GM23232*A

Certificate of Analysis

Product Description	Human Fibroblast reprogrammed with four factors (Oct 4, Sox 2, c-Myc and Klf-4) using retroviral vector	
Publication(s) describing iPSC establishment	Park et al., PMID **18691744**	
Parent Line and cell type	**GM01390**	Fibroblast
Diagnosis	Severe Combined Immunodeficiency, Autosomal recessive, T cell-negative, B cell-negative, NK cell-negative due to adenosine deaminase deficiency	
Fibroblast Freeze Passage	5	
Submitted Passage	13	
Freeze Passage (after recovery)	10	
Media	DMEM/F12 + 20% KOSR + 10 ng/ml bFGF	
Feeder	CF1 MEFs on 0.1% gelatin	
Passage method	Collagenase or TrypLE Express	
Split ratio	1:5; every 5 to 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	
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	Average Colony Diameter on day 5
2	406.88 microns	1181 microns

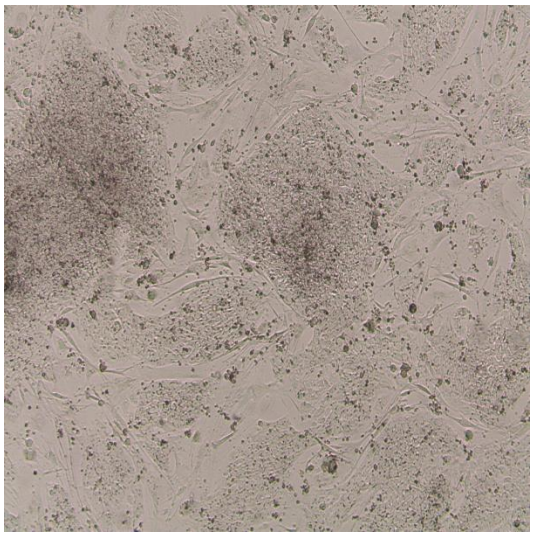


Figure 1A. Colony observed post thaw

Figure 1B. Colony 5 days after first observation

Karyotype Analysis

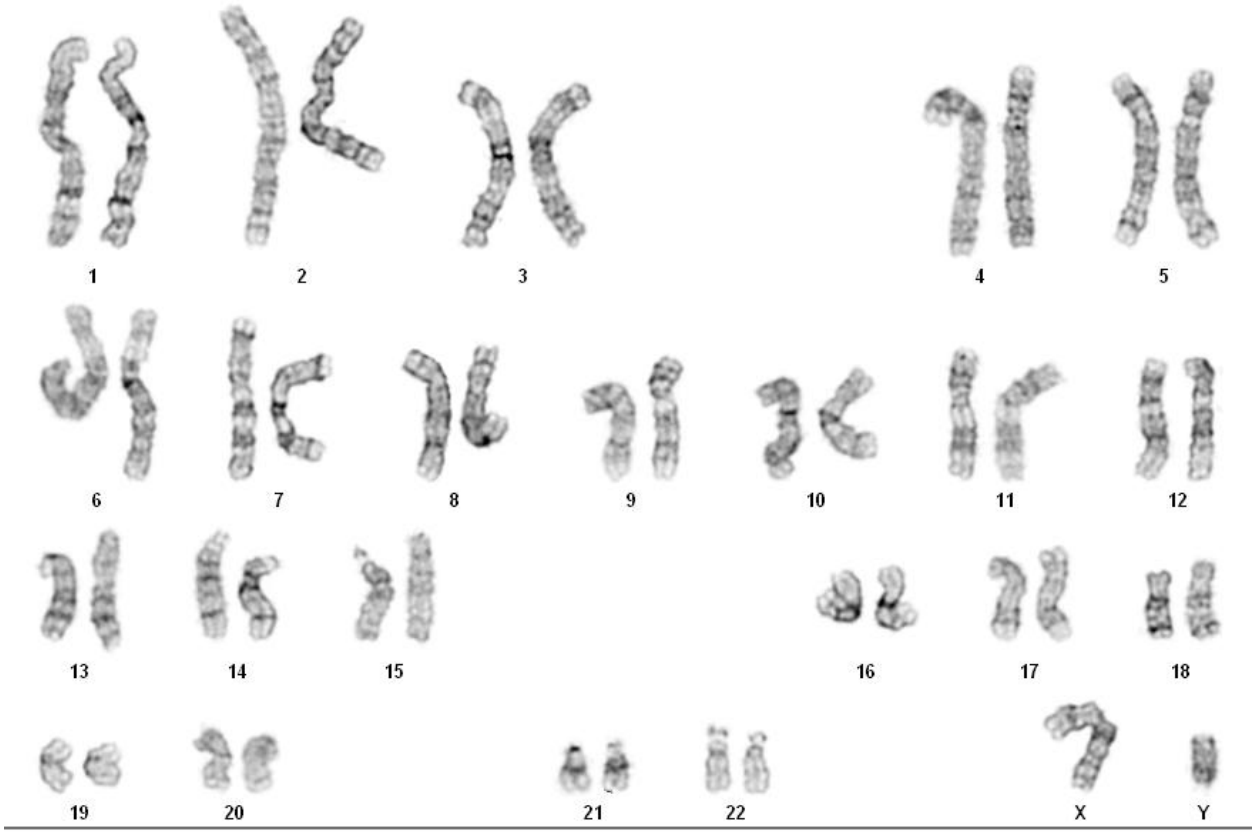


Figure 2: G-banded karyotype showing 46,XY

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.

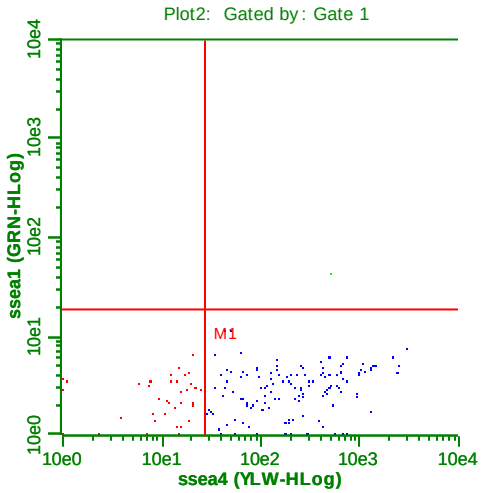


Figure 3A: Scatter plot of SSEA4 and SSEA1 double stained iPS cells.

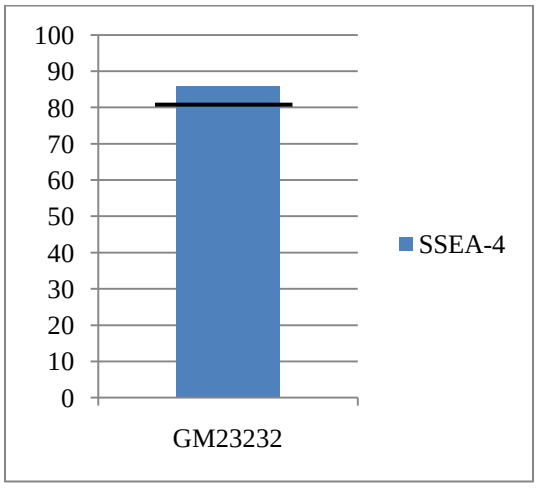


Figure 3B. Graph depicting percent SSEA1 positive cells in an undifferentiated cell culture

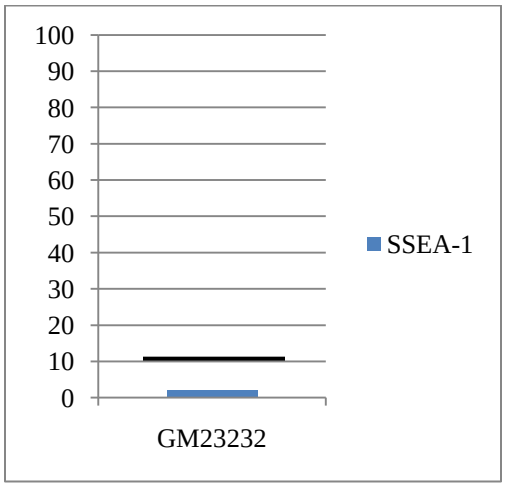


Figure 3C. Graph depicting percent SSEA4 positive cells in an undifferentiated cell culture.

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 compared to undifferentiated cells.

