



CORIELL INSTITUTE

FOR MEDICAL RESEARCH

GM 23240*B

Certificate of Analysis

Product description	Human fibroblast cell line reprogrammed with four factors (Oct4, Sox2, Nanog, Lin28) using lentivirus vector	
Publication(s) describing iPSC establishment	Ebert et al. PMID 19098894	
Parent cell line and cell type	GM03813	Fibroblast
Diagnosis	Spinal Muscular Atrophy I	
Parent cell line freeze passage		
Passage of iPSC reported at submission	20	
Number of passages at Coriell	13	
Media	DMEM/F12 + 20% KOSR + 100ng/ml bFGF	
Feeder	CF1 MEFs on 0.1% Gelatin	
Passage method	Collagenase	
Split ratio	1:6; every 5-7 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	In vitro differentiation (cardiac, pancreatic and neuronal)	Upregulation of genes appropriate to cell lineage	Pass
Teratoma Formation	<i>In Vivo</i> Teratoma formation	3 germ layer teratoma	Pass

Post-Thaw Viability

One vial of distribution lot was thawed. Cultures were observed daily. Colonies were photographed on the first day of appearance and then 5 days later. Colonies must double in diameter 5 days after first observation.

Days from Recovery to First Colony Observation	Average Colony Diameter (initial)	Average Colony Diameter (7 days post observation)
2 days	278	815

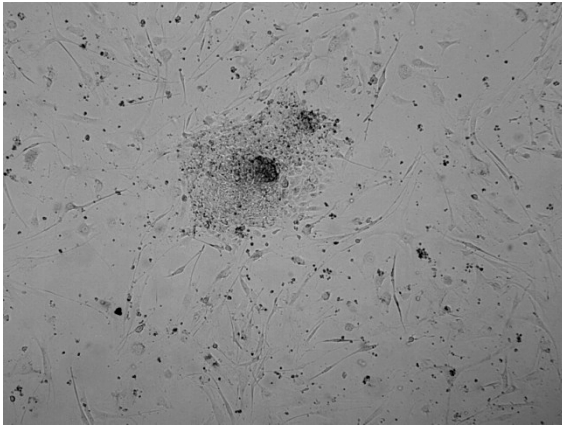


Figure 1A. Colony observed post thaw

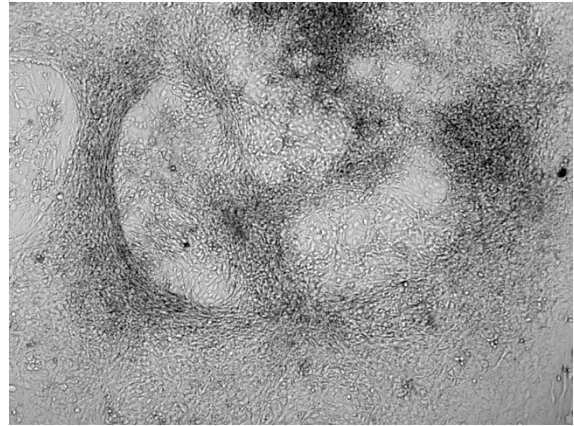


Figure 1B. Colony 7 days after first observation

Karyotype Analysis



Figure 2: G-banded karyotype showing 46 XY

Surface Antigen Expression of Stem Cell Markers

Undifferentiated cells are stained for the surface antigens, SSEA4 and SSEA1. SSEA4 (stage specific embryonic antigen 4) is expressed on undifferentiated human stem cells. SSEA1 (stage specific embryonic antigen 1) is expressed on differentiated stem cells.

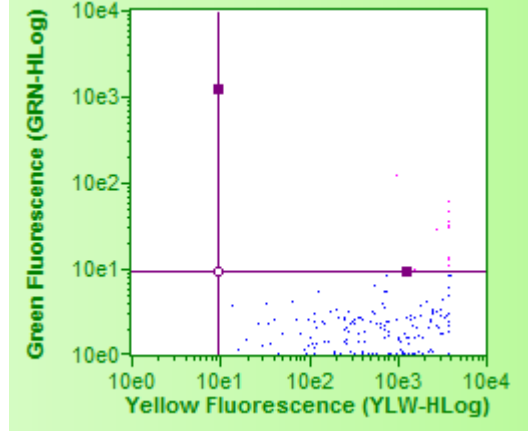


Figure 3A: Scatter plot of SSEA4 and SSEA1 double stained iPS cells.

