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Lab resource: Stem cell line

Derivation of Genea002 human embryonic stem cell line

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ABSTRACT

The Genea002 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 by CGH and male Allele pattern through STR analysis. Pluripotency of Genea002 was demonstrated with 75% of cells expressing Nanog, 93% Oct4, 83% Tra1-60 and 98% SSEA4, a Pluritest pluripotency score of 24.55, Novelty score of 1.39, teratomas with tissues from all embryonic germ layers and Alkaline Phosphatase activity. The cell line was negative for Mycoplasma and any visible contamination.

1. Resource table

Name of stem cell line	Genea002 (Alternate ID: SIVF002)
Institution	Genea Biocells
Person who created resource	Teija Peura
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	January, 2006
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. 1–8 below)
Link to related literature (direct URL links and full references)	1. Insights into the regulation of a common variant of HMGA2 associated with human height during embryonic development. Tay Y, Peter S, Rigoutsos I, Barahona P, Ahmed S, Dröge P. Stem Cell Rev. 2009 Dec.;5(4):328–33. http://www.ncbi.nlm.nih.gov/pubmed/?=20058197 2. Restricted ethnic diversity in human embryonic stem cell lines. Laurent LC, Nievergelt CM, Lynch C, Fakunle E, Harness JV, Schmidt U, Galat V, Laslett AL, Otonkoski T, Keirstead HS, Schork A, Park HS, Loring JF. Nat Methods. 2010 Jan.;7(1):6–7. http://www.ncbi.nlm.nih.gov/pubmed/term20038950 3. Derivation of three new human embryonic stem cell lines. Bradley CK, Chami O, Peura TT, Bosman A, Dumevska B, Schmidt U, Stojanov T. In Vitro Cell Dev Biol Anim. 2010 Apr.;46(3–4):294–9. http://www.ncbi.nlm.nih.gov/pubmed/?term=20198447 4. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Laurent LC, Ulitsky I, Slavin I, Tran H, Schork A, Morey R, Lynch C, Harness JV, Lee S, Barrero MJ, Ku S, Martynova M, Semechkin R, Galat V, Gottesfeld J, Izpisua Belmonte JC, Murry C, Keirstead HS, Park HS, Schmidt U, Laslett AL, Muller FJ, Nievergelt CM, Shamir R, Loring JF. Cell Stem Cell. 2011 Jan.;7;8(1):106–18. http://www.ncbi.nlm.nih.gov/pubmed/?term=21211785 5. A teratocarcinoma-like human embryonic stem cell (hESC) line and four hESC lines reveal potentially oncogenic genomic changes. Hovatta O, Jaconi M, Töhönen V, Béna F, Gimelli S, Bosman A, Holm F, Wyder S, Zdobnov EM, Irion O, Andrews PW, Antonarakis SE, Zucchelli M, Kere J, Feki A. PLoS One. 2010 Apr.;23;5(4):e10263 http://www.ncbi.nlm.nih.gov/pubmed/20428235 6. Proteomics of Huntington's disease-affected human embryonic stem cells reveals an evolving pathology involving mitochondrial dysfunction and metabolic disturbances. McQuade, L. R., Balachandran, A., Scott, H. A., Khaira, S., Baker, M. S., & Schmidt, U. (2014). Journal of Proteome Research, 13, 5648–5659. http://www.ncbi.nlm.nih.gov/pubmed/25316320
Information in public databases	Genea002 is a National Institutes of Health (NIH) registered stem cell line. NIH approval number is NIHhESC-12-0151 Genea002 is a UK Stem Cell Bank (UKSCB) registered stem cell line. UKSCB approval number is SCSC14-38 SNP data links http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM638396 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM638397

* Corresponding author.

http://dx.doi.org/10.1016/j.scr.2015.10.002

2. Resource Details

Date of derivation	21st December 2005
Karyotype	46, XY — no abnormalities detected (Fig. 2)
Sex	Male (Fig. 3)
Pluripotent	YES — by Nanog, Oct4, Tra1-60, and SSEA4 staining (Fig. 4) as well as tri-lineage teratoma formation (Fig. 5), Pluritest (Fig. 6) and Alkaline Phosphatase stain positivity (Fig. 7)
Disease status	Unaffected
Sterility	The cell line is tested and found to be negative for Mycoplasma and any visible contamination (Fig. 8)
Sibling lines available	YES — ESI017; ESI014; Genea048 (XY trisomy 5 NIHhESC-12-0152, SCSC14-38)

3. Materials and methods

3.1. Cell line derivation

The zona pellucida of a blastocyst-stage human embryo was manually removed using a small blade. The embryo was bisected and both ICM and trophectoderm were plated onto mitomycin C inactivated GMP grade (Ortec) human feeders (plated 200,000 cells per organ culture dish — 69,204 cells/cm^2) in 20% knockout serum in standard hESC culture medium with 50 ng/mL Fgf2 (Amit et al., 2000). CGH and karyotyping were performed at the first cryobanking step from ICM outgrowths maintained on feeders. This process was performed under GMP conditions, identical to those used in the creation of Embryonic Stem Cell International's GMP hESC lines (Crook et al., 2007), however not under a GMP control and documentation process. 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.

