GM 23450*B

Certificate of Analysis

Product description	Human Fibroblast reprogrammed with seven factors (Oct4, Sox2, Klf, c-Myc, Nanog, Lin-28, T-antigen) using MMLV vector	
Publication(s) describing iPSC establishment		
Parent cell line and cell type	Fibroblast	GM06113
Diagnosis	Apparently Healthy Non-Fetal Tissue	
Parent cell line freeze passage		
Passage of iPSC reported at submission	21	
Number of passages at Coriell	7	
Media	DMEM/F12 + 20% KOSR + 100ng/ml bFGF	
Feeder	CF1 MEFs on 0.1% Gelatin	
Passage method	Collagenase	
Split ratio	1:6; every 4-6 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	*In Vivo* 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 7 days)
2 days	229	1256

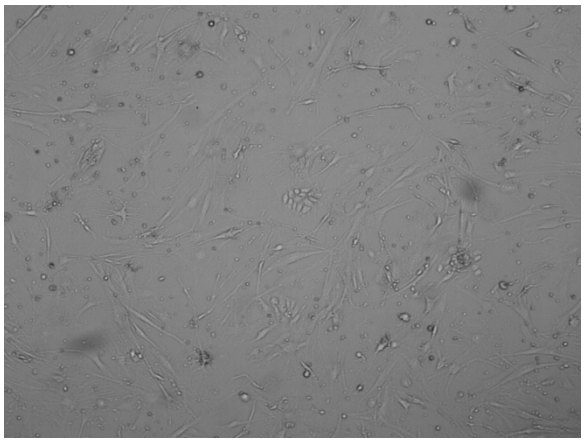

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Figure 1A. Colony observed post thaw