Embryoid Body (EB) Formation Assay

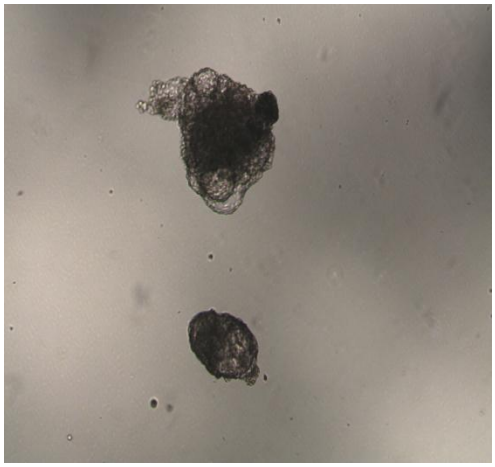


Figure 4A. Image of Embryoid Bodies, day 4

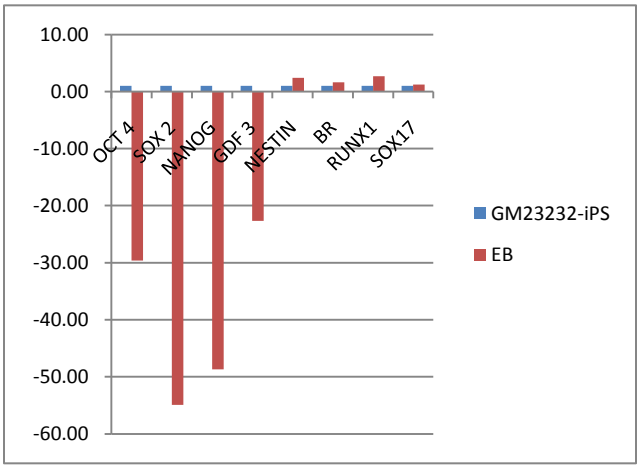


Figure 4B. Gene expression following EB differentiation. Fold difference is shown compared to undifferentiated cells.

	OCT 4	SOX 2	NANOG	GDF 3	NESTIN	BR	RUNX1	AFP	SOX 17
Undiff-erentiated	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
EB	-29.64	-54.92	-48.72	-22.65	2.39	1.61	2.69	16108.57	1.22

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

Neural Differentiation

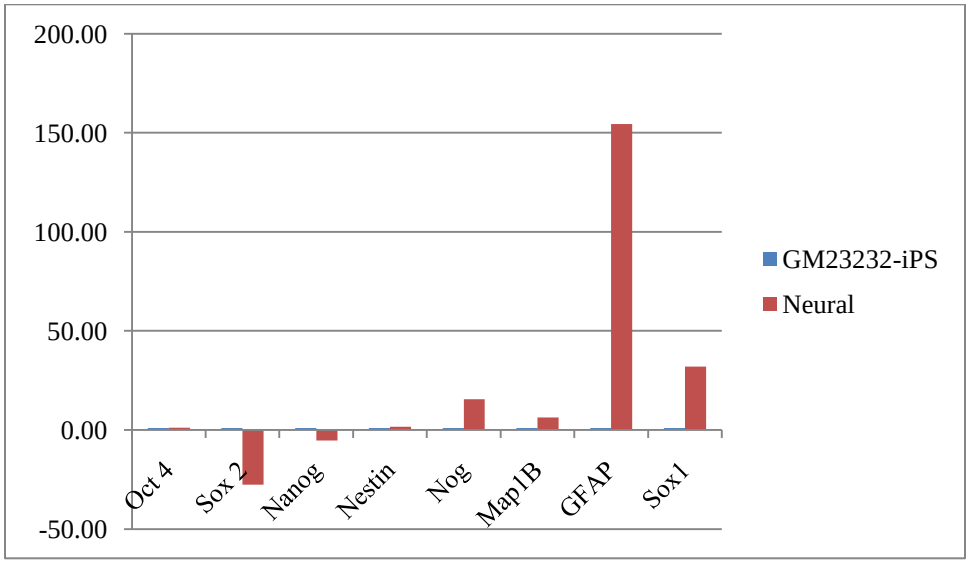


Figure 5. Gene expression following neuronal differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Cardiac Differentiation

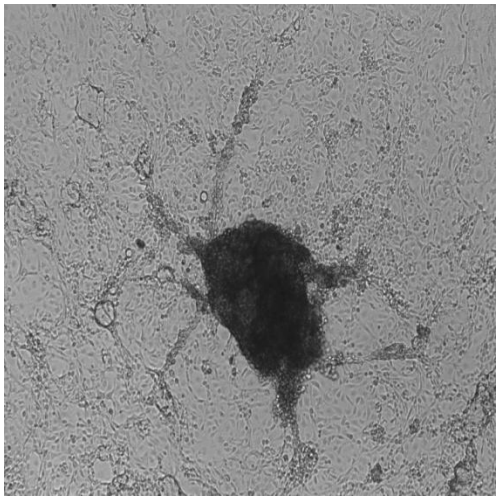


Figure 6A. Image of differentiated colony. Beating was observed.

