MISFISHIE Example 1

Revised: 2008-08-14

This document is a MISFISHIE-compliant dataset example.

1. Experiment Design:

- A. Experiment Description: An immunohistochemistry comparison of antibodies CD49a and CD90 in 4 different normal human prostate tissue sections.
- B. Assay Type(s): Immunohistochemistry
- C. Experiment Type: Comparison of multiple tissue specimens and antibodies
- D. Experimental factors: antibody; specimen
- E. Total number of assays performed: 4
- F. URL for more information: http://scgap.systemsbiology.net/data/
- G. Contact information: Alvin Liu, Institute for Systems Biology, 1441 N 34th St, Seattle WA 98103 (aliu systemsbiology.org)

2. Biomaterials and Treatments:

- A.i. Attributes of the individuals

Specimen Designator	Tissue Type	Organism	Sex	Age
99-010E	Normal Prostate Glands	Homo sapiens	Male	55-65
99-047D	Normal Prostate Glands	Homo sapiens	Male	65-75
99-066C	Normal Prostate Glands	Homo sapiens	Male	45-55
99-068D	Normal Prostate Glands	Homo sapiens	Male	55-65

- A.ii. Physiological state: Normal
- A.iii. Relevant exogenous factors: None
- A.iv. Anatomic source of specimens: Human prostate
- A.v. Provider of the specimens: All specimens obtained from the University of Washington Medical Center:
- B. Physiological state: normal
- Assay preparation protocol: Upon receipt of the radical prostatectomy specimen 3 mm thick transverse sections are made after inking the exterior surface (the surgical margin). Tissue blocks from the posterior aspect of each alternate transverse section are embedded in OCT that is immersed in isopentane that is precooled in liquid nitrogen. This procedure, which takes 10 to 20 minutes, maximizes the rate of cooling of the tissue. The frozen tissue blocks are assigned an anonymized code. To assess the feasibility of sorting cancer cell populations by flow cytometry, only tumors with a relatively large volume (approximately 1 cc) in a single focus are used. Tissue enriched for cancer, where at least 85% of the cells in the corresponding frozen section are cancer cells and the tissue sample weighs at least 100 mg, is dissected from the opposing aspect of the non-fixed section that is adjacent to the block of tissue that was frozen. For immunohistochemistry, multiple 5-micron thin serial sections are cut and fixed in cold acetone for 10 minutes. After air drying these are kept in a -20C freezer until used for immunostaining.

3. Reporter information:

- A. Unambiguous reporter identification: See table below
- B. Full sequence or clone if of the reporters: See table below
- C. Protocol for obtaining exaect reporters: Monoclonal antibodies purchased from BD Pharmingen http://www.bdbiosciences.com/pharmingen/
- D. Other important attributes: Monoclonal

Antibody Name	Ref DB	Accession	Origin	Vendor Details
CD49a	Entrez Gene	3672	BD Pharmingen	http://www.bdbiosciences.com/external_files/pm/doc/tds/ihc/live/web_enabled/75311E_550568.pdf
CD90	Entrez Gene	7070	BD Pharmingen	http://www.bdbiosciences.com/external_files/pm/doc/tds/ihc/live/web_enabled/74851E_550402.pdf

4. Staining protocols and parameters:

- A. Detection method: See protocol
- B. Staining protocol: 1. Let frozen slides equilibrate at room temperature for 15 min. 2. Fix in cold acetone for 10 min. 3. Rinse with PBS [10X = 1.3M NaCl (38g), 20mM KCl (0.75g), 80mM Na2HPO4 (5.7g), 10mM KH2PO4 (0.68G) pH 7.4 w/NaOH in 500mL]. 4. Quench in H2O2, 1uL stock 30% H2O2 in 1mL PBS, 15min. 5. Rinse with PBS 6. Add blocking

