



CORIELL INSTITUTE

FOR MEDICAL RESEARCH

GM 23446*B

Certificate of Analysis

Product description	Human fibroblast reprogrammed with six factors (OCT4, SOX2, KLF4, CMYC, NANOG, LIN28) using retroviral vectors	
Publication(s) describing iPSC establishment		
Parent cell line and cell type	GM00038	Fibroblast
Diagnosis	Apparently Healthy Non-Fetal Tissue	
Parent cell line freeze passage		
Passage of iPSC reported at submission	10	
Number of passages at Coriell	8	
Media	DMEM/F12 + 20% KOSR + 100ng/ml bFGF	
Feeder	CF1 MEFs on 0.1% Gelatin	
Passage method	Collagenase or TrypLE Express	
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 (post 5 days)
2 days	296	1045

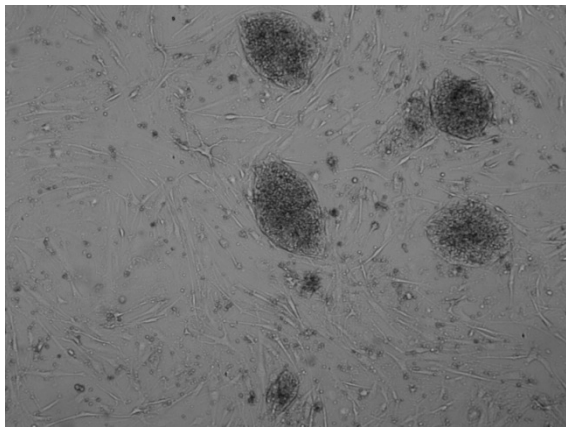


Figure 1A. Colony observed post thaw

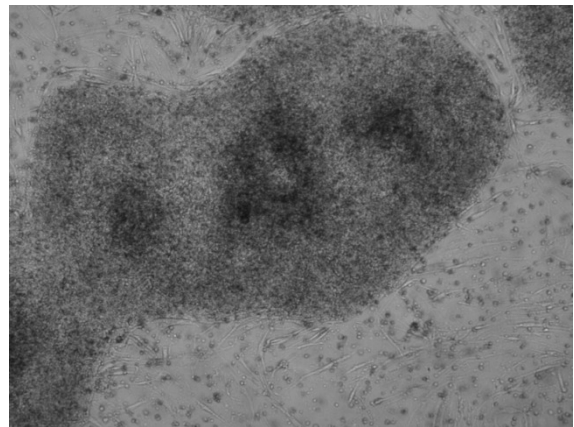


Figure 1B. Colony 5 days after first observation

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 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.

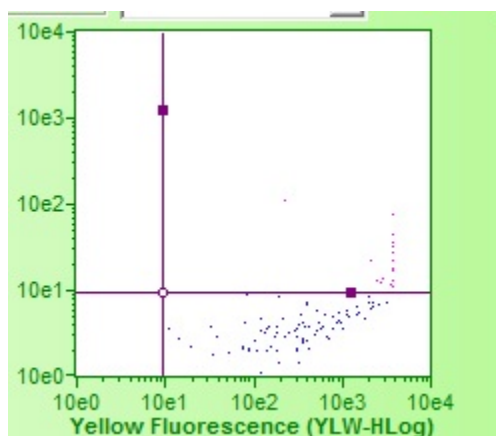


Figure 3A: Scatter plot of SSEA4 stained iPS cells.

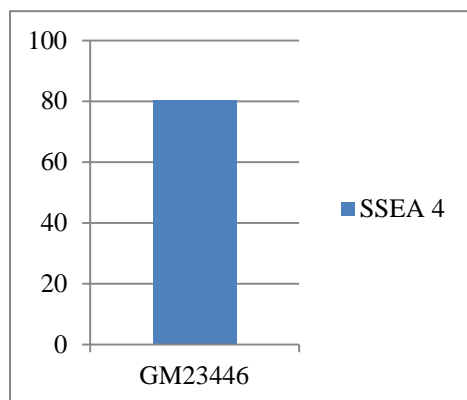


Figure 3B. Graph depicting percent SSEA4 positive cells in an undifferentiated cell culture.

