Assessing the Quality of Tissue

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I. Standards for assessing preservation of macromolecules (RNA, protein) in tissue

II. A specification for tissue localization experiments (MISFISHIE)

NCI Biospecimen Best Practices Forums
Seattle, WA, January 28, 2008

Lifecycle of a Biospecimen

Factors potentially having significant effect on gene expression

Uncontrollable variables

Time 0

Controllable variables

Patient
Age
Diet

Surgery
Ischemia
Anesthetic





Patient

Medical/ Surgical Procedures

Tissue Acquisition

Handling/ Processing

Storage

Pre-acquisition

Post-acquisition

Time 0

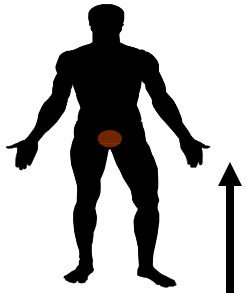
Design of experiments to evaluate effect of variables on gene expression

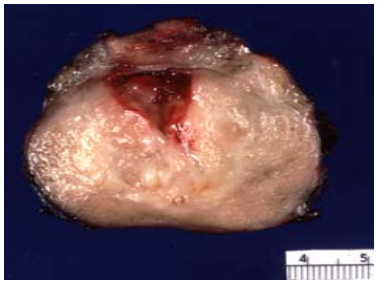
Patient
Age
Diet

External factors
Temperature
Time of day

Surgery
Tissue ischemia
Anesthetic

Tissue handling
Time on bench
Storage
Laser capture

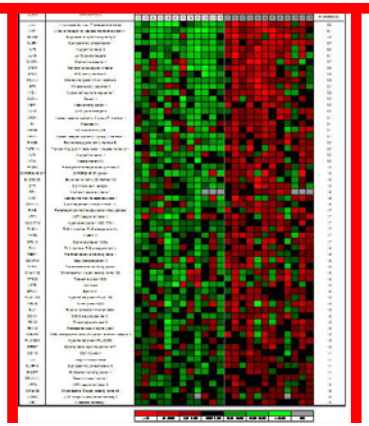




Prostate biopsies
Diagnosis:
Cancer

Prostate biopsies
Gene expression profile

Comparative gene expression analysis



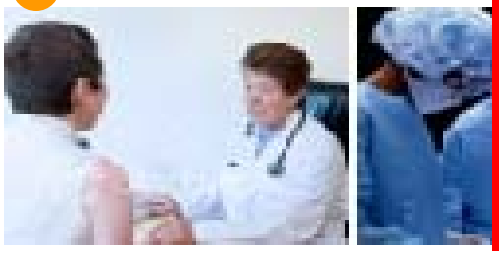
Prostate biopsies
Gene expression profile

Expression profiling
Lab of Peter Nelson, MD

Effect of 6 weeks of high-caloric diet

Uncontrollable variables → **Time 0**

Patient
Age
Diet



HUGO	NAME	PRE-DIET 1	PRE-DIET 2	PRE-DIET 3	PRE-DIET 4	POST-DIET 1	POST-DIET 2	POST-DIET 3	POST-DIET 4	AVERAGE RELATIVE EXPRESSION
MMP7	Matrix metalloproteinase 7									13.1
OLFM4	Olfactomedin 4									6.9
BF	B-factor properdin									6.4
IGF-2R	Insulin-like growth factor-2 receptor									3.5
VMP1	Likely ortholog of rat vacuole membrane protein 1									3.3
TGFB1I4	Transforming growth factor beta 1 induced transcript 4									3.2
IER3	Immediate early response 3									3.2
CCT2	Chaperonin containing TCP1, subunit 2 (beta)									3.1
HLA-DRB5	Major histocompatibility complex class II DR beta 4									3.1
CXCR4	Chemokine (C-X-C motif) receptor 4									2.8

Patient | Medical/ Surgical Procedures | Acquisition | Handling/ Processing | Storage