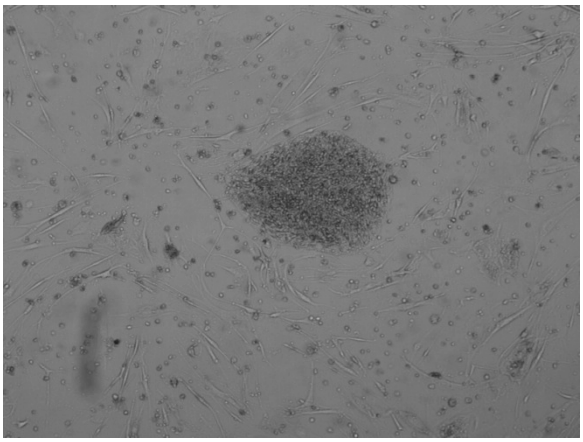


Figure 1B. Colony 7 days after first observation

Karyotype Analysis

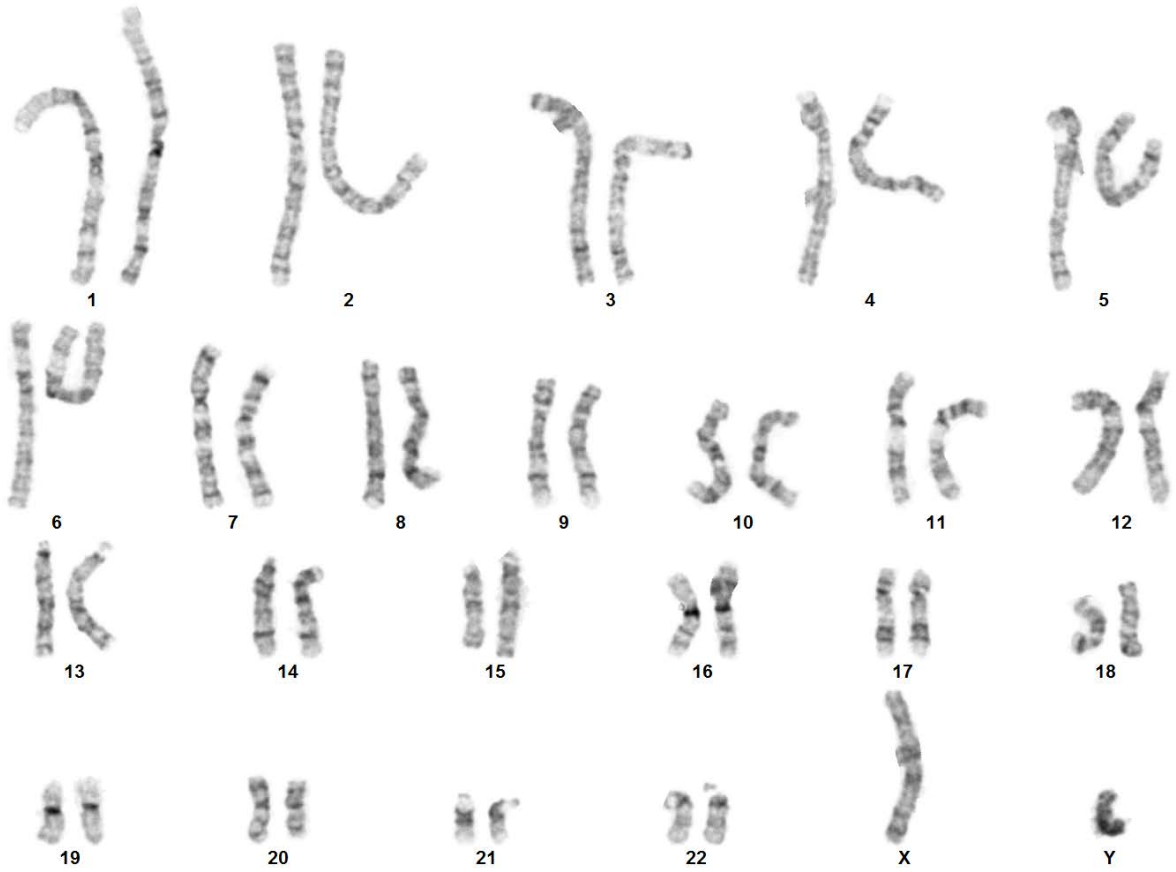


Figure 2: G-banded karyotype showing 46 XY

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.

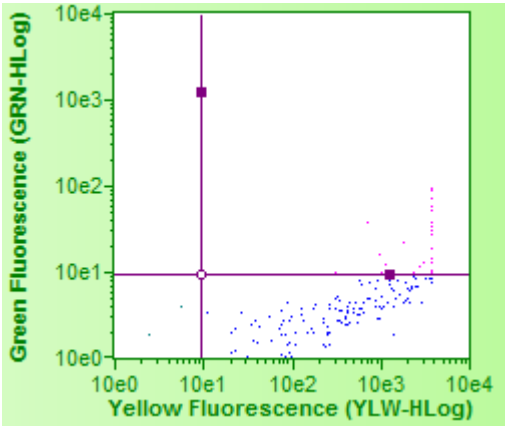


Figure 3A: Scatter plot of SSEA4 and SSEA1 double stained iPS cells.

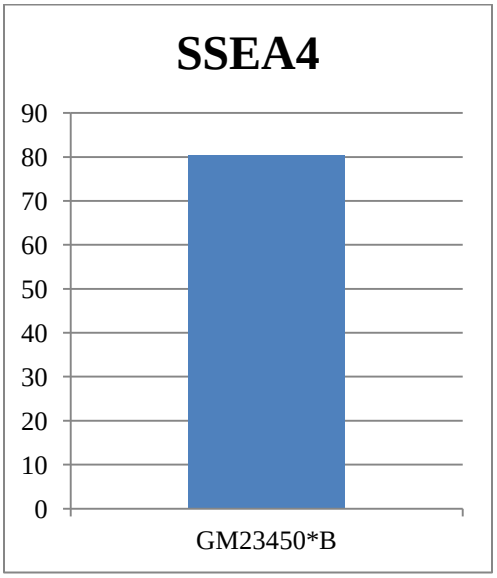


Figure 3B. Graph depicting percent SSEA4 positive cells in an undifferentiated cell culture.

