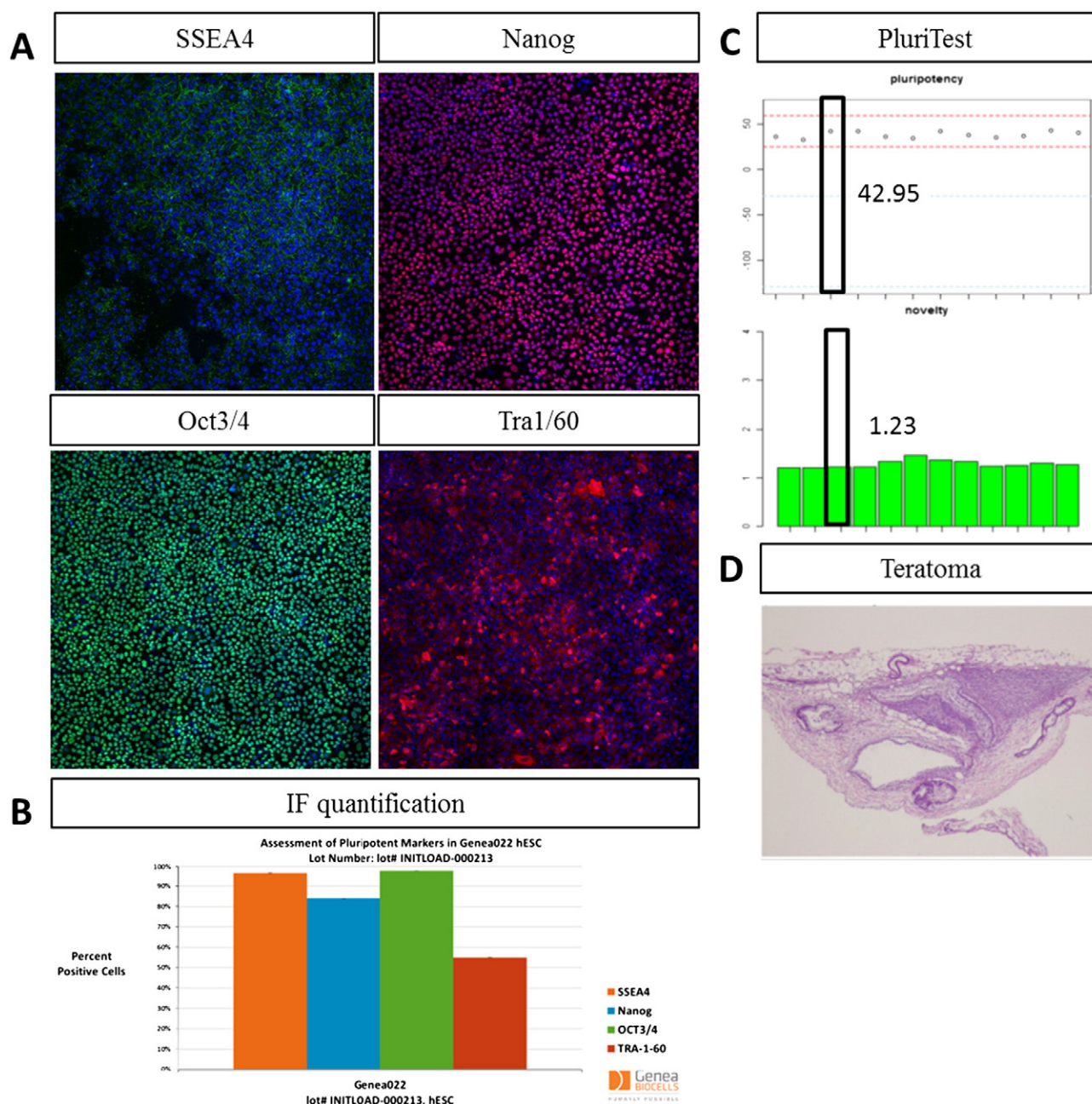


Fig. 2. Pluripotency validation of Genea022. A) Immunofluorescent staining ($10\times$) of pluripotent cell markers SSEA4 (green), Nanog (red), Oct3/4 (green) and Tra1/60 (red), (passage 8, 2 enzymatic). B) Quantitation of expression of pluripotent markers. C) Pluritest pluripotency (left) and novelty (right) scores with Genea022 (passage 9, 3 enzymatic) outlined in black. D) Tri-lineage teratoma formation (passage 24) with gut, squamous and immature mesenchyme.

Genea021 to be siblings with Genea022 and Genea023 (Table 3, Supplementary Fig. 4).

Disease Status

Unaffected.

Pluripotency

GENEA022 is pluripotent by;

1. Alkaline Phosphatase stain positivity (Fig. 1A)
2. Immunofluorescence with 84% Nanog positive, 98% Oct4, 55% Tra1-60 and 97% SSEA4 positive (Fig. 2A, quantified in 2B).
3. Pluritest with a Pluripotency score of 42.95 and Novelty score of 1.23 (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. 5).

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scr.2016.02.011>.

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