



CORIELL INSTITUTE

FOR MEDICAL RESEARCH

GM 24474*D

Certificate of Analysis

Product description	Human Fibroblast reprogrammed with 4 factors (hOCT3/4 with shp53, hSOX2, hKLF4, hL-MYC) using episomal vector.	
Publication(s) describing iPSC establishment	None, Coriell, in house generated line.	
Parent cell line and cell type	GM03814	Fibroblast
Diagnosis	Spinal Muscular Atrophy I; SMA1	
Parent cell line freeze passage	5	
Passages of iPSC at freezing	20	
Media	Essential 8	
Feeder	Matrigel	
Passage method	0.02% EDTA in PBS or Versene®	
Split ratio	1:6 every 3-5 days	

The following testing specifications have been met for the specified product lot:

Test Description	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	Colony Doubling	Colony formation and diameter doubling within 5 days	Pass
Sterility	Growth on agar	Negative	Pass
Mycoplasma	PCR	Negative	Pass
Karyotype	G-banding	Normal Karyotype	Pass
Identity Match	STR (THO-1, D22S417, D10S526, vWA31, D5S592, and FES/FPS)	Match parent fibroblast line	Pass
Surface Antigen Expression of Stem Cell Markers	Immunostaining	> 80% expression of SSEA-4 < 10% expression of SSEA-1	Pass
Pluripotency	Illumina Array and PluriTest Software (www.pluritest.org)	Pluripotency Score greater than 20 and a Novelty Score less than 1.67	Pluripotency: 26.84 Novelty: 1.391
Episomal integration	Genomic PCR	Plasmid specific primers	No integration detected

Post-Thaw Viability

One vial of distribution lot was thawed. Cultures were observed daily. Colonies were photographed when they first appeared, then 2 days later (Colonies must double in diameter within 5 days).

Day 1	364 μm
Day 3	1082 μm

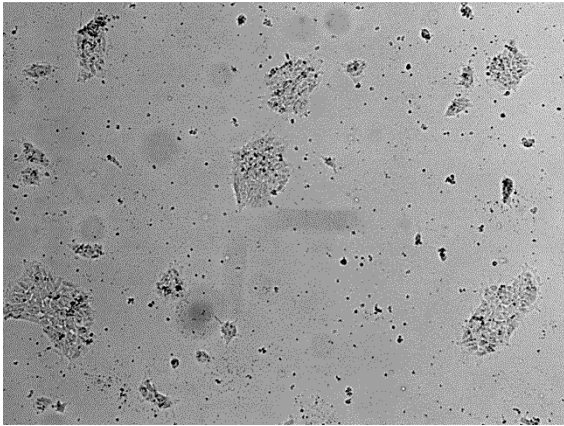


Figure 1A. Colony observed post thaw

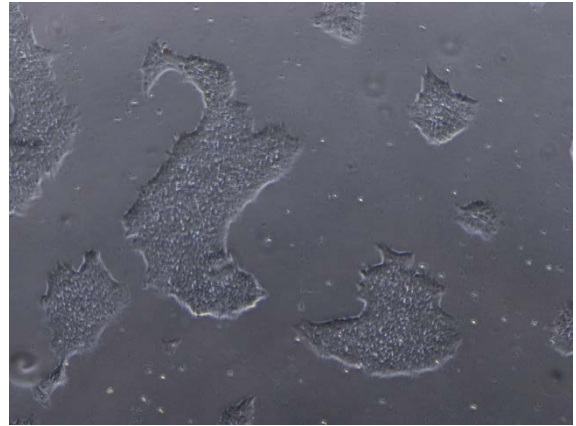


Figure 1B. Colony, 2 days after first observation

Karyotype Analysis



Figure 2: G-banded karyotype showing 46,XX

Surface Antigen Expression of Stem Cell Markers

Undifferentiated cells are stained for the surface antigens, SSEA4. SSEA4 (stage specific embryonic antigen 4) is expressed on undifferentiated human stem cells.

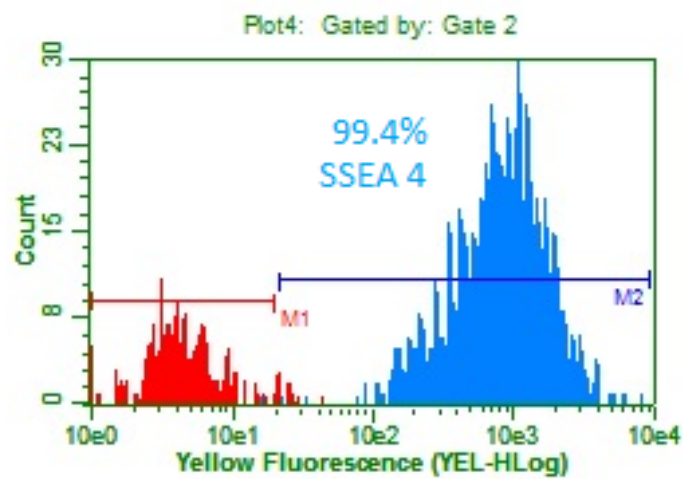


Figure 3: Representative histogram of SSEA-4 positive population. Histogram is an overlay of isotype control (red) and SSEA-4 positive population (blue).

