# GM23226*A

## Certificate of Analysis

| | | |
|---|---|---|
| Product Description | Human fibroblast line 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 | **GM02416** | Fibroblast |
| Diagnosis | DIABETES MELLITUS, JUVENILE-ONSET INSULIN-DEPENDENT; IDDM | |
| Fibroblast Freeze Passage | 3 | |
| Submitted Passage | 11 | |
| Freeze Passage (after recovery) | 9 | |
| Media | DMEM/F12 + 20% KOSR + 10 ng/ml bFGF | |
| Feeder | Yes, 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 | 46 XX |
| Identity Match | TR (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<br>< 10% expression of SSEA-1 | Pass |
| Pluripotency | In vitro differentiation (cardiac, pancreatic and neuronal) | Expression of differentiation 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 5 days) |
|---|---|---|
| 5 | 165.8 microns | 642.8 microns |

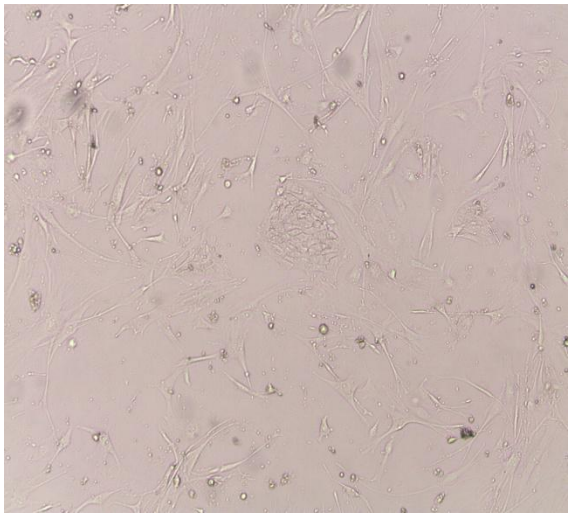


Figure 1A. Colony observed post thaw

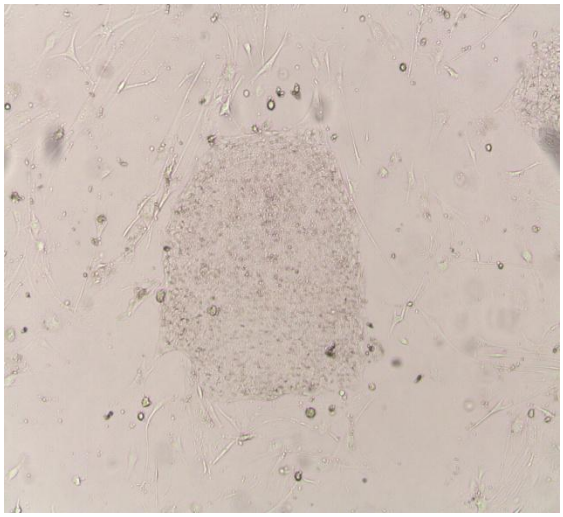


Figure 1B. Colony 5 days after first observation

## Karyotype Analysis

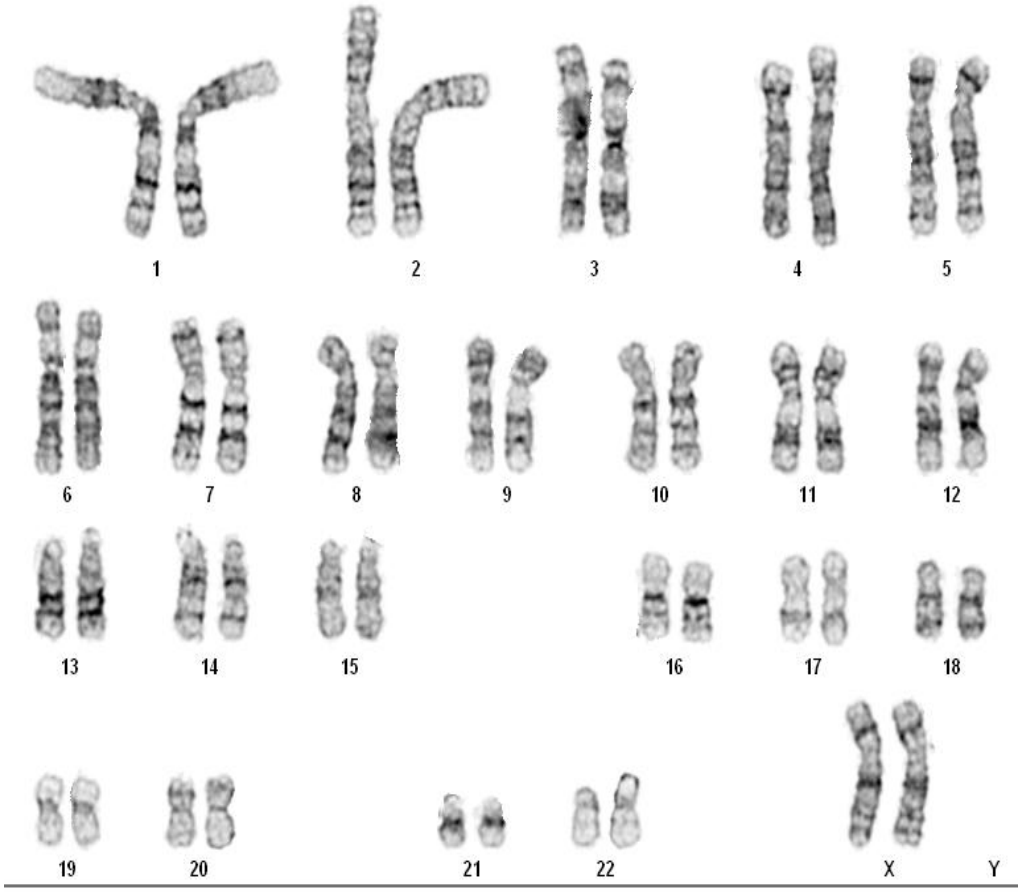



Figure 2: G-banded karyotype showing 46,XX

## 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.

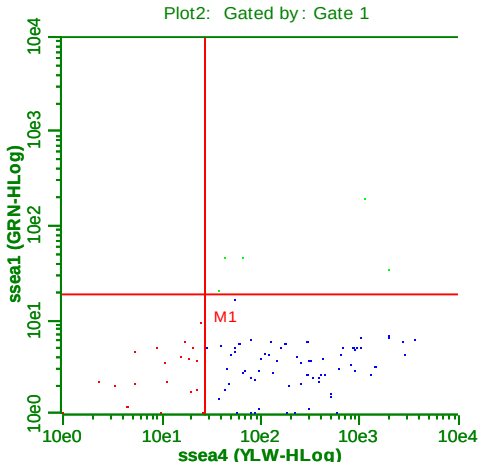



Figure 3A: Scatter plot of SSEA4 and SSEA1 double stained iPS cells.

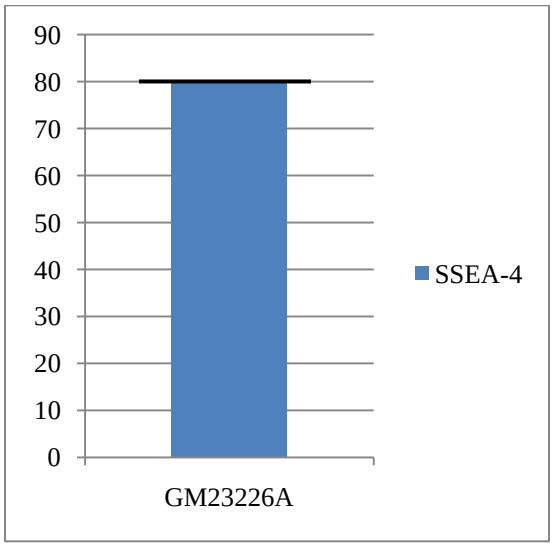



Figure 3B. Graph depicting percent SSEA4 positive cells in undifferentiated cell culture.