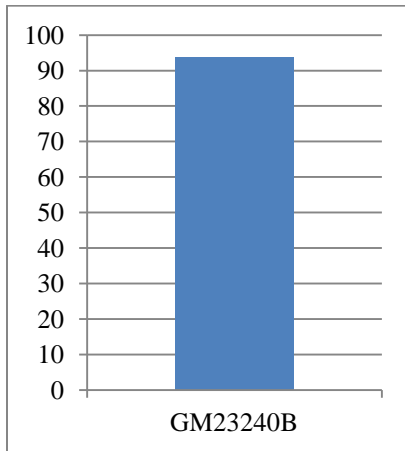


Figure 3B. Graph depicting percent SSEA4 positive cells in an undifferentiated cell culture.

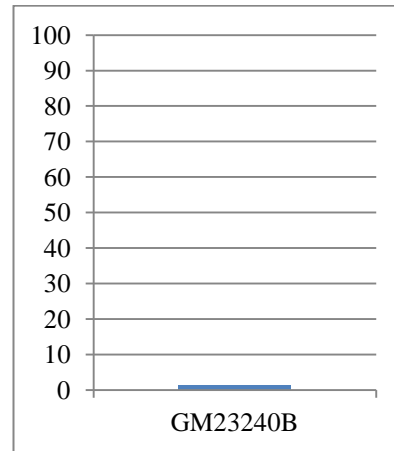


Figure 3C. Graph depicting percent SSEA1 positive cells in an undifferentiated cell culture