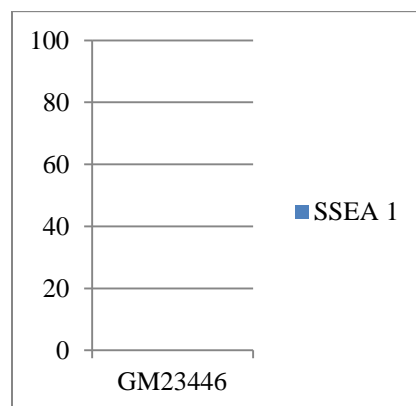


Figure 3C. Graph depicting percent SSEA1 positive cells in undifferentiated cell culture

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

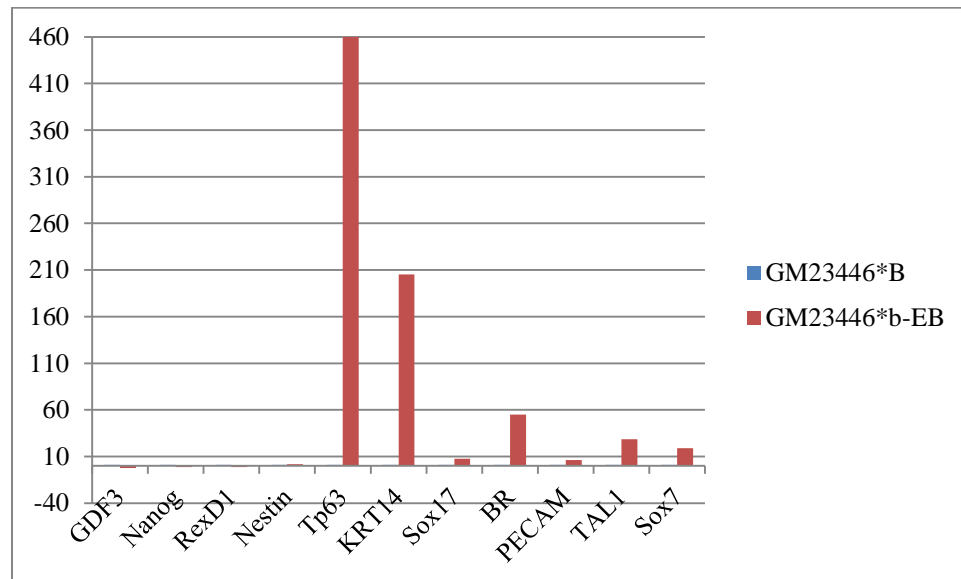


Figure 4. Gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

	GDF3	Nanog	RexD1	Pax6	Nestin	Tp63	KRT14	Sox17	BR	PECAM	TAL1	Sox7	AFP
GM23446*B	1	1	1	1	1	1	1	1	1	1	1	1	1
GM23446*B-EB	-3	-1	-1	3120	2	461	205	7	55	6	29	19	338029