Assessment of Pluripotency of a Cell Line

Cells are directed to differentiate to assess the pluripotency of the cell line. RNA is harvested and gene expression is analyzed by real-time PCR. Ct values are adjusted for loading using a housekeeping gene. Gene expression is shown as fold difference to undifferentiated cells.

Embryoid Body (EB) Formation Assay

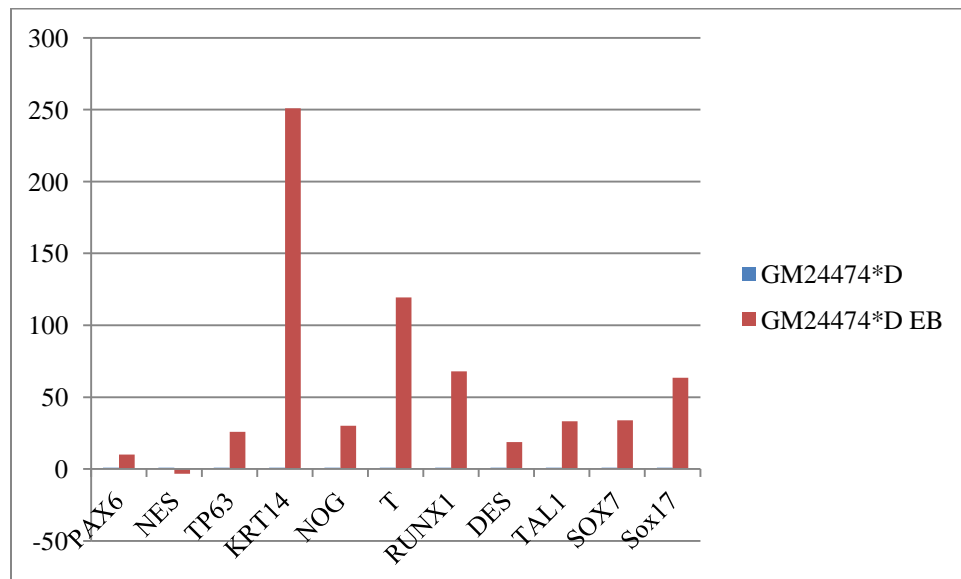
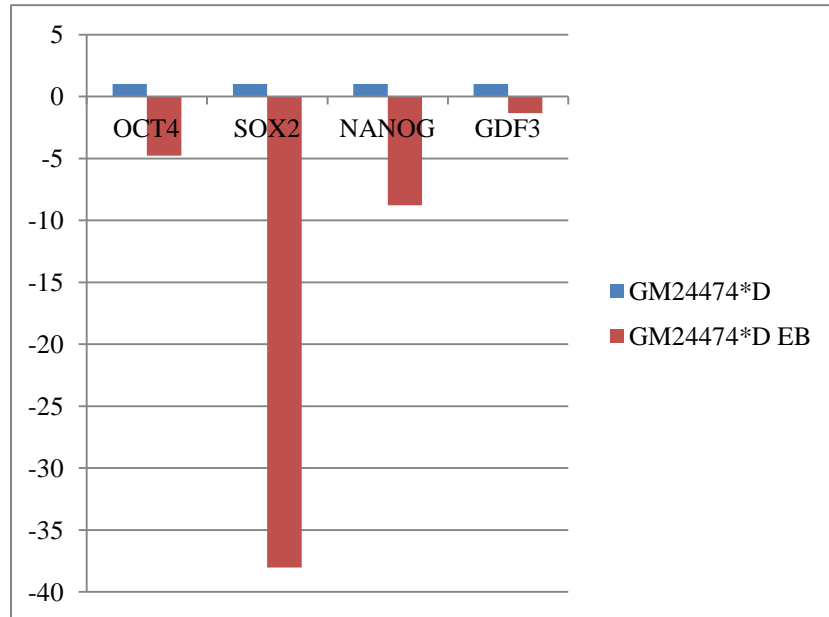


Figure 4. Gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Pluripotency Markers

	OCT 4	SOX 2	NANOG	GDF 3
GM24474*D EB	-5	-38	-9	-1

Ectoderm

	PAX 6	NES	TP 63	KRT 14	NOG
GM24474*D EB	10	-3	26	251	30

Mesoderm

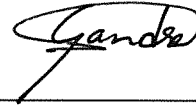
	T	RUNX 1	DES	TAL 1
GM24474*D EB	119	68	19	33

Endoderm

	SOX 7	SOX 17	AFP
GM24474*D EB	34	63	22471628

Table 1. Fold difference values of gene expression of EB. Fold difference is shown relative to undifferentiated cells.

- Pass
- Fail
- Other:



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