Pre-acquisition | **Post-acquisition**

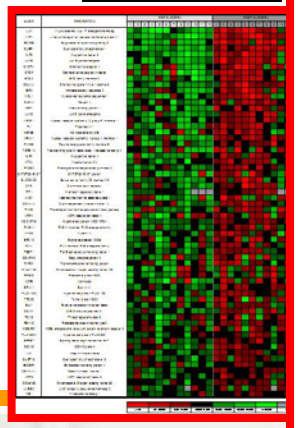
Time 0

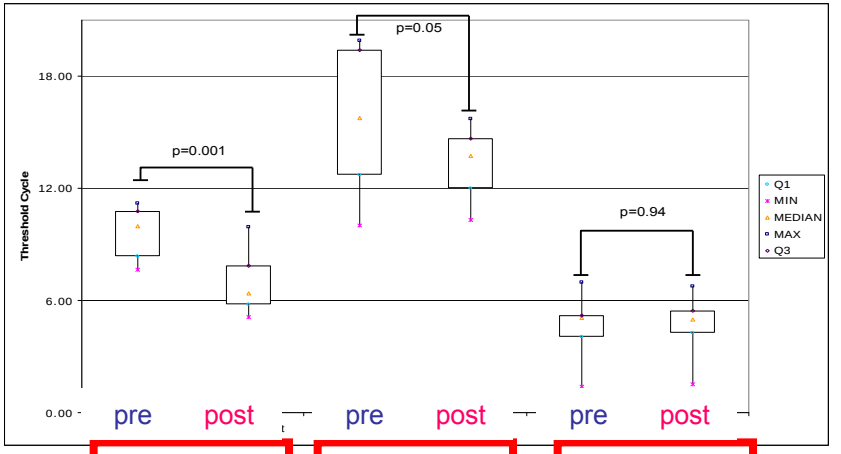
Lin, D. et al. Cancer Epidemiol Biomarkers Prev 2007;16:2150

Effect of ischemia during surgery on tissue

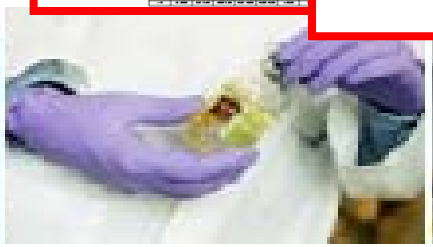
Uncontrollable variables → **Time 0**

Surgery
Ischemia
Anesthetic









Patient | Medical/ Surgical Procedures | Acquisition | Handling/ Processing | Storage

Pre-acquisition | **Post-acquisition**

Time 0

Lin, Coleman, Hawley..JCO '06; 10;24(23):3763-70

Lifecycle of a Biospecimen

Uncontrollable variables

Record the data & develop a molecular "fingerprint"

Time 0

Controllable variables

Optimize handling & processing procedures

Patient
Diet
⇧ MMP7
⇧ IGF2R

Surgery
Ischemia
⇩ DUSP1
⇩ KLF6

Patient | Medical/ Surgical Procedures | Acquisition | Handling/ Processing | Storage

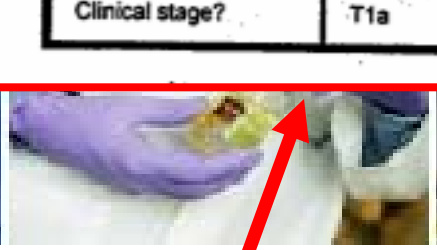
Pre-acquisition

Post-acquisition

Lifecycle of a Biospecimen

Type of surgery	RRP	Laparoscopy	Cystoprostatectomy	
Serum PSA	6.92	———	———	———
Prior therapy (circle)	None	Hormone?	ChemoRx ?	Other
Nerve sparing (circle if yes)	Right ? no	Left ? no	——— ———	——— ———
Times of day tissue	Out of pt	First sample frozen	Last sample frozen	———
Time	6:40	7:15	7:20	———
Nodular parenchyma	None	Rare Multiple	Small Medium	Large
Clinical stage?	T1a	T1b T1c	T2	





Patient

Medical/ Surgical Procedures

Acquisition

Handling/ Processing

Storage

Pre-acquisition

Post-acquisition

Time 0

Factors affecting immunohistochemical stains

Controllable variables

Tissue handling, storage

Antigen retrieval

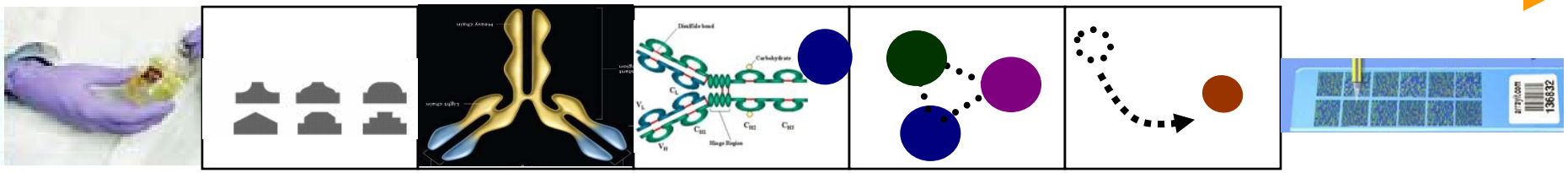
Primary antibody Specificity, affinity, saturation of binding sites