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

Neural Differentiation

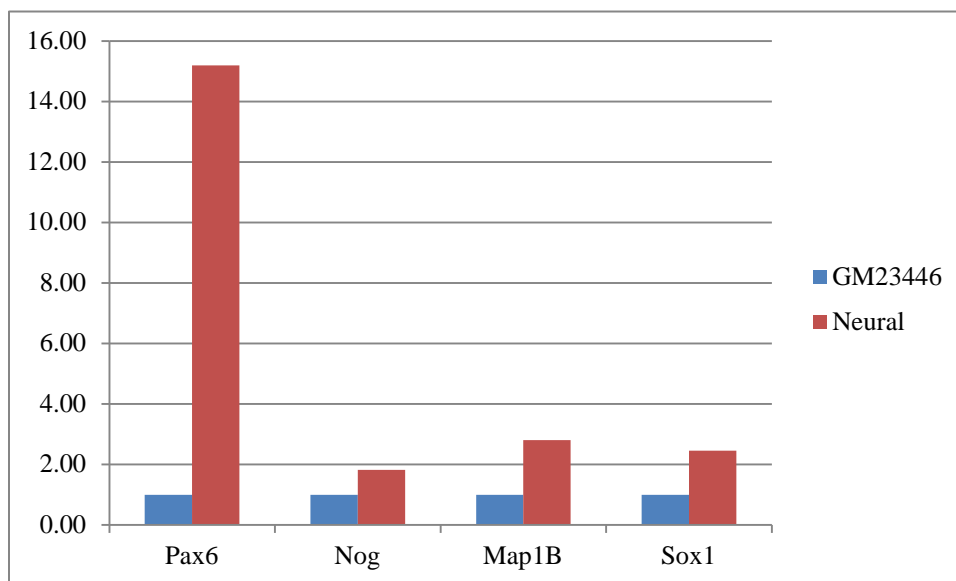


Figure 5. Gene expression following neuronal differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Cardiac Differentiation

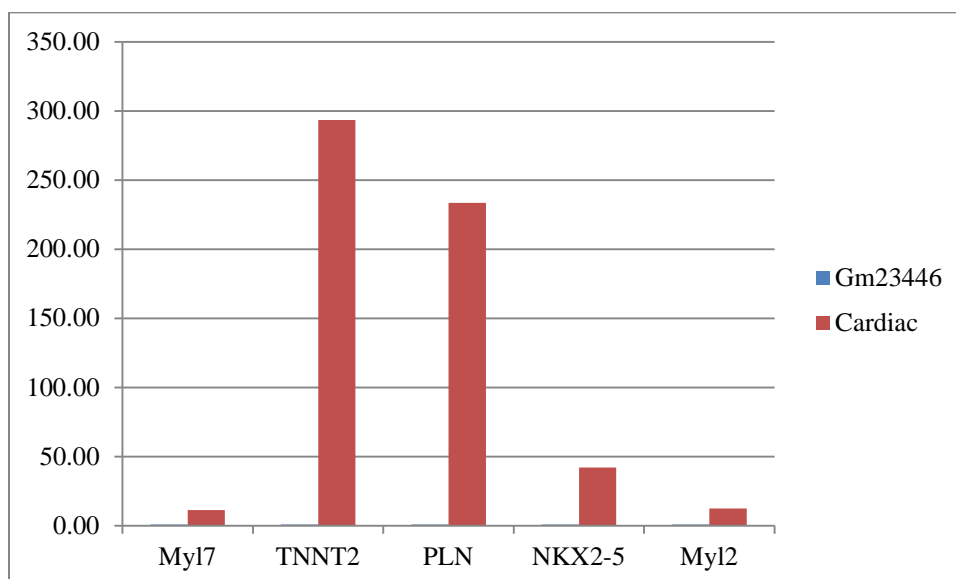


Figure 6. Gene expression following cardiac differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Definitive Endoderm Differentiation

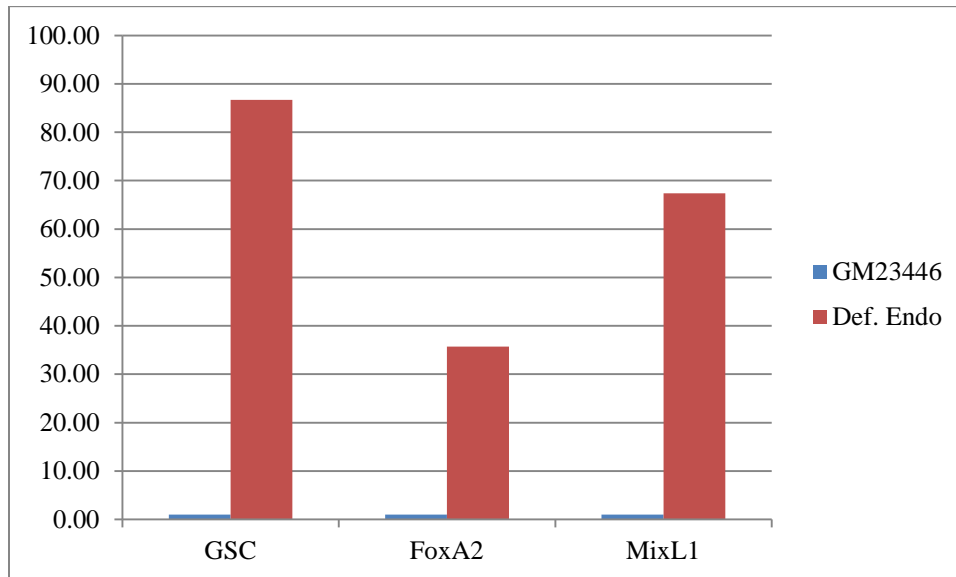


Figure 7. Gene expression following definitive endoderm differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