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

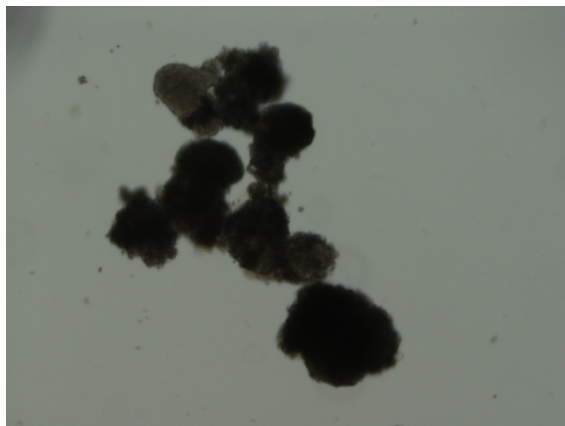


Figure 4A. Image of Embryoid Bodies

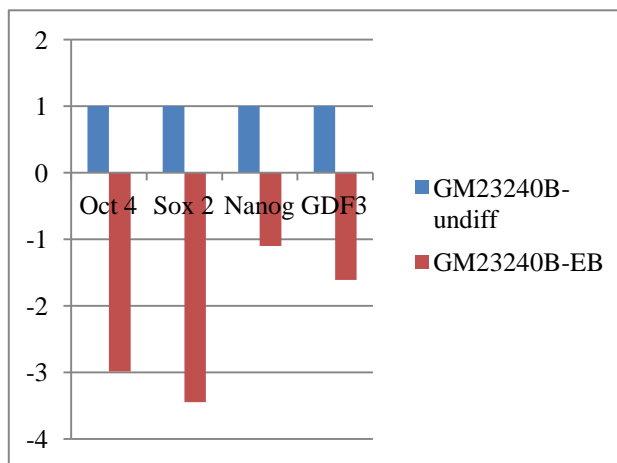


Figure 4B. Gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

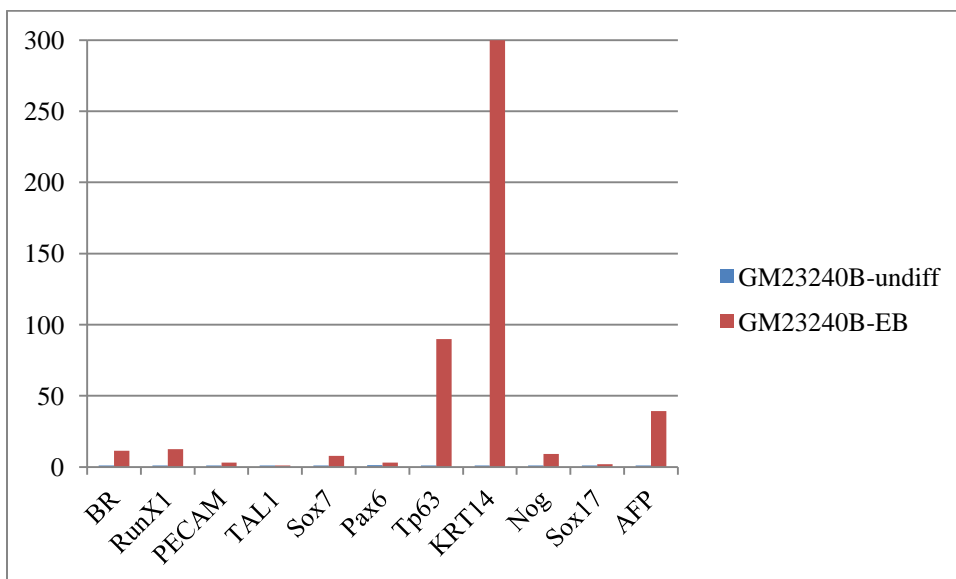


Figure 4C. Gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

	BR	RunX1	PECAM	TAL1	Sox7	Pax6	Tp63	KRT14	Nog	Sox17	AFP
GM23240B-undiff	1.00	1.00	1.00	1.00	1.00	1.18	1.00	1.00	1.00	1.00	1.00
GM23240B-EB	11.4	12.5	3.14	1.13	7.68	3.14	89.9	342	9.19	1.91	39

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

Neural Differentiation

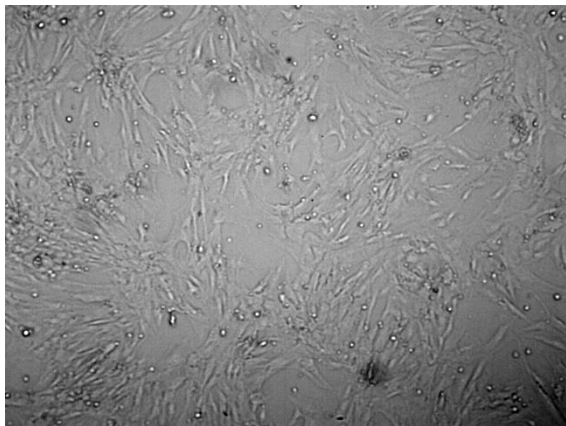


Figure 5A. Image of Neuronal Differentiation

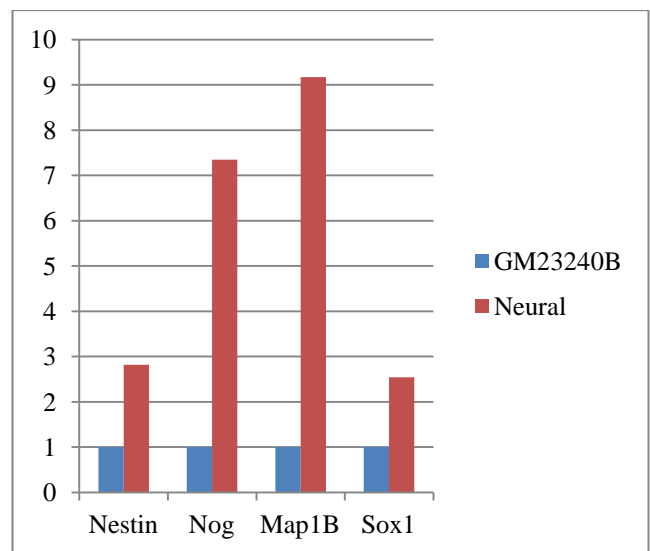


Figure 5B. Gene expression following neuronal differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Cardiac Differentiation