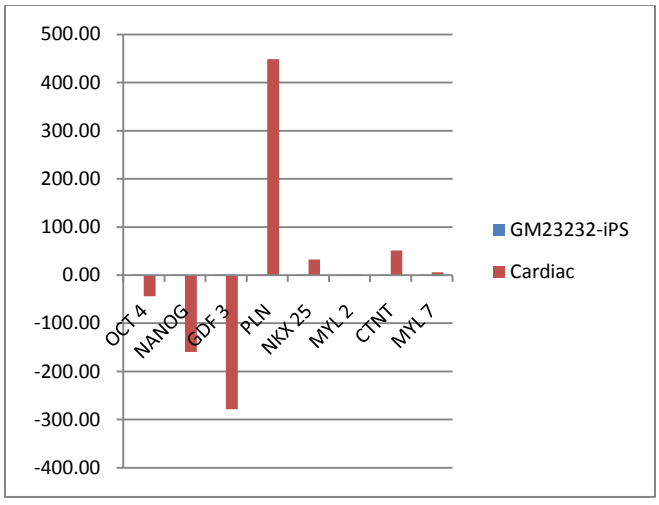


Figure 6B. Gene expression following cardiac differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Pancreatic Differentiation

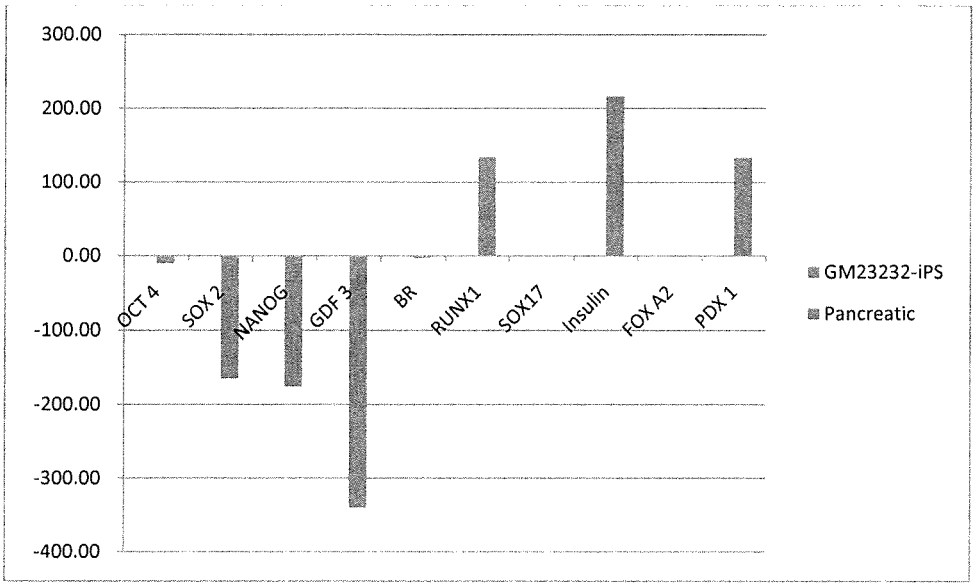


Figure 7. Gene expression following pancreatic differentiation. Fold difference is shown relative to undifferentiated iPS cell line. Insulin production and release was confirmed by ELISA.

☑ Pass
☐ Fail
☐ Other: _____

Margaret A. Keller, PhD
Director, Stem Cell Biobank
April 11, 2011

Teratoma Formation Analysis Report

Project Information

Service Title: Teratoma Formation Analysis
Customer: Coriell Institute
PI/Contact Person: Karen Fecenko-Tacka
Purchase Order Number: MS510