solution, 200uL, 30min. 7. Rinse with PBS. 8. Add primary antibody 0.4ug in 50uL 0.1% BSA-PBS. 30 min. 9. Rinse with PBS. 10. Add secondary antibody 1uL biotinylated anti-mouse Ig + 180uL PBS + 20uL horse serum, 100uL per slide, 30 min. 11. Rinse with PBS. 12. Make Vector detection solution 30 min before use, 5uL bottle A + 5uL bottle B + 500uL PBS, 30 min, then dilute with 2mL PBS. 13. Add Vector ABC, 30 min. 14. Rinse with PBS. 15. Add DAB solution (aliquot 0.5mL from -20C 5 min before use), <8 min. 16. Rinse in H2O. 17. Counterstain 30 sec in hematoxylin. 18. Rinse in H2O. 19. 5 sec in bluing. 20. rinses (10 dips) in H2O, 95% EtOH (2X), 100% EtOH (3X), xylene (2X). 21. Mount with coverslip.

Slide Designator	Specimen Designator	Number of Antibodies	Antibodies
CD90 99-010E 1	99-010E	1	CD90
CD90 99-047D 1	99-047D	1	CD90
CD49a 99-066C 1	99-066C	1	CD49a
CD49a 99-068D 1	99-068D	1	CD49a

5. Imaging data and parameters:

- A. Digital images for each assay: See table below
- B. Detection Method: See full protocol below
- C. Image acquisition protocol: 1. Turn on power to the digital camera (Spot Insight Color, Model 3.2.0., No. 206510) and microscope (B Olympus BH2 Compound Microscope, No. 4D02712). 2. Open "SpotAdvanced" imaging application (Ver 3.5.2) from the desktop (the software will warn you if the camera is not on. If the camera is on, a LIVE window will pop up). 3. Place slide under scope to determine its tissue type (labeling on the slide itself will most often disclose the tissue type, but it is best to make sure). 4. Examine specimen under low magnification to get a feel for which cell types were captured and which are visible. 5. Position slide so that multiple cell types (or a specific cell type if you're looking for a particular type) are visible through the eye piece. Compare this image with that in the Spot LIVE window, and focus the scope according to the live image. 6. Use the "SNAP" button in the Spot application window to capture images. 7. Take both low and high magnification images of cell types of interest. Typically, one image on low (40x) magnification, and three to five images on higher (100,200,400x) magnification will suffice. (The magnifications are the objective power multiplied by the eyepiece, which is typically 10x). Use your judgement and capture images of interesting cell types: for example, intensely stained cells, lightly-stained cells, cells that are not stained, areas that contain cancer, take high mag images of areas that are indistinguishable under low mag, etc. 8. Save image according to the "Immunostain Image Filename Convention", to a folder titled "IMAGES TO EDIT", so that the images may be selected at a later time to crop or edit in Adobe Illustrator. 9. Once all images have been taken for a particular slide, remove the slide from the scope and use a marking pen to write "IS" for "image saved" on the slide itself. This way, one will be sure not to take images of the same slide twice. 10. When finished, exit Spot and turn off the microscope and camera.

Image Designator	Slide Designator	Magnification	Image
CD90 99-010E HP a- 40	CD90 99-010E 1	40	IMAGE URL
CD90 99-047D HP a- 40	CD90 99-047D 1	40	IMAGE URL
CD49a 99-066C HP a- 40	CD49a 99-066C 1	40	IMAGE URL
CD49a 99-068D HP a- 40	CD49a 99-068D 1	40	IMAGE URL

6. Image Characterizations:

- A. Definition of structural units:

Structural Unit	Definition
Atrophic glands	Benign glands lined by an apparent single layer of predominantly basal cells that form a flat epithelium.
Hyperplastic glands	Glands that exhibit luminal cell hyperplasia forming micropapillary protrusions into gland.
Normal glands	Neither atrophic nor hyperplastic glands.
Basal Epithelial Cells	A layer of small, often inconspicuous epithelial cells adjacent to the basement membrane and subjacent to the luminal/secretory epithelial cells. The basal cells are the presumed progenitor cells of the prostate epithelium.
Stromal Fibromuscular Cells	The spindle cells that comprise the interstitium in which the prostate ducts and glands are embedded.
Stromal Endothelial Cells	The cells that line the lumen of all blood vessels.
Stromal Perineural Cells	The outermost layer of stromal cells that surround peripheral nerves
Stromal Nerve Sheath Cells	Spindle cells that surround axonal processes of nerves.