☒ Pass
☐ Fail
☐ Other: _____

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April 5, 2013

Teratoma Formation Analysis Report

Project Information

Service title: Teratoma Formation Analysis
 Customer: Coriell Institute
 PI/Contact person: Dr. Karen Fecenko-Tacka
 Report date: November 19, 2012
 Project manager: Qi Zheng
 Contact person: Tianmin "Ivy" Zhang

Service Detail

Cell type: human iPS cells
 Cell line & passage: GM23446/P3
 Feeder layer: Cf1 MEF
 Mouse type: Fox Chase SCID-beige, male, 6 week old, from Charles River
 Cell concentration: 1-3 million/site, in 30% Matrigel
 3 H&E slides
 Injection date: September 12, 2012

	Mouse #1	Mouse #2	Mouse #3	Positive Control
Injection sites	kidney capsule testis	kidney capsule testis	kidney capsule testis	kidney capsule testis
Tissue harvested	one kidney tumor and one testis tumor	one kidney tumor and one testis tumor	one kidney tumor and one testis tumor	one kidney tumor and one testis tumor
Days post-injection	61	61	61	49

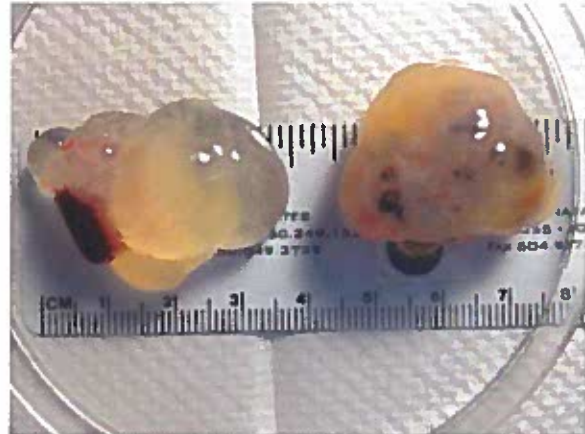
H&E Histology Instruction

Histology: 10% Formalin fixed over night, embedded in paraffin, cut into 5- μ m serial sections, H&E staining
 Three embryonic germ cell layers: endoderm, mesoderm and ectoderm
 Endoderm: digestive system (includes liver and pancreas), respiratory system, most glands
 Mesoderm: muscle, blood vessels, much of the genital-urinary system, skeletal system
 Ectoderm: skin, hair, nails, sweat and mammary glands, nervous system (including hypothalamus and both lobes of the pituitary gland), oral and nasal cavities, portions of the vagina, vestibule, penis and clitoris

Tumor and organ pictures



Mouse#1: one kidney tumor (left) and one testis tumor (right) harvested on day 61 after injection



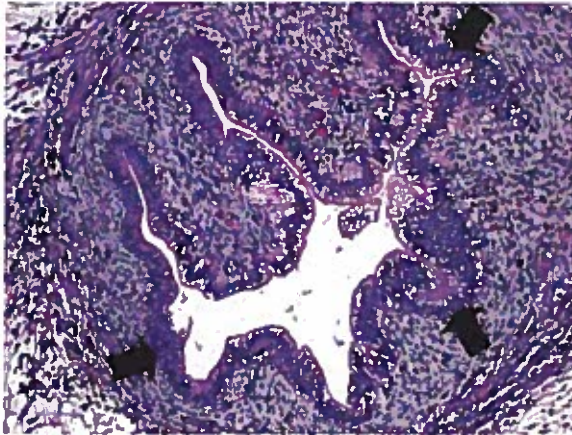
Mouse#2: one kidney tumor (left) and one testis tumor (right) harvested on day 61 after injection



Mouse#3: one kidney tumor(left) and one testis tumor (right) harvested on day 61 after injection