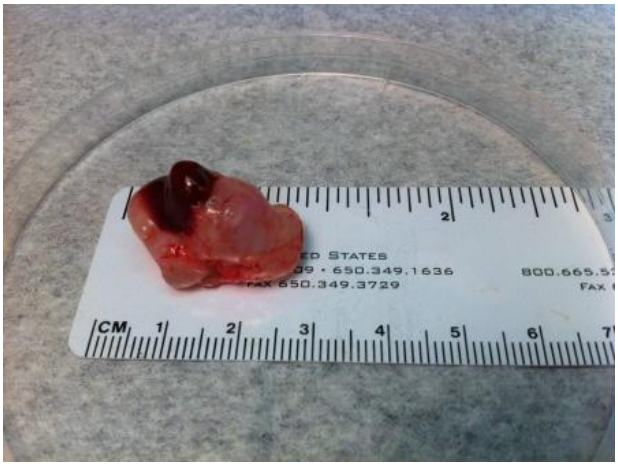
Service Detail

Cell type: human iPS cells
Cell line & Passage: GM23232A, P6 and P10
Feeder layer: MEF , 1 million cells per $10cm^2$
Mouse type: Fox Chase SICD-beige, male, 6 week old, from Charles River
Injection sites: 8 kidney capsules and 2 testes
Cell concentration: 1.5 to 3 Million/site, in 30% Matrigel
Injection date: November 24, 2010 and December 12, 2010
Mice monitoring: November 24, 2010 – March 2, 2011, monitor 2-3 times/week
Tissue harvested: February 11, 2011 (day 61), March 2, 2011 (day 80 and day 98), take pictures
Histology: 10% Formalin fixed over night, embedded in paraffin, cut into 5-μm serial sections, H&E staining
Imaging: Nikon Eclipse E1000 with motor macro slide (microscope) and Nikon photohead V-TP (camera)
6 H&E slides
Report date: March 22, 2011
Project manager: Qi Zheng
Contact person: Esther Tang

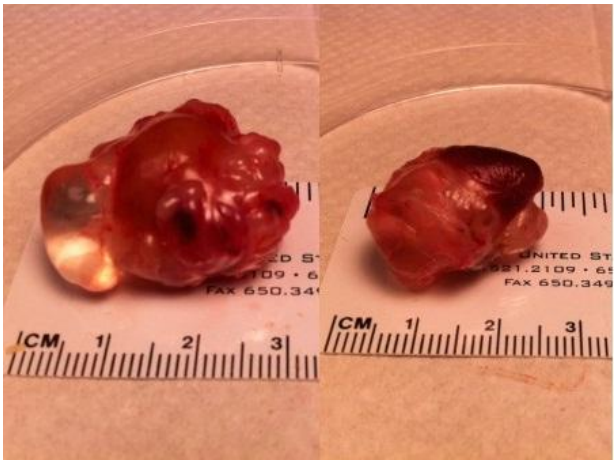
H&E Histology Instruction

Wheater's Functional Histology (B. Young and J.W. Heath), 4th edition
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

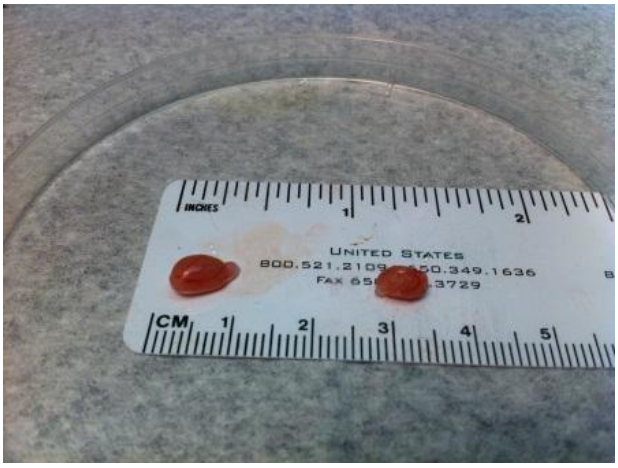
Tumor and organ pictures



One kidney tumor harvested on day 98 after injection



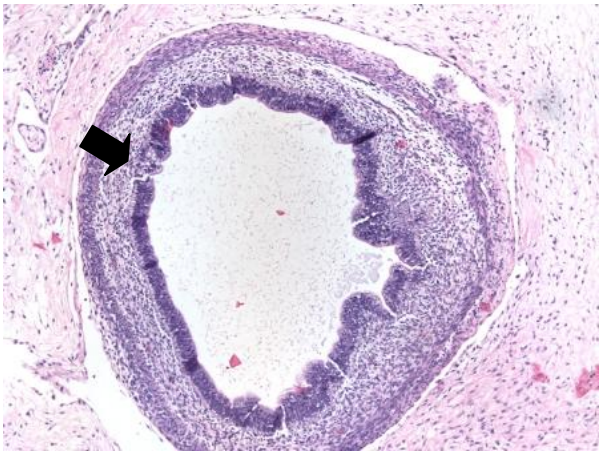
Two kidney tumors harvested on day 61 after injection



