



GM23262*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	GM04981	Fibroblast
Diagnosis	Muscular Dystrophy, Becker type; BMD	
Fibroblast Freeze Passage	9	
Submitted Passage	8	
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	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	Average Colony Diameter on day 5
2	189 microns	545 microns

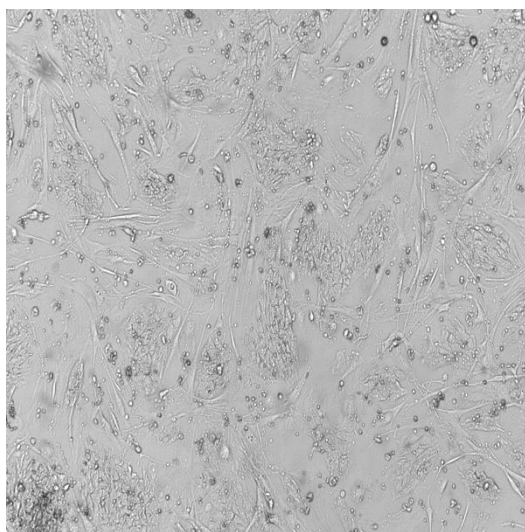


Figure 1A. Colony observed post thaw

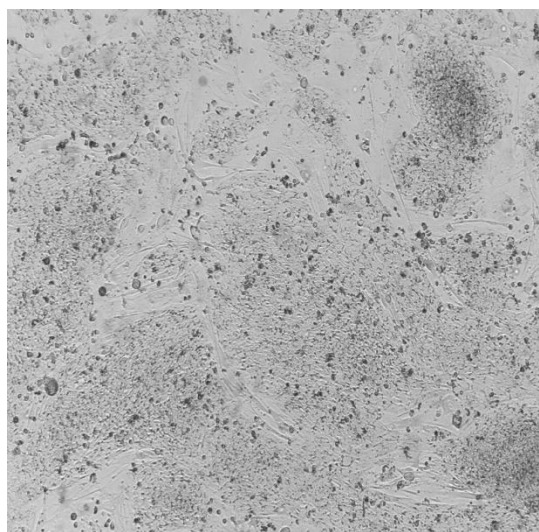


Figure 1B. Colony 5 days after first observation

Karyotype Analysis