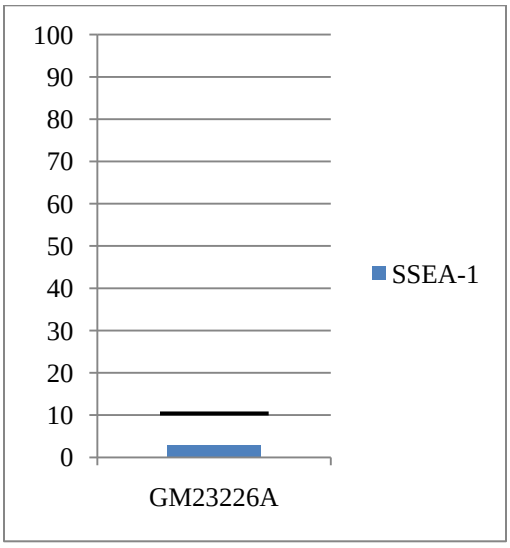



Figure 3C. Graph depicting percent SSEA1 positive cells in undifferentiated cell culture

## 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 compared to undifferentiated cells.

## Embryoid Body (EB) Formation Assay

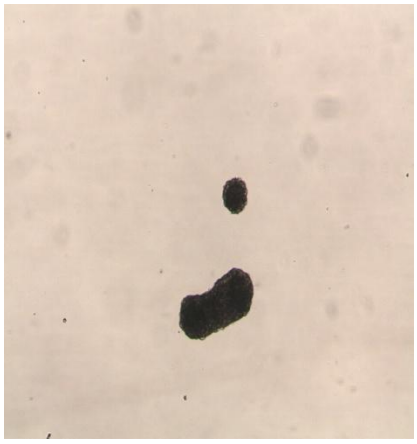


Figure 4A. Image of Embryoid Bodies, day 4

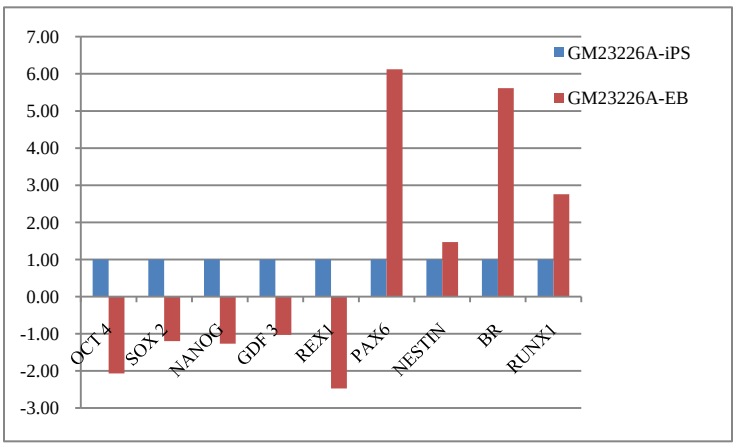



Figure 4B. Gene expression following EB formation. Fold difference is shown relative to undifferentiated iPS cell line.

| | OCT 4 | SOX 2 | NANO G | GDF 3 | REX 1 | PAX 6 | NESTI N | BR | RUNX 1 | AFP | SOX1 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GM23226A undifferentiated | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.0 0 | 1.00 | 1.0 0 | 1.00 | 1.00 | 1.00 |
| GM23226A EB | - 2.07 | - 1.20 | -1.27 | - 1.03 | - 2.47 | 6.1 2 | 1.47 | 5.6 2 | 2.76 | 288868. 56 | -2.14 |

Table 1. Fold difference values of gene expression in EB relative to in undifferentiated cells.