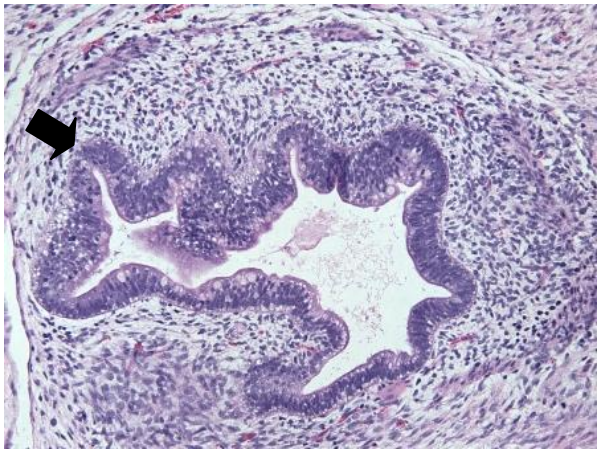
Two testis tumors harvested on day 80 after injection

H&E staining result of kidney and testis tumors:

Endoderm:

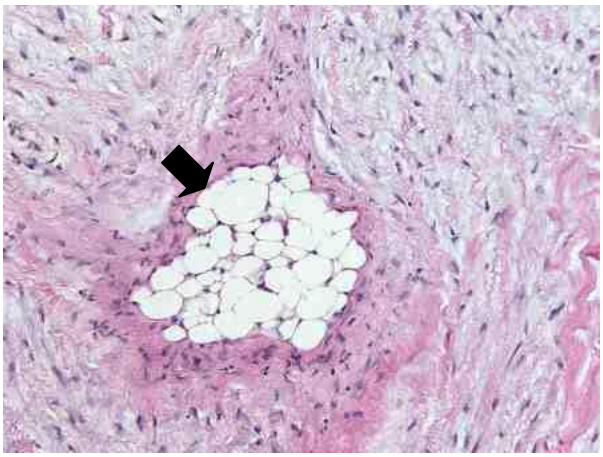


Gland (100x)

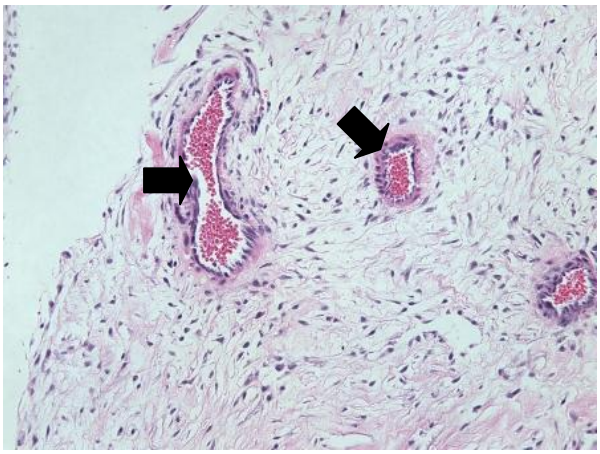


Gland (200x)

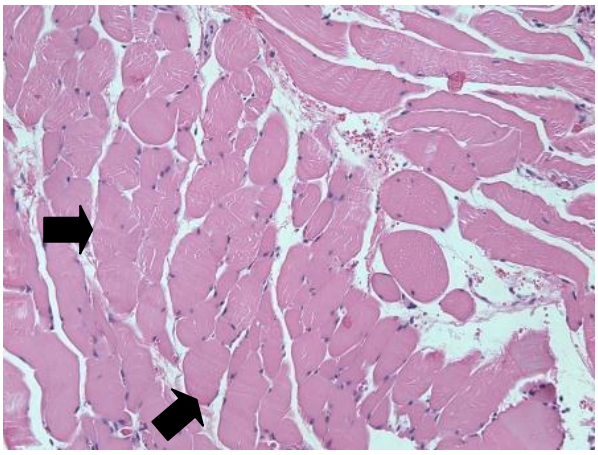
Mesoderm:



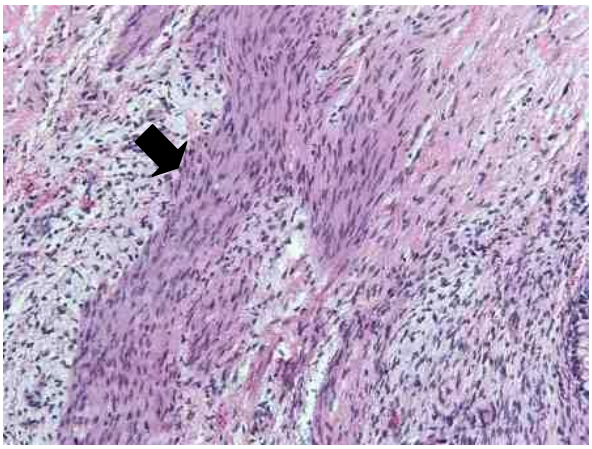
White adipose tissue (200x)



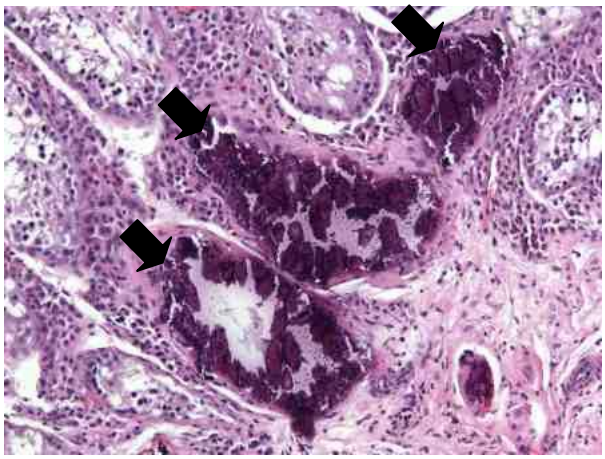
Blood vessel (200x)



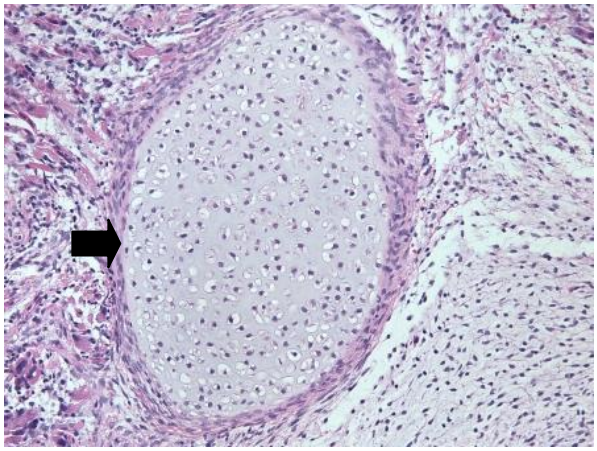
Skeletal muscle (200x)



Smooth muscle (200x)

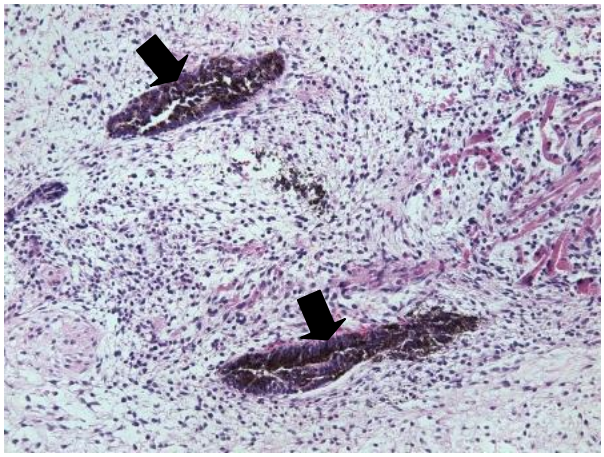


Bone (200x)



Cartilage (200x)

Ectoderm:



Melanocyte (200x)

Summary

Two kidney tumors harvested on day 61 and one kidney tumor on day 98 are composed of scattered regions of differentiated cells and a population of undifferentiated neoplastic cells. Three germ lays were identified in kidney tumors. For two testis tumors harvested on day 80, bone structure can be found. The tissues listed above indicate that small areas of what might be these kinds of tissues were noted within the tumors. Overall, there is some degree of differentiation of these cells with organized structures, suggesting that some of these cells are pluripotent.