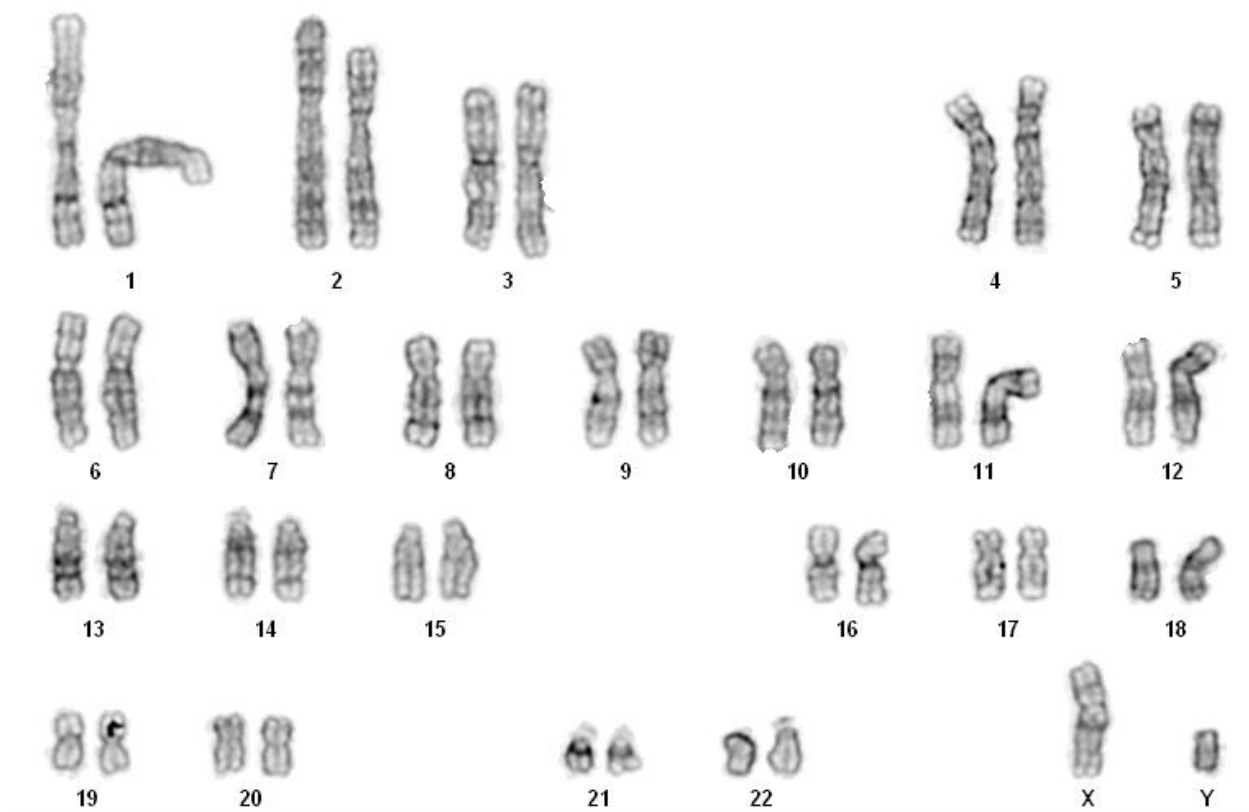


Figure 2: G-banded karyotype showing 46,XY

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.

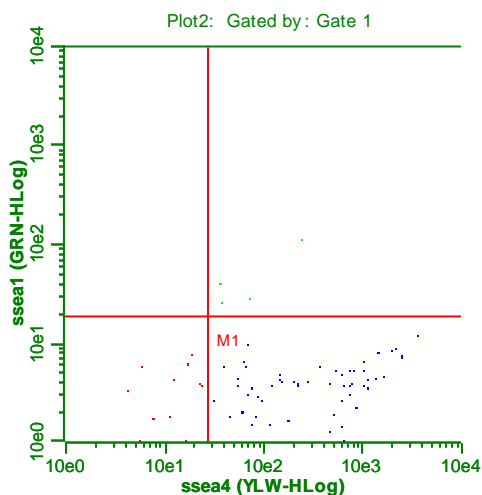


Figure 3A: Scatter plot of SSEA4 and SSEA1 double stained iPS cells.

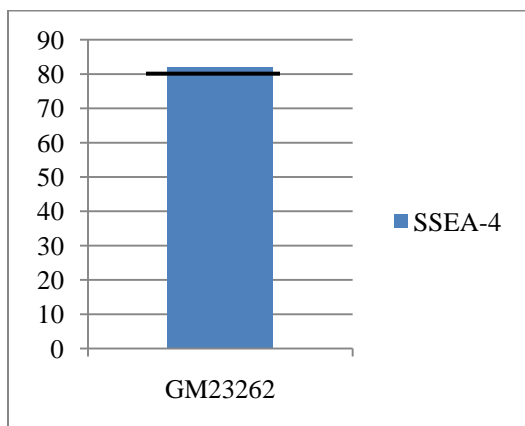


Figure 3B. Graph depicting percent SSEA4 positive cells in an undifferentiated cell culture.

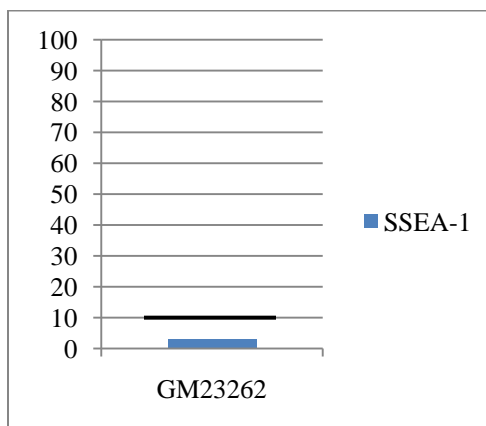


Figure 3C. Graph depicting percent SSEA1 positive cells in undifferentiated cell culture