See also: http://scgap.systemsbiology.net/ontology/Glossary.php

- B. Definition of intensity scale:

Intensity Unit	Definition
Intense	Immunoreaction deposit is distinctly more optically dense than background and than tissue that does not express the antigen.
Equivocal	Immunoreaction deposit is either similar enough in optical density to the background and/or to tissue that does not express the antigen, or is so focal, i.e. < 5% of cells, that there is reasonable uncertain regarding whether the cells express the antigen.
None	There is either no immunoreaction deposit or reaction product is no more optically dense than background.

- C. Characterization of results in tabular form:

Stain Name	Antibody Name	Structural Unit	Intense Staining Percent	Equivocal Staining Percent	None Staining Percent
CD49a 99-066C 1	CD49a	Atrophic glands	0	0	100
CD49a 99-066C 1	CD49a	Normal glands	0	0	100
CD49a 99-066C 1	CD49a	Hyperplastic glands	0	0	100
CD49a 99-066C 1	CD49a	Basal Epithelial Cells	0	0	100
CD49a 99-066C 1	CD49a	Stromal Fibromuscular Cells	95	5	0
CD49a 99-066C 1	CD49a	Stromal Endothelial Cells	0	0	100
CD49a 99-066C 1	CD49a	Stromal Perineural Cells	0	50	50
CD49a 99-066C 1	CD49a	Stromal Nerve Sheath Cells	0	50	50
CD49a 99-066C 1	CD49a	Gleason Pattern 4	0	0	100
CD49a 99-066C 1	CD49a	Gleason Pattern 3	0	0	100
CD49a 99-068D 1	CD49a	Atrophic glands	0	0	100
CD49a 99-068D 1	CD49a	Normal glands	0	0	100
CD49a 99-068D 1	CD49a	Hyperplastic glands	0	0	100
CD49a 99-068D 1	CD49a	Basal Epithelial Cells	0	0	100
CD49a 99-068D 1	CD49a	Stromal Fibromuscular Cells	70	30	0
CD49a 99-068D 1	CD49a	Stromal Endothelial Cells	0	0	100
CD49a 99-068D 1	CD49a	Stromal Perineural Cells	0	0	100
CD49a 99-068D 1	CD49a	Stromal Nerve Sheath Cells	0	0	100
CD49a 99-068D 1	CD49a	Gleason Pattern 4	0	0	100
CD49a 99-068D 1	CD49a	Gleason Pattern 3	0	0	100
CD90 99-010E 1	CD90	Atrophic glands	0	0	100
CD90 99-010E 1	CD90	Normal glands	0	0	100
CD90 99-010E 1	CD90	Hyperplastic glands	0	0	100
CD90 99-010E 1	CD90	Basal Epithelial Cells	0	0	100
CD90 99-010E 1	CD90	Stromal Fibromuscular Cells	10	0	90
CD90 99-010E 1	CD90	Stromal Endothelial Cells	0	0	100
CD90 99-010E 1	CD90	Stromal Perineural Cells	0	0	100
CD90 99-010E 1	CD90	Stromal Nerve Sheath Cells	95	5	0
CD90 99-010E 1	CD90	Gleason Pattern 3	0	0	100
CD90 99-047D 1	CD90	Atrophic glands	0	0	100
CD90 99-047D 1	CD90	Normal glands	0	0	100
CD90 99-047D 1	CD90	Hyperplastic glands	0	0	100
CD90 99-047D 1	CD90	Basal Epithelial Cells	0	0	100
CD90 99-047D 1	CD90	Stromal Fibromuscular Cells	10	0	90
CD90 99-047D 1	CD90	Stromal Endothelial Cells	0	0	100

CD90 99-047D 1	CD90	Stromal Perineural Cells	0	0	100
CD90 99-047D 1	CD90	Stromal Nerve Sheath Cells	95	5	0
CD90 99-047D 1	CD90	Gleason Pattern 4	0	0	100
CD90 99-047D 1	CD90	Gleason Pattern 3	0	0	100