Secondary antibody Specificity, affinity, saturation of binding sites

Avidin-biotin complex Affinity, saturation biotin sites

Chromogen Incubation time, temp., [H2O2), [DAB]

Immuno-stains/ FISH images



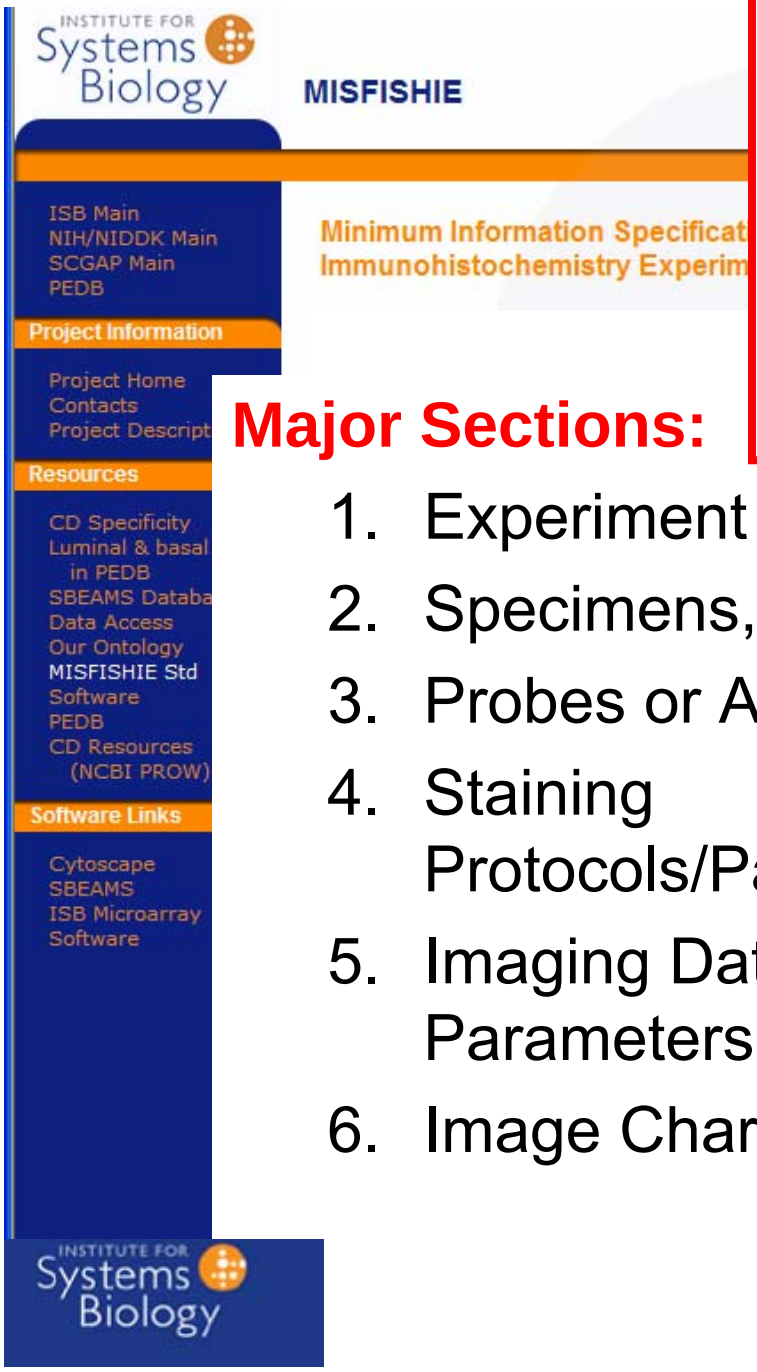
tissue | antigen | 1º Ab | Biotin-2º Ab | ABC complex | DAB + H_2O_2 | Tissue microarray