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

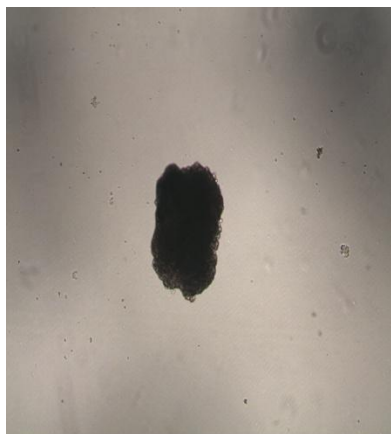


Figure 4A. Image of Embryoid Bodies, day 4

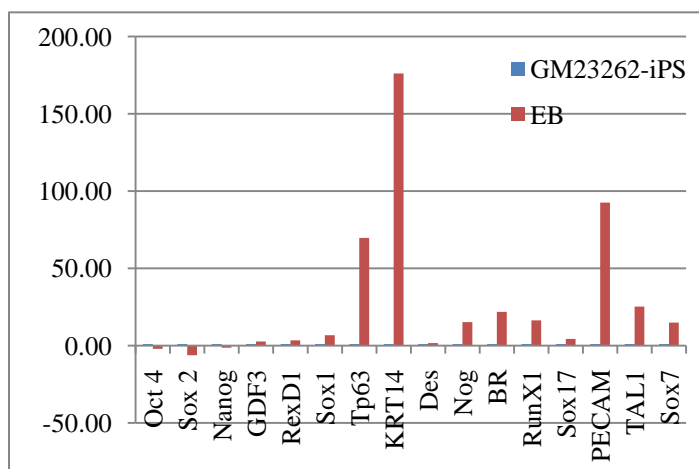


Figure 4B. Gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

	Oct 4	Sox 2	Nanog	Sox1	Tp63	KRT14	Des	Nog	BR	AFP	RunX 1	Sox17	PECAM	TAL1	Sox7
GM23262 iPS	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GM23262 EB	-2	-6	-1	7	70	176	2	15	22	2.E+07	16	4	93	25	15

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

Neural Differentiation

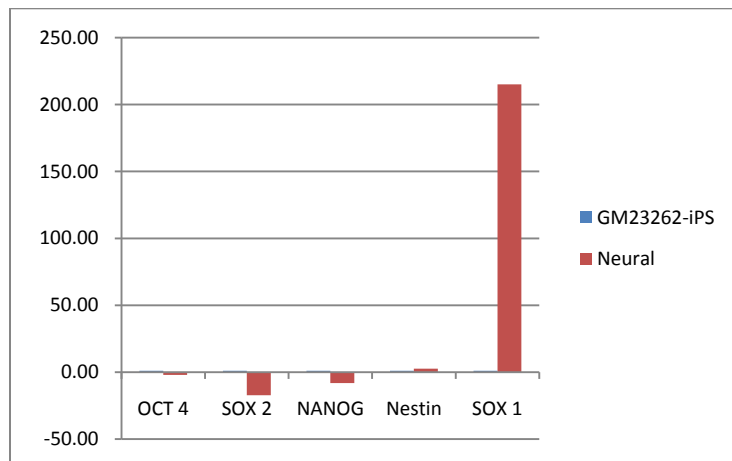


Figure 5. Gene expression following neuronal differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Cardiac Differentiation

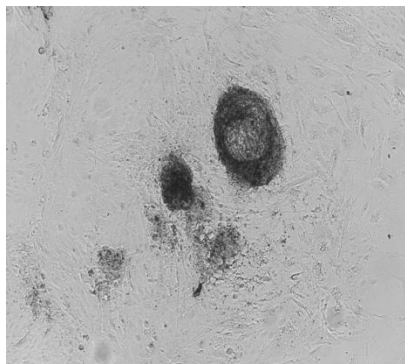


Figure 6A. Image of differentiated colony. Beating was observed.