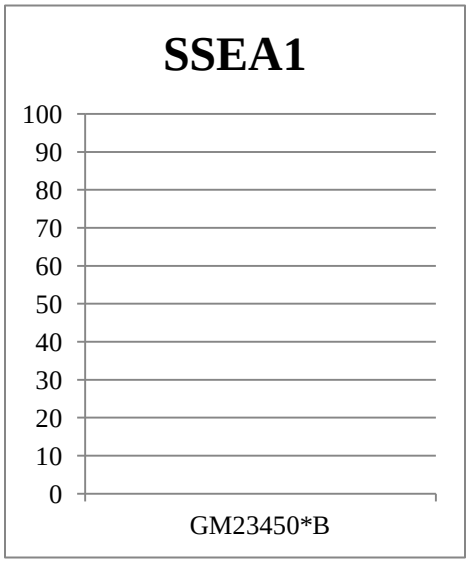


Figure 3C. Graph depicting percent SSEA1 positive cells in undifferentiated cell culture

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

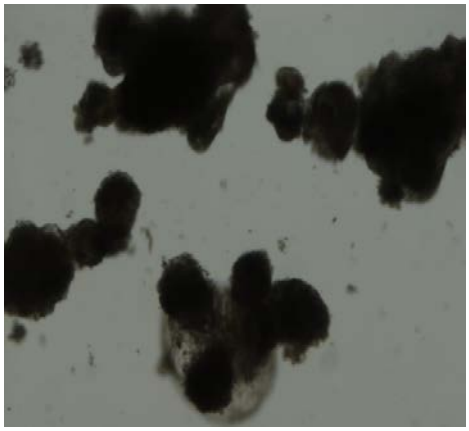


Figure 4A. Image of Embryoid Bodies, day 2

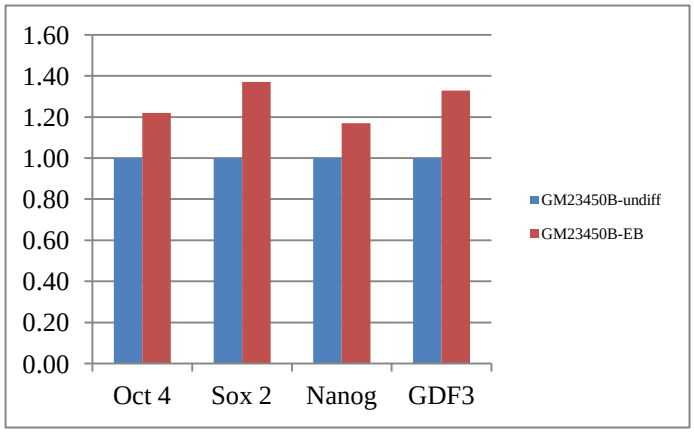


Figure 4B. Gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

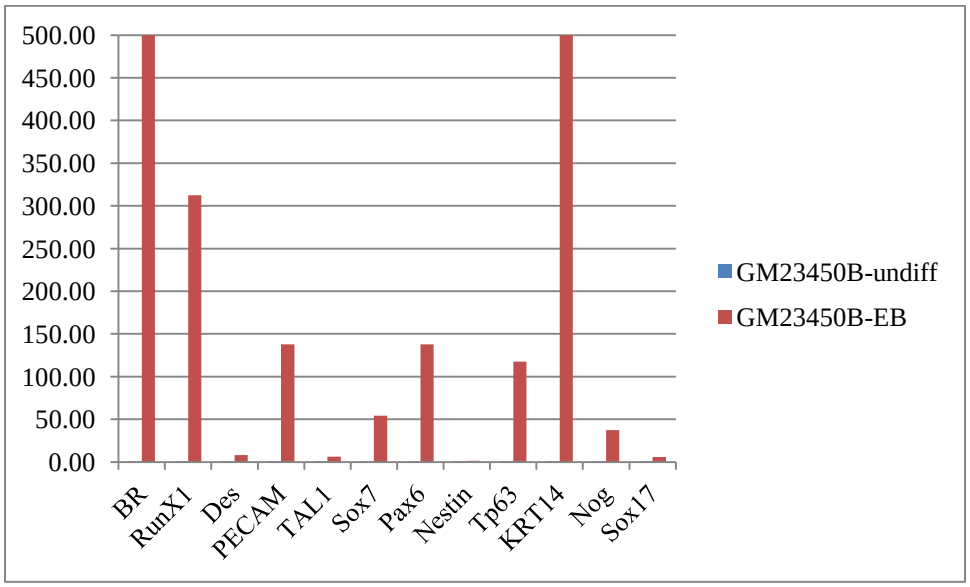


Figure 4C. Gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

	BR	RunX1	Des	PECAM	TAL1	Sox7	Pax6	Nestin	Tp63	KRT14	Nog	Sox17	AFP