True LD, Am J Clin Path 1988;90:324

MISFISHIE – *report all data in your experiments*

- Minimum Information Specification For *In Situ* Hybridization and Immunohistochemistry Experiments (MISFISHIE)
- The purpose is to **"Insure that the minimum information that a researcher at a different lab needs to reproduce or evaluate the experiment is provided."**
- MISFISHIE does ***not*** specify the data format, merely ***what*** information must be communicated
- Based on principles of MIAME (specification for reporting expression microarray data)

Deutsch E, et. al. OMICS. 2006;10(2):205-8 (PMID: 16901227); Nature Biotechnology (in press)



Major Sections:

1. Experiment Design
2. Specimens, Treatments
3. Probes or Antibodies
4. Staining Protocols/Parameters
5. Imaging Data and Parameters
6. Image Characterizations

32 articles from:

Am J Clin Path
Am J Surg Path
Cancer Res
J Clin Path
Lab Invest
Modern Path
Molecules & Cells
Nature
Science

Section 3. Reporters (Antibodies, Probes)

Unambiguous reporter identification, ideally genomic
Full sequence or clone id of the reporters
Protocol for obtaining exact reporter (purchase from..., create, etc.)
Other important attributes (e.g., mono- or polyclonal, organism in which antibody generated)

Information not supplied (25% of articles):

Antibody clone numbers and/or catalogue numbers

Conditions of antigen retrieval (buffer, heat duration)

Antibody Name	Locus Link ID	Origin	Vendor Details
CD49a	3672	BD Pharmingen	http://www.bdbiosciences.com/external_files/pm/doc/tds/ihc/live/web_enabled/75311E_550568.pdf
CD90	7070	BD Pharmingen	http://www.bdbiosciences.com/external_files/pm/doc/tds/ihc/live/web_enabled/74851E_550402.pdf

Section 4. Staining

Detection Method (number of reporters, detection reagent & systems)
Staining protocol (enough to reproduce?)
Details about positive and negative controls

Information not supplied (25% of articles):

Was nonspecific protein binding blocked?

Was endogenous peroxidase inhibited?

Negative controls?

http://scgap.systemsbiology.net/resources/protocols.php

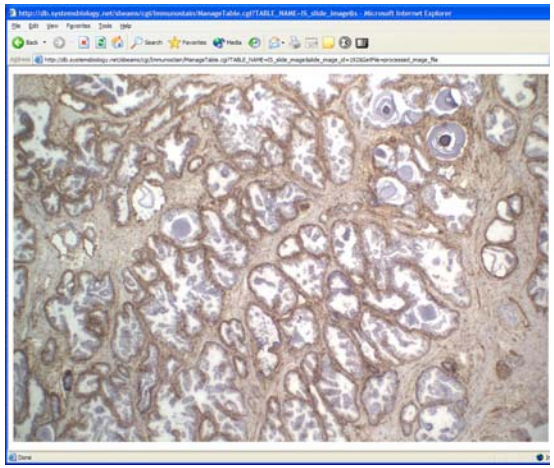
Section 5. Imaging Data

The digital images for each assay (can the images be downloaded to your computer and evaluated ?)

Information not supplied (85+ % of articles):

Images of all specimens (for reader to evaluate for her/himself)

