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Lab Resource: Stem Cell Line

Derivation of human embryonic stem cell line Genea023

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ABSTRACT

The Genea023 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 Genea023 was demonstrated with 85% of cells expressed Nanog, 98% Oct4, 55% Tra1-60 and 98% SSEA4, gave a Pluritest Pluripotency score of 42.76, Novelty of 1.23, demonstrated Alkaline Phosphatase activity and tri-lineage teratoma formation. The cell line was negative for *Mycoplasma* and visible contamination.

Resource table

Name of stem cell line	Genea023 (alternate ID: SIVF023)
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)
Link to related literature (direct URL links and full references)	(Laurent et al., 2010) http://www.ncbi.nlm.nih.gov/pubmed/?term=20038950 (Laurent et al., 2011) http://www.ncbi.nlm.nih.gov/pubmed/?term=21211785 (McQuade et al., 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 number, GEO: GSM638435

Resource details

Date of derivation	December 2004
Karyotype	46, XY — hemizygous loss of approximately 735 kb on chromosome 1
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), Genea022 (XY, UKSCB: SCSC14-60)

Materials and methods

Cell line derivation

A fresh, blastocyst stage embryo was bisected and plated onto Mitomycin C inactivated human feeders (Detroit 551 HFF — 130,000/well in 4-well) in 20% KSR medium (Amit et al., 2000) with 50 ng/mL FGF added fresh. CGH karyotyping and STR profiling was 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.

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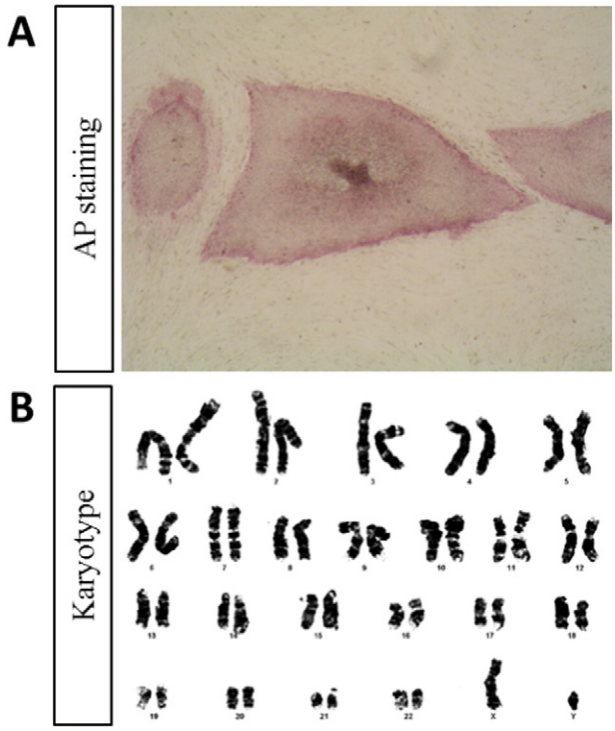

Fig. 1. Morphology and karyotype of Genea023. A) Alkaline Phosphatase activity (passage 14) on human inactivated feeders. B) Karyotypic analysis (passage 38) showing 46, XY normal, male karyotype.

Table 1
CGH analysis summary; Genea023 (passage 15, 4 enzymatic) reporting a male cell line with hemizygous loss of approximately 735 kb on chromosome 1.

	CGH summary
Sample name	Genea023p15_TE4
Date reported	8th October 2012
Hybridisation balance	Balanced hybridization was observed for chromosomes 2–22 and sex chromosomes, relative to reference DNA Unbalanced hybridization was observed for chromosome 1
Copy number change	Loss, 1q21.2 approximately 735 kb
Interpretation	Male cell line — a hemizygous loss of approximately 735 kb on chromosome 1

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 15; 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).
3. *DNA Profiling*: Passage 8; 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
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 of 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 14 (11 on feeders, 3 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 14 (11 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; Genea023 grown on feeders were stained as per manufacturers protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004).
4. *Teratoma formation*: Passage 41; 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 × 10^6 cells (1 × T25) in 50 μL 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 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

Table 2
STR profile; Genea023 (passage 8) demonstrating male allele pattern.

	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
SIVF023p8	14	30,32.2	11,13	11,13	15,16	9.3	11	11,13	17,24	13,14	14,19	8,9	13,16	11,12	22,24