## Neural Differentiation

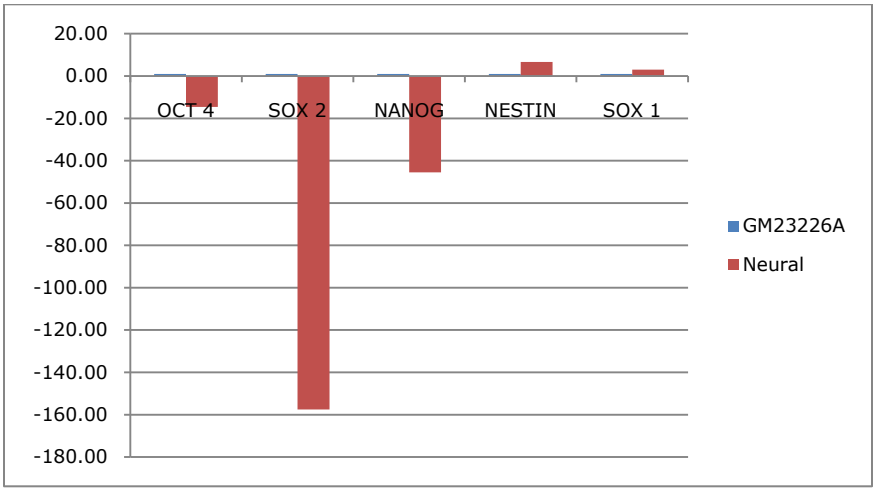



Figure 5. Gene expression following neuronal differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

## Cardiac Differentiation

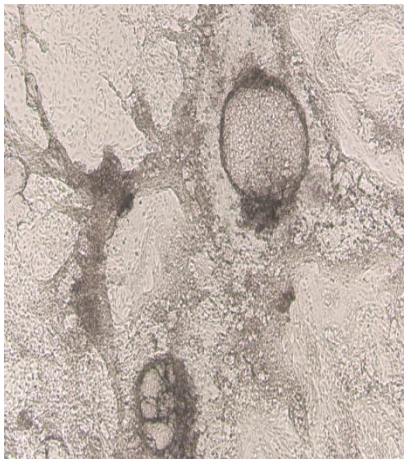


Figure 6A. Image of differentiated colony. Beating was observed.

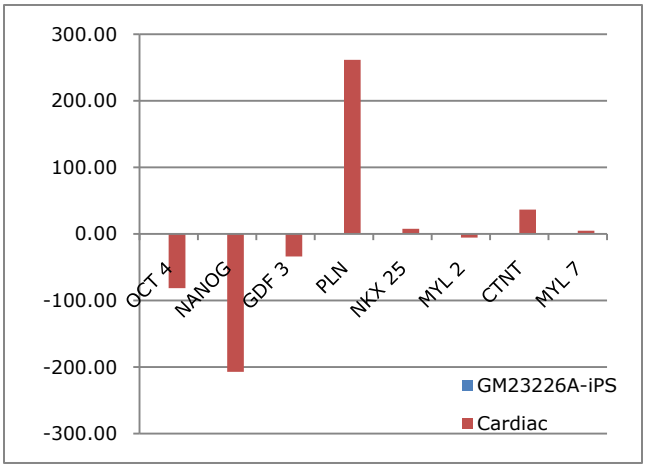



Figure 6B. Gene expression following cardiac differentiation. Fold difference is shown relative to undifferentiated iPS cell line.

## Pancreatic Differentiation

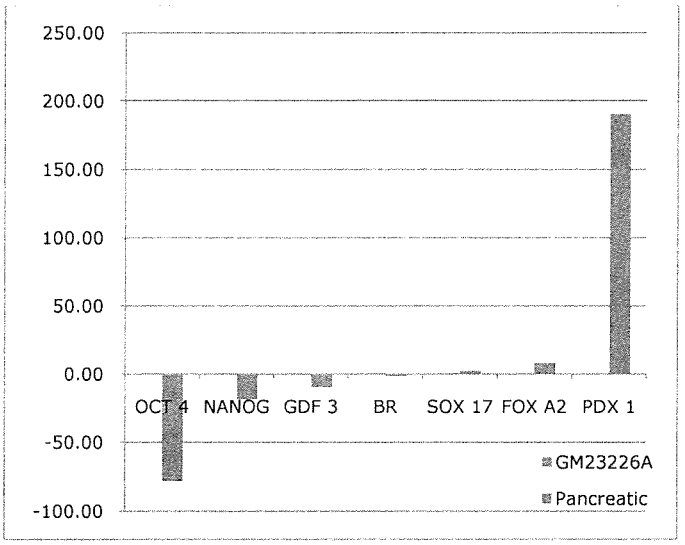



Figure 7. Gene expression following pancreatic differentiation. Fold difference is shown relative to undifferentiated iPS cell line. Insulin production and release were detected by ELISA.