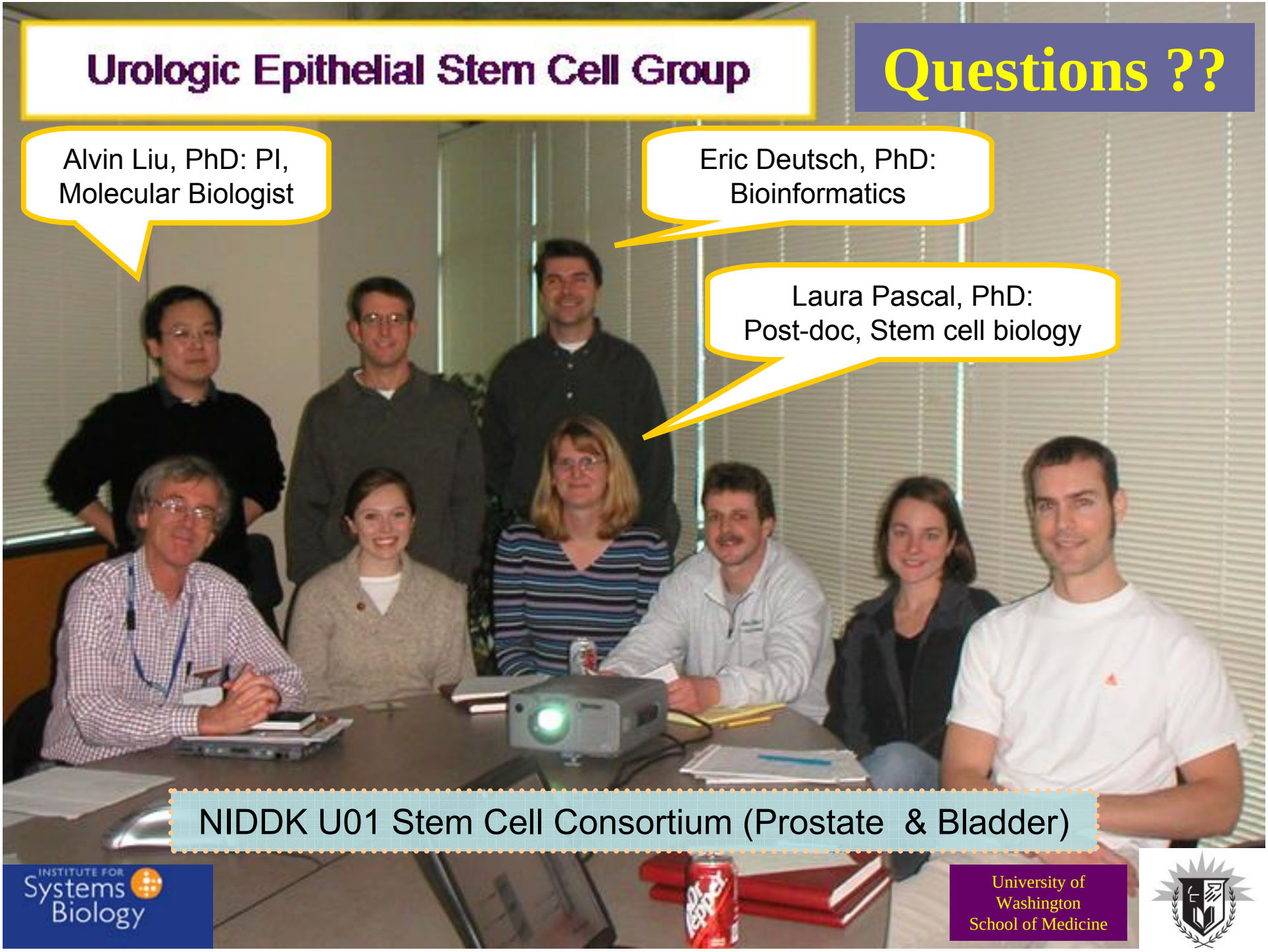
(for example) Was “nonspecific staining” expression of an antigen by cells not expected to stain?