3.2. Genetic analysis

CGH and DNA profiling were performed after acquisition of sufficient cell numbers from derivation and then again following the thawing of banked vials of frozen stem cells (passage 17: 14 on feeders, 3 enzymatic), ensuring consistency in the genetic profile. CGH was used to screen targeted regions of the genome for gains and losses associated with chromosomal imbalances such as aneuploidy, deletions and duplications. Microarray analysis was performed using 8X 60k ISCA format (Sure print G3, Agilent) with average 70 kb probe spacing. DNA profiling using DNA Devyser Compact v3 QF-PCR method was used to study the Allele pattern for the analysis of STR markers.

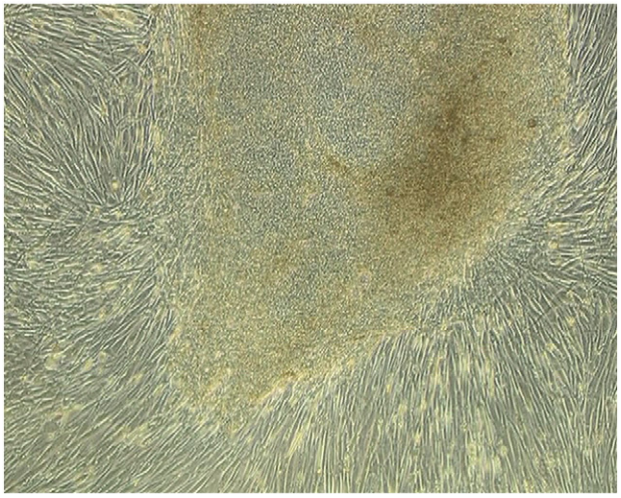

Fig. 1. Brightfield morphology of Genea002 on human inactivated feeders.

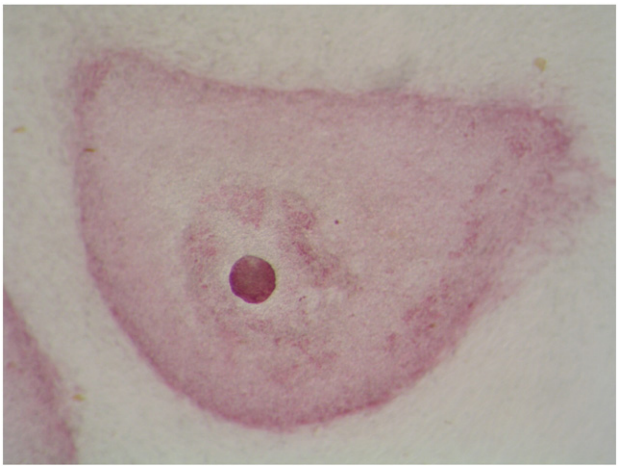

Fig. 2. CGH array analysis certification.

4. Pluripotency assessment

1. 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 #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. Teratoma formation: Passage 38; Genea002 were injected intramuscularly to immuno-deficient mice and resultant teratomas excised, fixed, sectioned and stained for assessment of tissues from each of the embryonic germ layers.
3. Pluritest: Passage 17 (14 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).
4. Alkaline Phosphatase: Passage 10; Genea002 were stained as per manufacturers protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004).

5. Sterility testing

1. Mycoplasma: Passage 20; testing was performed as per manufacturer's instructions using the MycoAlert Mycoplasma Detection Kit from LONZA.

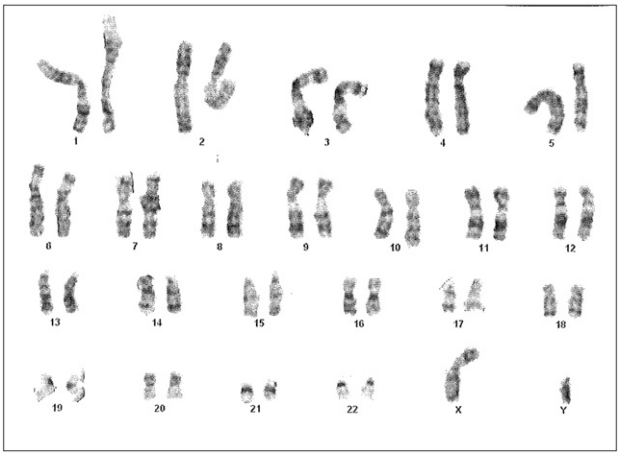

Fig. 3. STR analysis certification.