☑ Pass
☐ Fail
☐ Other: _____

Margaret A. Keller, PhD
Director, Stem Cell Biobank
March 28, 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: GM23226A, P4 and P8
Feeder layer: MEF, 1 million cells per $10cm^2$
Mouse type: Fox Chase SICD-beige, male, 6 week old, from Charles River
Injection sites: 4 kidney capsules and 1 testis
Cell concentration: 2 or 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: January 26, 2011 (day 45) and February 7, 2011 (day 57), March 2, 2011 (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 21, 2011
Project manager: Qi Zheng
Contact person: Esther Tang

## H&E Histology Instruction

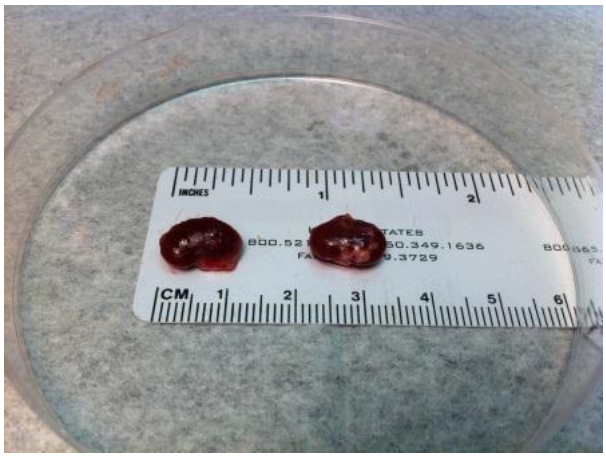
Wheater's Functional Histology (B. Young and J.W. Heath), 4th edition
Three embryonic germ cell layers: endoderm, mesoderm and ectoderm
- Endoderm: digestive system (includes liver and pancreas), respiratory system, most glands
- Mesoderm: muscle, blood vessels, much of the genital-urinary system, skeletal 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**

Two kidney tumors harvested on day 45 after injection

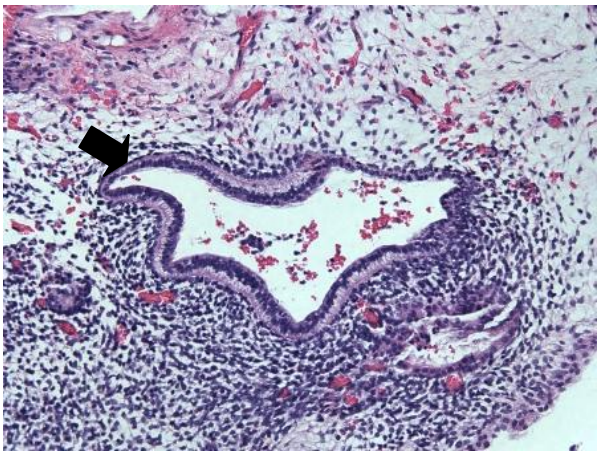
One testis tumor harvested on day 57 after injection



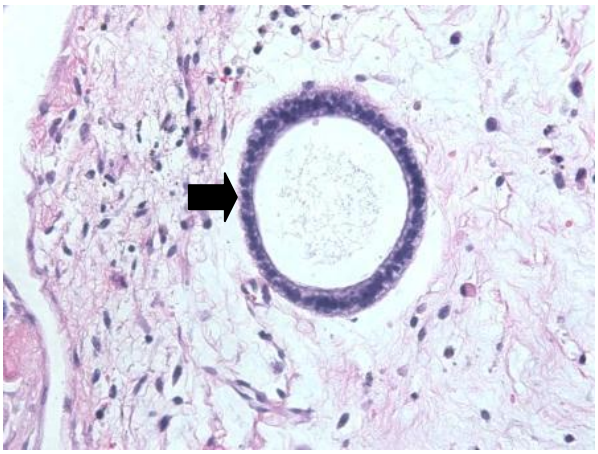
Two kidneys without tumor formation harvested on day 98 after injection