GM23450B-undiff	1	1	1	1	1	1	1	1	1	1	1	1	1
GM23450B-EB	722	312	8	138	6	54	138	1	118	1678	37	6	37046519

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

Neural Differentiation

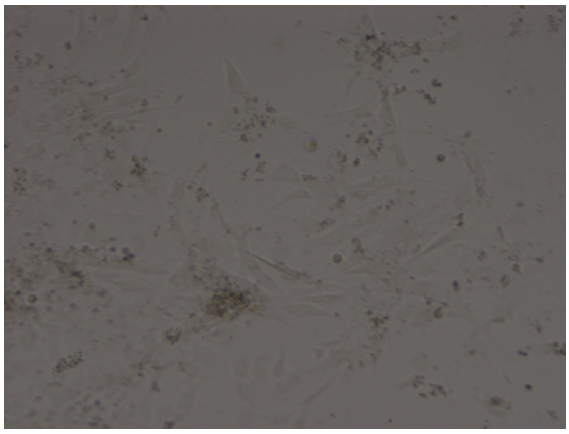


Figure 5A. Image of Neuronal Differentiation

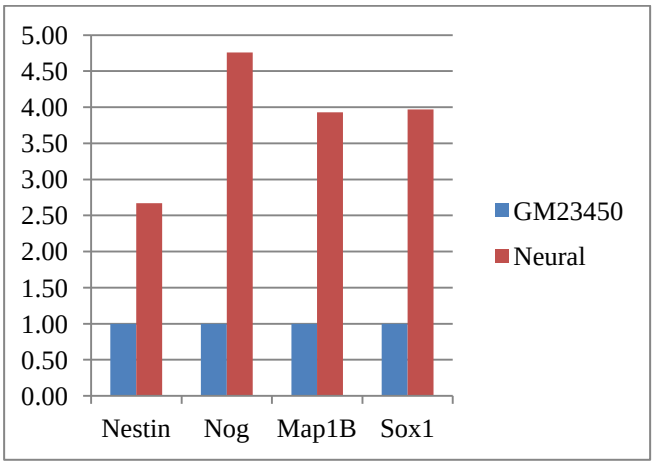


Figure 5B. Gene expression following neuronal differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Cardiac Differentiation

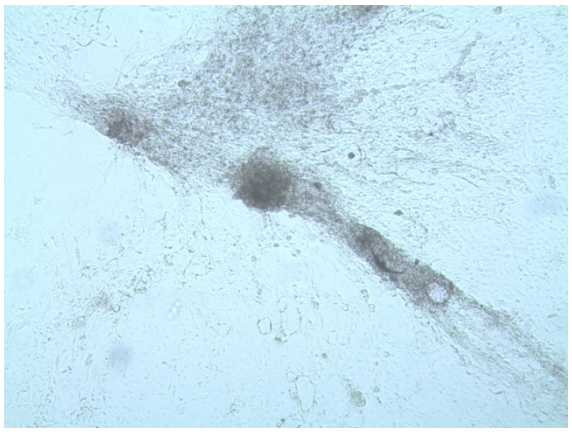


Figure 6A. Image of differentiated colony.