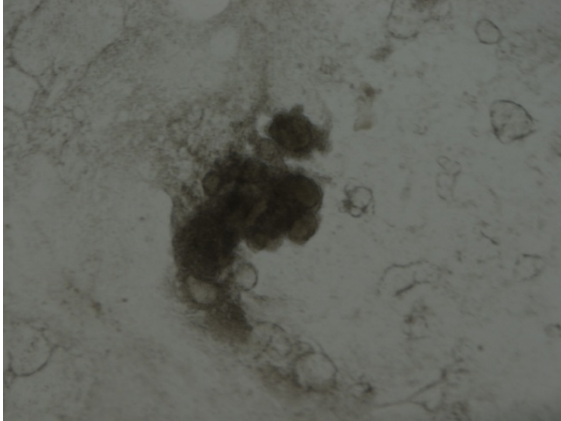


Figure 6A. Image of differentiated colony.

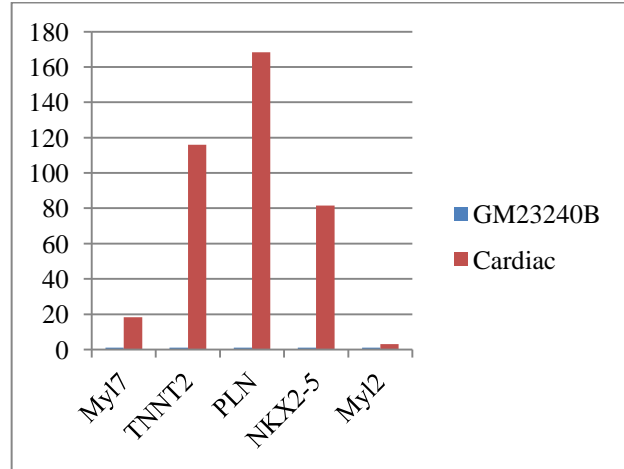


Figure 6B. Gene expression following cardiac differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Definitive Endoderm Differentiation

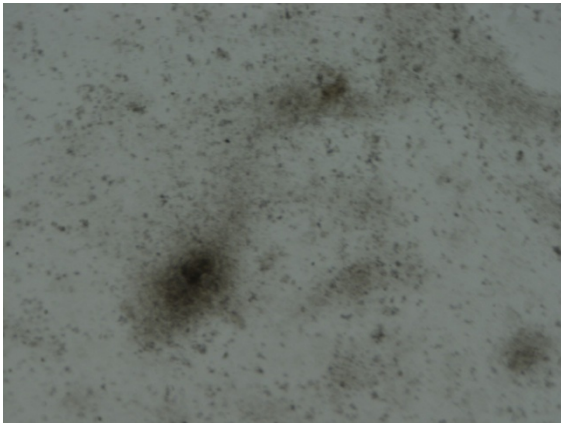


Figure 7A. Image of Definitive Endoderm Differentiation

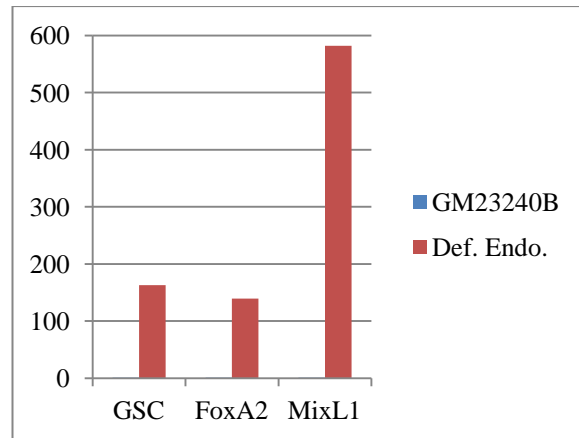


Figure 7B. Gene expression following Definitive Endoderm differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

- Pass
- Fail
- Other: _____



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Date 7.25.13