**H&E staining result of kidney tumor:**

Endoderm:

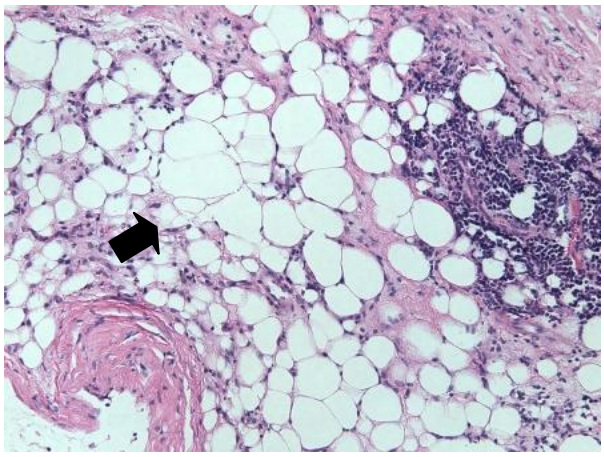


Gland (200x)

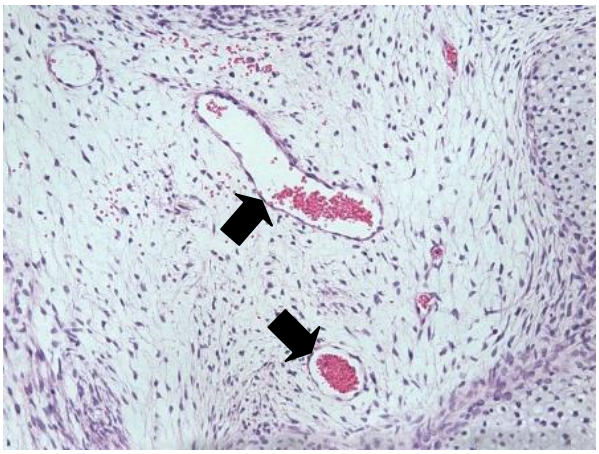


Duct (200x)

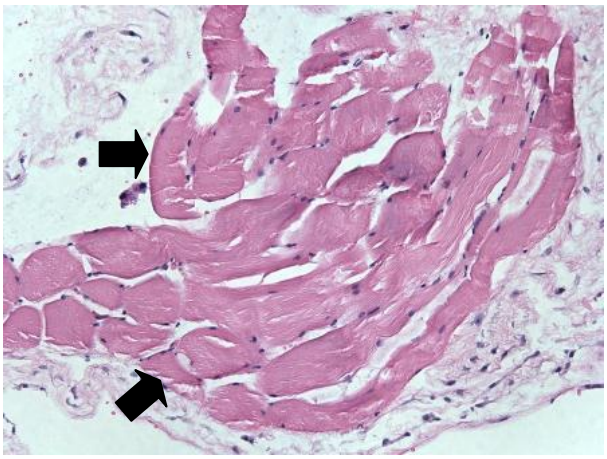
Mesoderm:



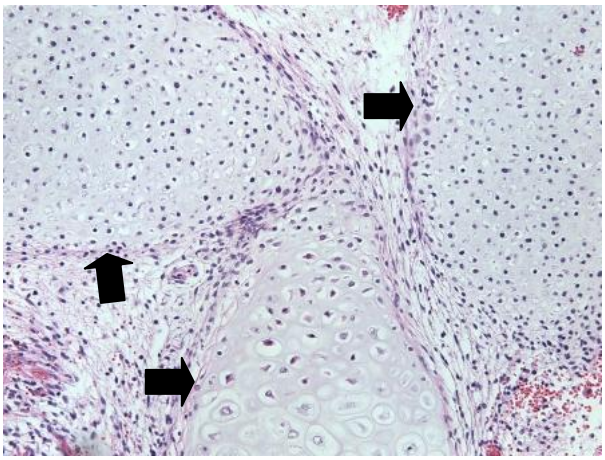
White adipose tissue (200x)