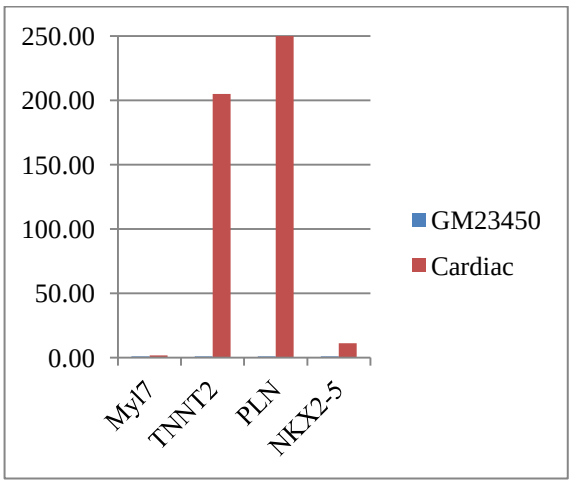


Figure 6B. Gene expression following cardiac differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Definitive Endoderm Differentiation

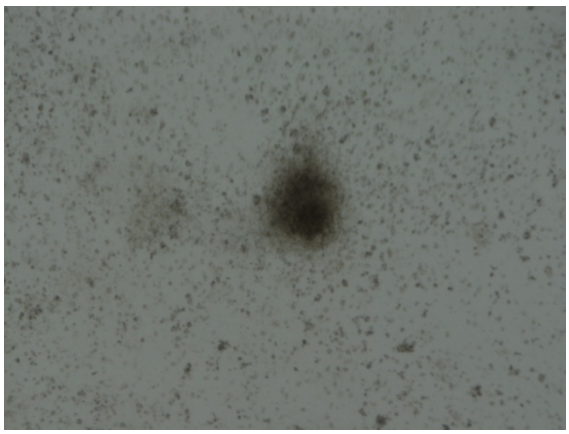


Figure 7A. Image of Definitive Endoderm Differentiation

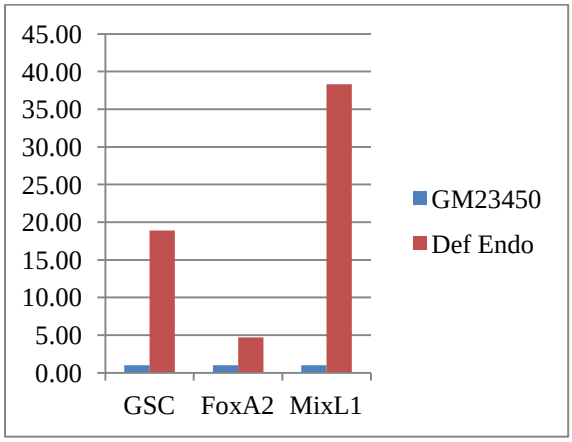
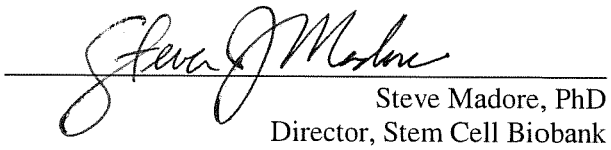


Figure 7B. Gene expression following Definitive Endoderm differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

☒ Pass
☐ Fail
☐ Other: _____



Steve Madore, PhD
Director, Stem Cell Biobank
December 12, 2012

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

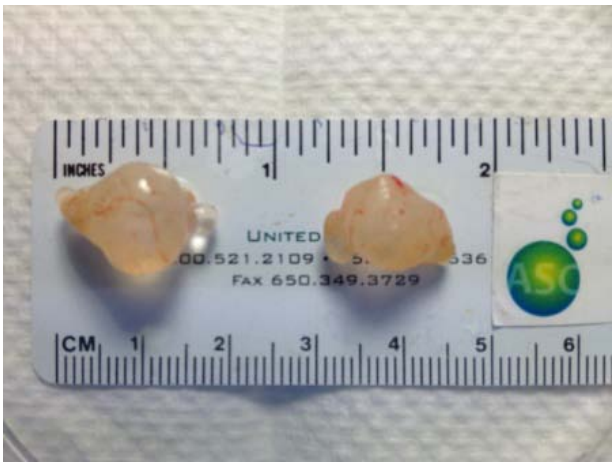
Cell type: human iPS cells
Cell line & passage: GM23450/P3
Feeder layer: Cf1 MEF
Mouse type: Fox Chase SCID-beige, male, 6 week old, from Charles River
Cell concentration: 3 million/site, in 30% Matrigel
3 H&E slides
Injection date: September 12, 2012

