Stem Cell Research 16 (2016) 401–404

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Stem Cell Line

Derivation of Trisomy 21 affected human embryonic stem cell line Genea021

Biljana Dumevska *[,1], Alexis Bosman [1], Robert McKernan, Heather Main, Uli Schmidt, Teija Peura

Genea Biocells, Sydney, Australia

ARTICLE INFO

Article history:
Received 26 January 2016
Accepted 1 February 2016
Available online 11 February 2016

ABSTRACT

The Genea021 human embryonic stem cell line was derived from a donated, fully commercially consented ART blastocyst, carrying Trisomy 21, indicative of Down Syndrome. Following ICM outgrowth on inactivated human feeders, CGH and STR analyses demonstrated a 47, XY, +21 karyotype and male allele pattern. The hESC line had pluripotent cell morphology, 71% of cells expressed Nanog, 84% Oct4, 23% Tra1–60 and 95% SSEA4, gave a Pluritest Pluripotency score of 21.85, Novelty of 1.42, 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	Genea021 — Trisomy 21 affected (Alternate ID: SIVF021)
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	Down Syndrome affected human pluripotent cell line
Key marker expression	Nanog, Oct4, Tra1–60, and SSEA4
Authentication	Identity and purity of cell line confirmed (Figs. 1–2, 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 (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: GSM638431, GEO: GSM638432

Resource details

Date of derivation	December 2004
Karyotype	47, XY, +21
Sex	Male
Pluripotent	YES — by Nanog, Oct4, Tra1–60, and SSEA4 staining, Pluritest, Alkaline Phosphatase and tri-lineage teratoma formation
Disease status	Trisomy 21, Down Syndrome affected, OMIM: 190685
Sterility	The cell line is tested and found negative for Mycoplasma and any visible contamination
Sibling lines available	YES — Genea022 (XY, UKSCB:SCSC14-60), Genea023 (XY, UKSCB: SCSC14-60)

Materials and methods

Cell line derivation

The fresh, blastocyst stage embryo was plated whole 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 were 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.

* Corresponding author.
[1] Equal contributions.

http://dx.doi.org/10.1016/j.scr.2016.02.010

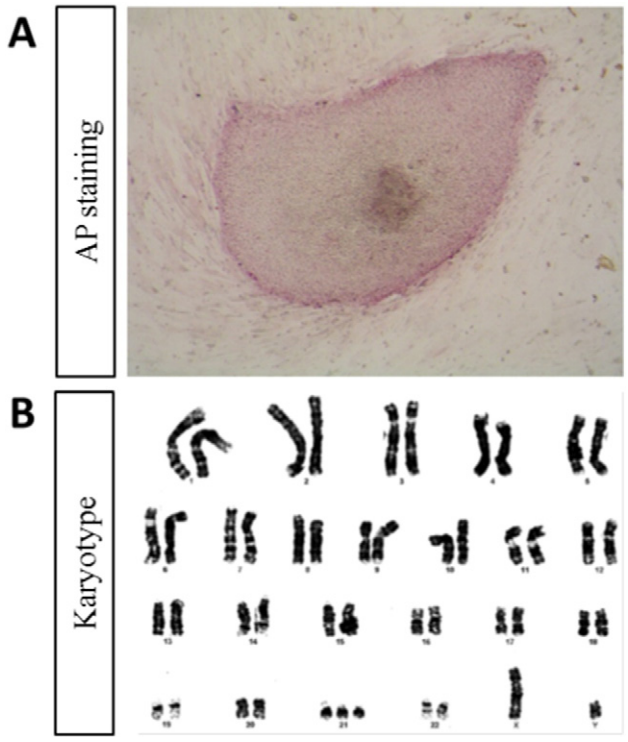

Fig. 1. Morphology and karyotype of Genea021. A) Alkaline phosphatase activity (passage 14) on human inactivated feeders. B) Karyotypic analysis (passage 44, 2 enzymatic) showing 47, XY, +21, Trisomy 21 affected male karyotype.

Genetic analysis

1. Karyotyping: passage 44 (42 on feeders, 2 enzymatic); 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 were performed for 5 and 10 of the metaphases respectively.
2. Comparative Genomic Hybridisation (CGH) based chromosomal analysis: passage 12; 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 the 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.

Table 1
CGH analysis summary; Genea021 (passage 12, 4 enzymatic) reporting a Trisomy 21 affected male cell line.

	CGH summary
Sample name	Genea021p12_TE4
Date reported	8th October 2012
Hybridisation balance	Balanced hybridisation was observed for chromosomes 1–20, 22 and sex chromosomes, relative to reference DNA. Unbalanced hybridisation was observed for chromosome 21, relative to reference DNA.
Copy number change	Gain, chromosome 21, approximately 32,605 Kb
Interpretation	ABNORMAL — male, trisomy 21

Table 3
Summary of siblingship testing; de-identified Genea021, Genea022 and Genea023 share identical maternal and paternal lines and with variable locus 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

Pluripotency assessment

1. Immunofluorescence: passage 10 (8 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 an In Cell Developer Software (GE).
2. Pluritest: passage 12 (8 on feeders, 4 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; Genea021 grown on feeders was stained as per manufacturers protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004).
4. Teratoma formation: Passage 33; 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 (1xT25) 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

Table 2
STR profile; Genea021 (passage 8) demonstrating male allele pattern and trisomy at D21S11.

	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
SIVF021p8	13, 14	29, 30, 32.2	11, 13	11, 13	15	6, 9.3	11, 12	11, 13	17, 24	14.2, 15	14, 18	8, 11	13, 19	11, 12	22, 24