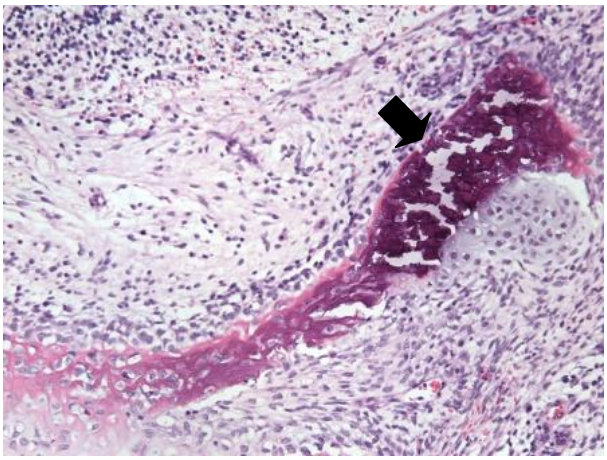
Blood vessel (200x)



Skeletal muscle (200x)

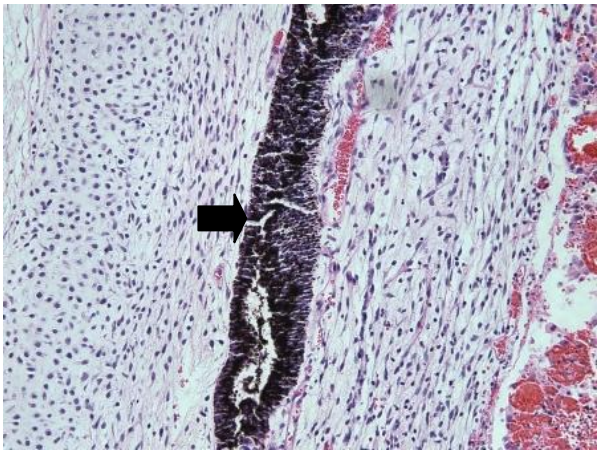


Cartilage (200x)

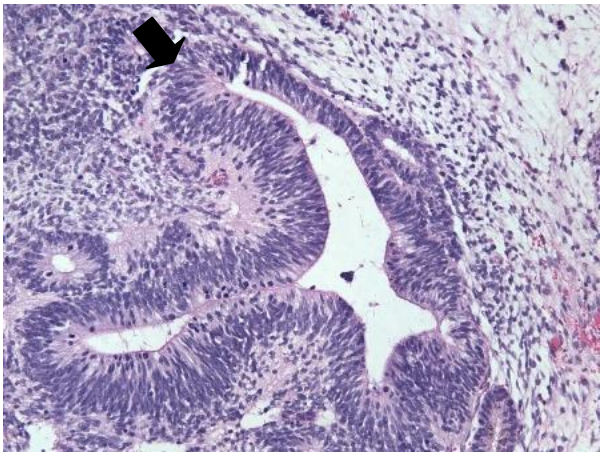


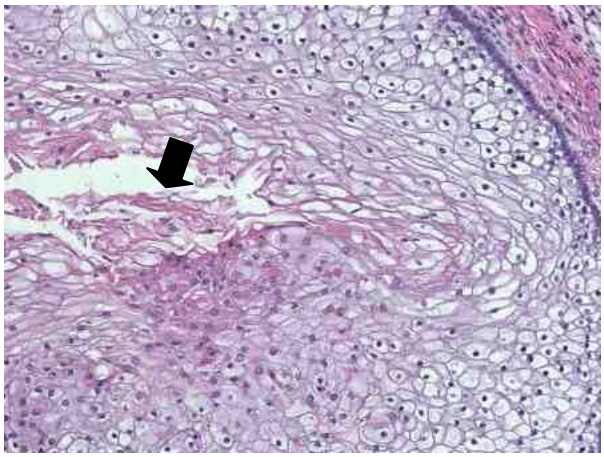
Bone (200x)

Ectoderm:



Melanocyte (200x)





Stratified squamous epithelium (200x)

**Summary**

Two kidney tumors harvested on day 45 and one testis tumor on day 57 are composed of scattered regions of differentiated cells and a population of undifferentiated neoplastic cells. 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.