	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

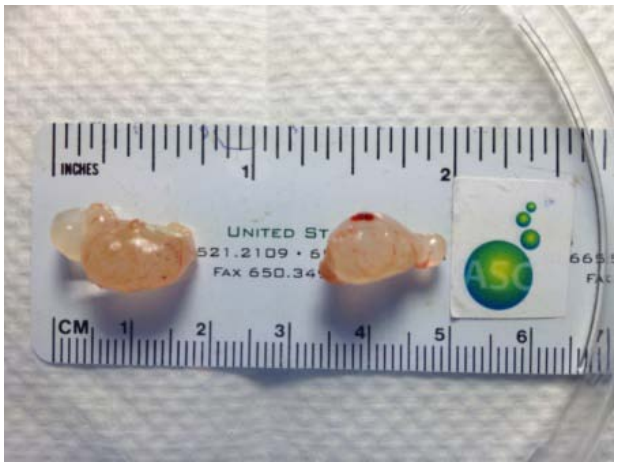
H&E Histology Instruction

Histology: 10% Formalin fixed over night, embedded in paraffin, cut into 5-μm 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

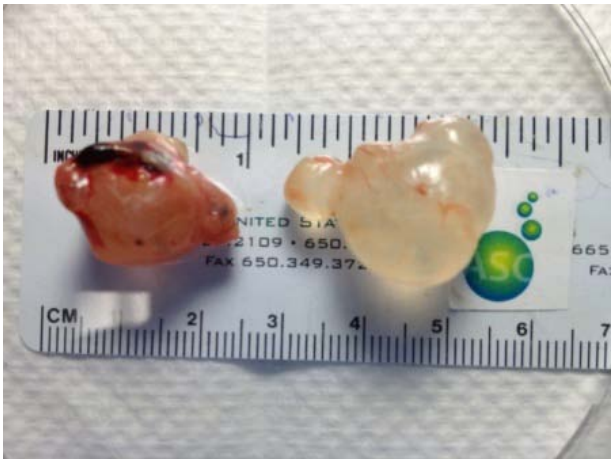
Tumor and organ pictures



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



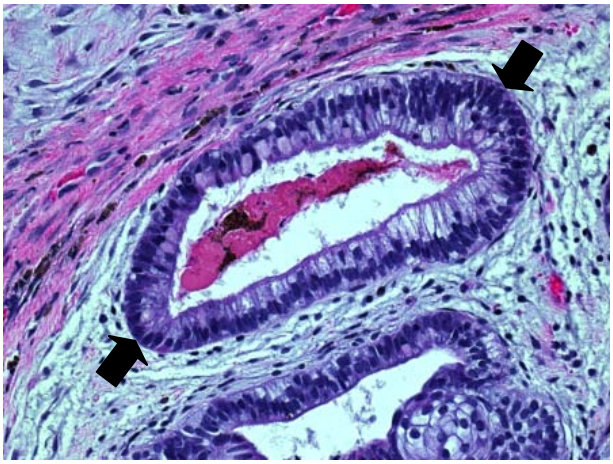
Mouse#2: one kidney tumor (left) and one testis tumor (right) harvested on day 61 after injection