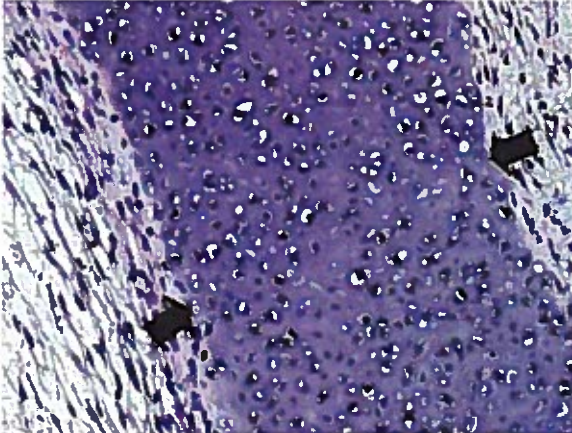
H&E staining results of kidney and testis tumors:

Endoderm

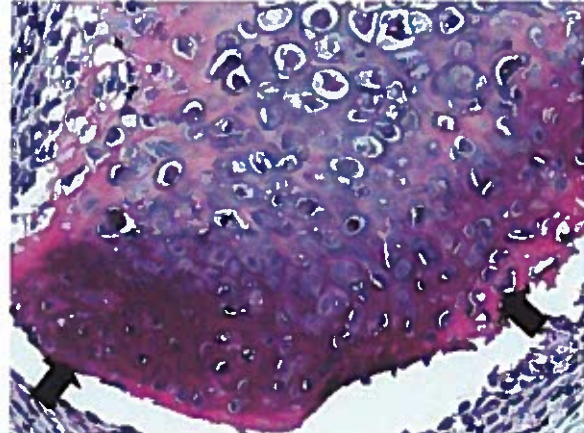


Gland (100x)

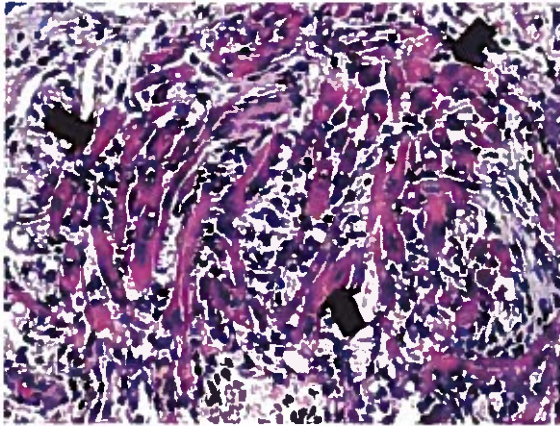
Mesoderm



Cartilage (200x)

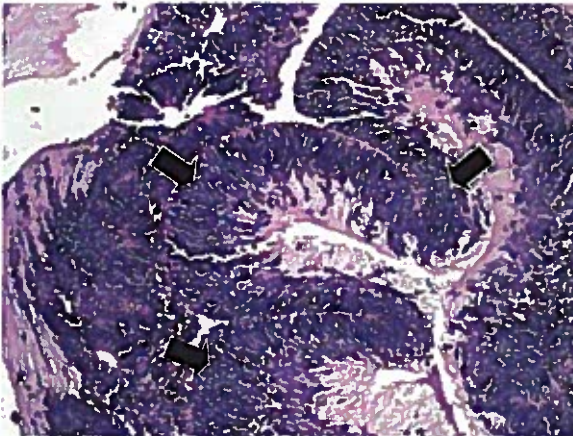


Ossifying cartilage (200x)



Muscle (200x)

Ectoderm



Neuronal rosette (200x)

Summary

Three kidney tumors and three testis tumors are composed of scattered regions of differentiated cells and a large population of undifferentiated neoplastic cells. Three germ layers were clearly identified in histology analysis. The tissues listed above indicate that small areas of what might be these kinds of tissues were noted within the tumor. Overall, there is some degree of differentiation of these cells with organized structures, suggesting that some of these cells are pluripotent.

Project managerSignature: Date: 11/19/2012Qi Zheng, Ph.D.
Senior Scientist**Reviewed and proved by**Signature: Date: 11/19/2012Steve Yu, Ph.D.
Director of Service Department