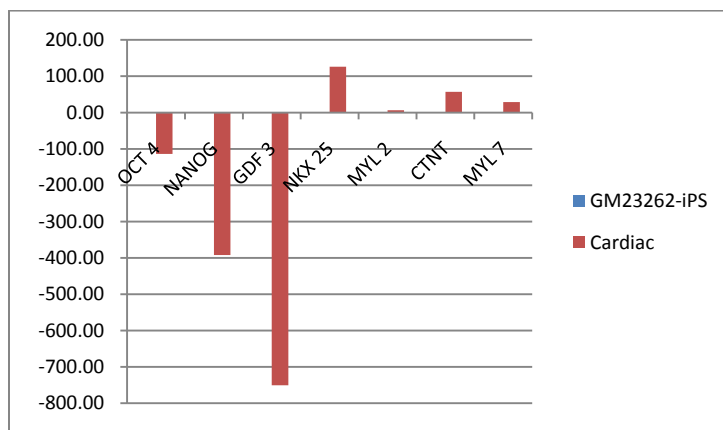


Figure 6B. Gene expression following cardiac differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

Pancreatic Differentiation

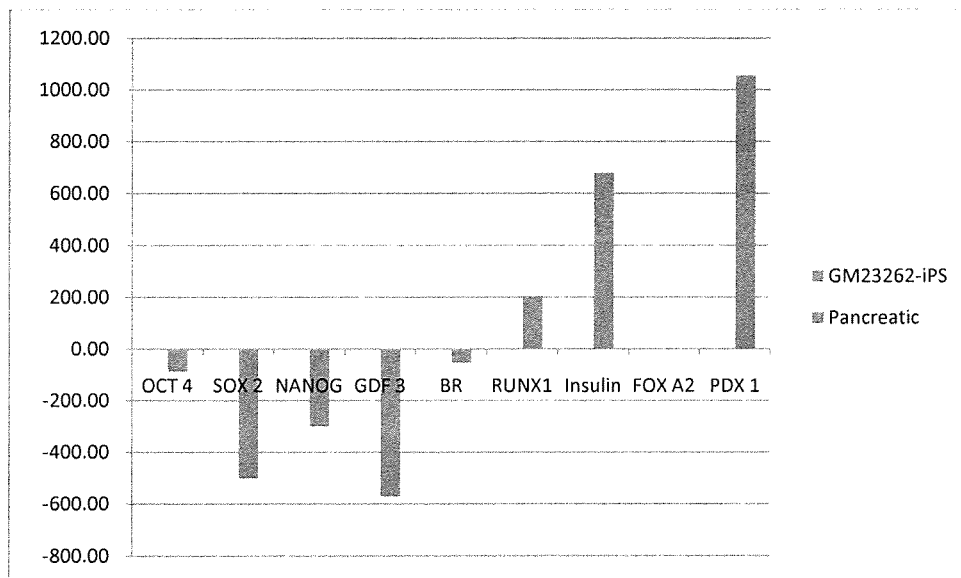


Figure 7. Gene expression following pancreatic differentiation. Fold difference is shown relative to undifferentiated iPS cell line. Insulin production and release was confirmed by ELISA.

☒ Pass
☐ Fail
☐ Other


Margaret A. Keller, Director Stem Cell Biobank

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: GM23262A, P6

Feeder layer: MEF, 1 million cells per 10cm²

Mouse type: Fox Chase SICD-beige, male, 6 week old, from Charles River

Kidney injection: 4 to 6 Million/site, in 30% Matrigel

Mice monitoring: Nov 29, 2010 – Feb 1, 2011, monitor 2-3 times/week

Tumor tissue harvested: Four kidney tumors on Jan 17, 2011 (day 50) and two kidney tumors on Feb 1, 2011 (day 64), 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)

12 H&E slides

Report date: Feb 13, 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 pictures



Two kidney tumors harvested on day 50 after injection



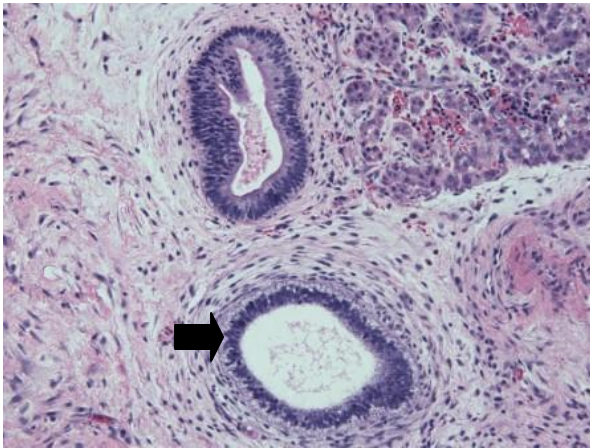
Two kidney tumors harvested on day 50 after injection