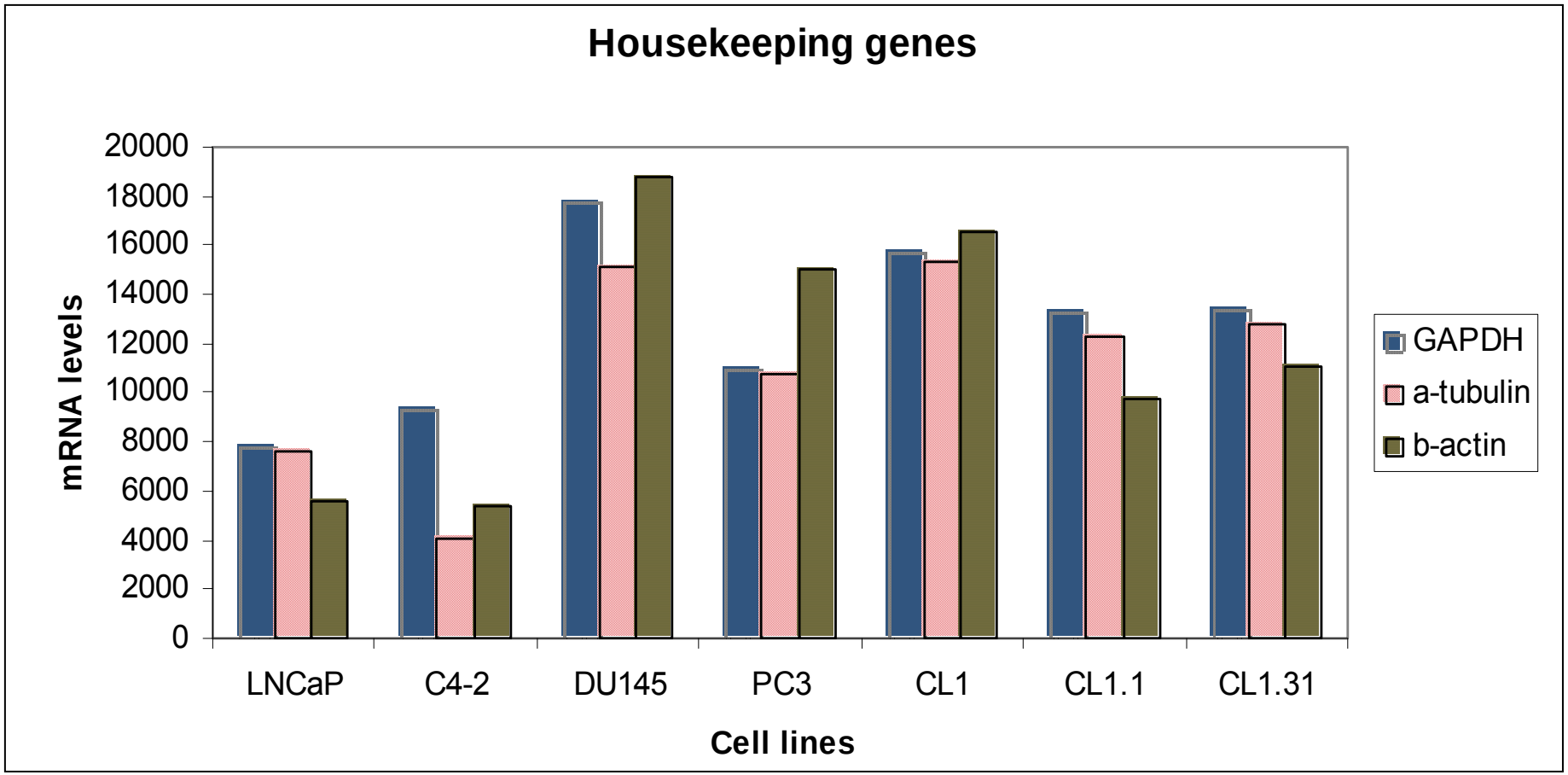
	# Specimens	# Stains	# Images	
Summary by Organism:				
Human	135	687	2222	[Immunostain Summary]
Mouse	11	72	163	[Immunostain Summary]
Summary by Tissue Type:				
Human Bladder	28	315	1025	[Immunostain Summary]
Mouse Bladder	3	24	75	[Immunostain Summary]
Human Prostate	107	372	1197	[Immunostain Summary]
Mouse Prostate	8	48	88	[Immunostain Summary]

Conclusions

- To determine if a molecular profile of experimental tissue is abnormal:
  - For **uncontrollable variables** (diet, anesthesia, patient age, duration of surgery, tissue ischemia) we need to develop a molecular fingerprint and subtract that fingerprint profile from the experimental gene expression profile
  - For **controllable variables** (rapidity of processing, freezing, storage), we need to optimize handling of specimens to minimize the effect of steps that most affect gene expression profiles
- To achieve these goals, we need to
  - Obtain all relevant information from all experimental data sets (MISFISHIE specification)


Urologic Epithelial Stem Cell Group
Questions ??
Alvin Liu, PhD: PI, Molecular Biologist
Eric Deutsch, PhD: Bioinformatics
Laura Pascal, PhD: Post-doc, Stem cell biology
NIDDK U01 Stem Cell Consortium (Prostate & Bladder)
INSTITUTE FOR Systems Biology
University of Washington School of Medicine

Is there a set of “housekeeping” genes/proteins that can be used as a metric for preservation?



Range of mRNA:
β-actin: 5,500 – 19,000
α tubulin: 4,000 – 15,500
GAPDH: 8,000 – 18,000

There is no single “housekeeping gene” and probably not a generic set of genes that can be used as a general metric for RNA preservation.