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

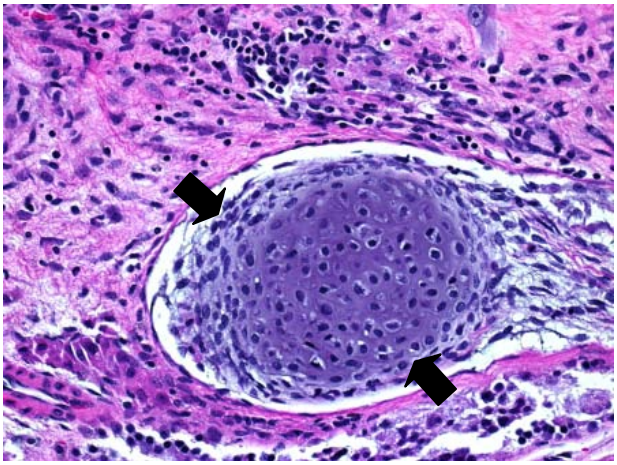
H&E staining results of kidney and testis tumors:

Endoderm

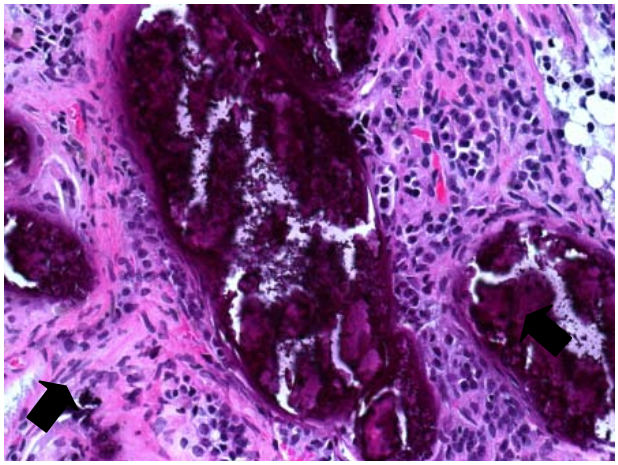


Gland (200x)

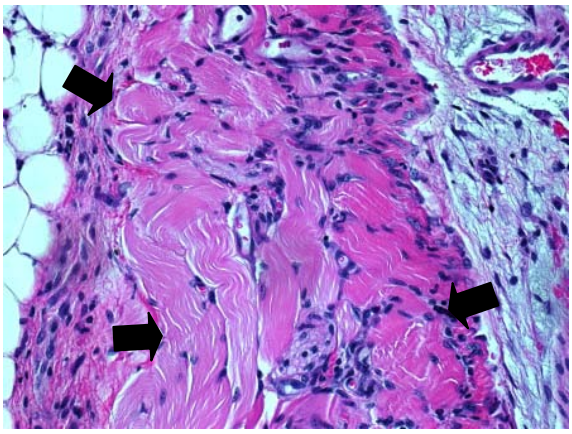
Mesoderm



Cartilage (200x)

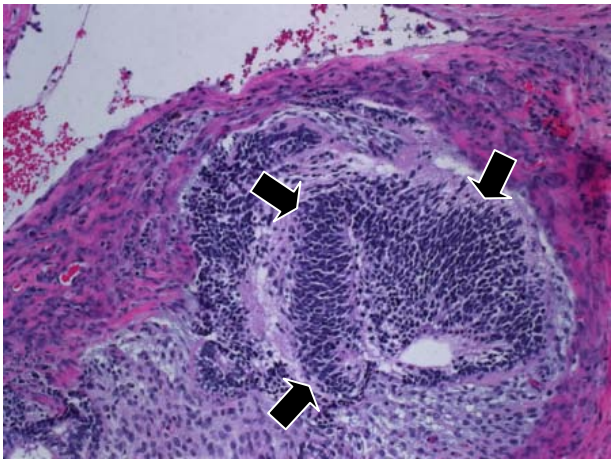


Bone (200x)



Muscle (200x)

Ectoderm



Neuronal rosette (100x)

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 manager

Signature:  Date: 11/19/2012

Qi Zheng, Ph.D.
Senior Scientist

Reviewed and proved by

Signature:  Date: 11/19/2012

Steve Yu, Ph.D.
Director of Service Department