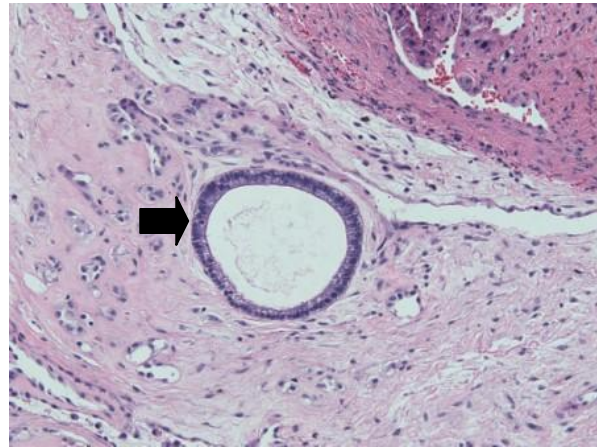
Two kidney tumors harvested on day 64 after injection

H&E staining result:

Endoderm:

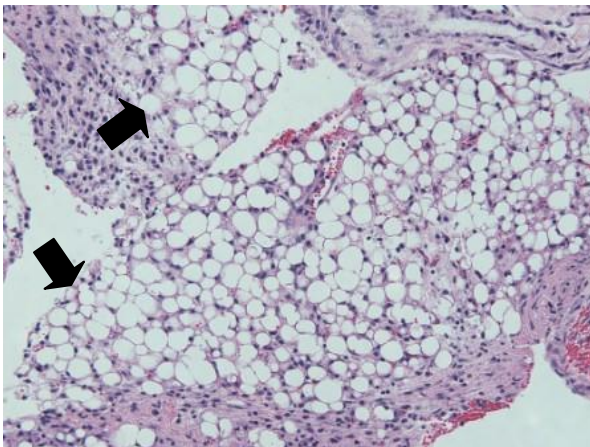


Gland (200x)

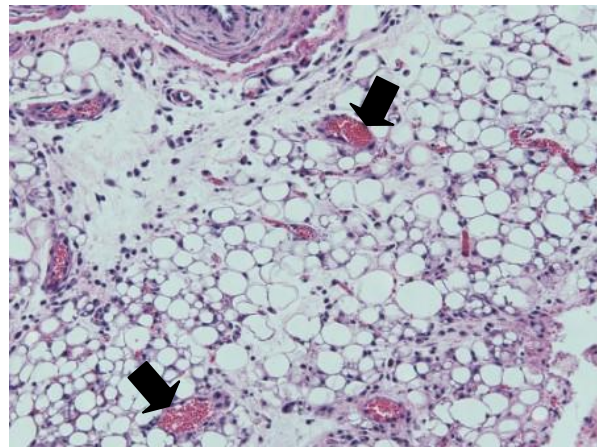


Gland (200x)

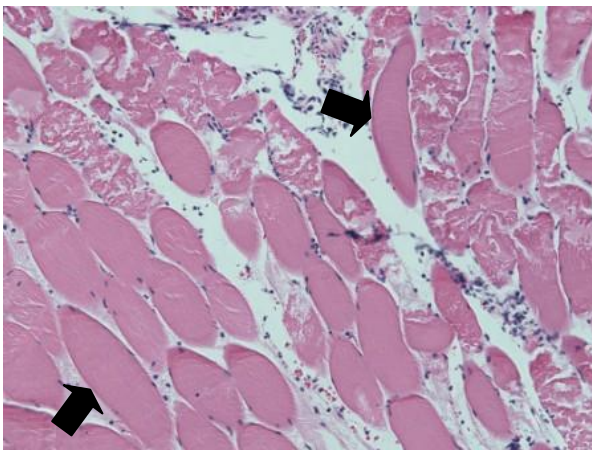
Mesoderm:



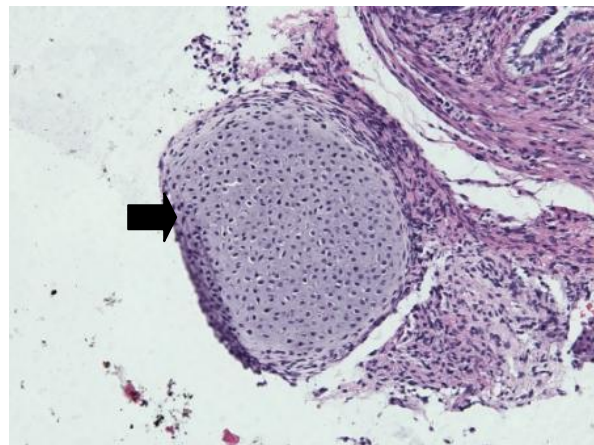
Adipose tissue (200x)



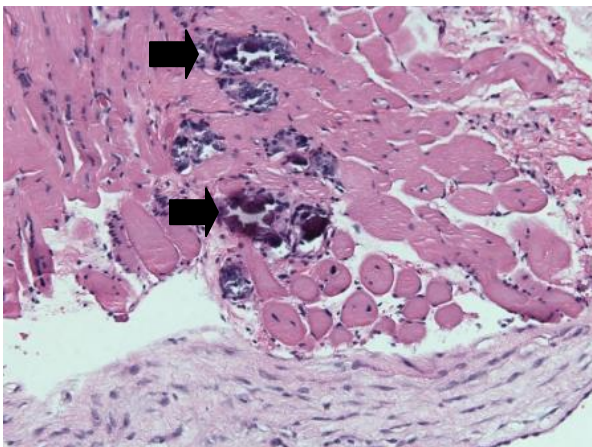
Blood vessel (200x)



Skeletal muscle (200x)

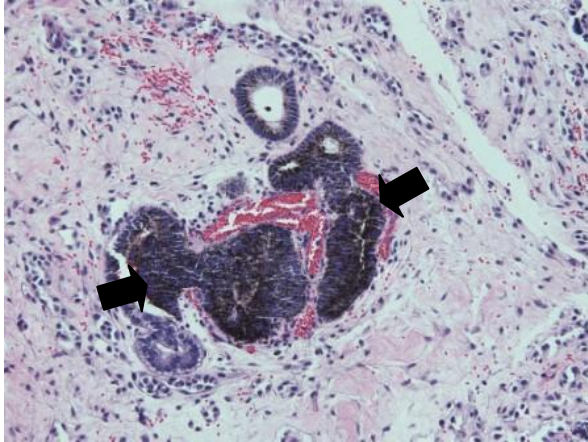


Cartilage (200x)

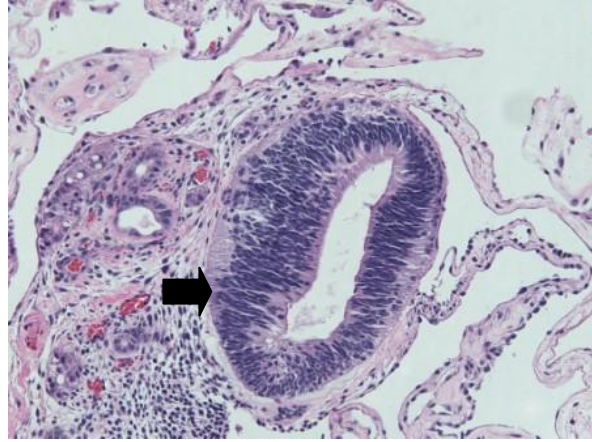


Bone (200x)

Ectoderm:



Melanocyte (200x)



Neuron (200x)

Summary

Four kidney tumors harvested on day 50 and two kidney tumors on day 64 are composed of scattered regions of differentiated cells and a population of undifferentiated neoplastic cells. The tumors derived from all three germ layers are observed in the histology slides. 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.