Data Acquisition and Management

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Responsible Conduct for Research Program - 08
Building a Solid Foundation for Research Integrity

Summary of Ethical Issues in Science

- *Scientific research effort must be built on trust.*
  - That the reports by other scientists are valid.
  - That sources of novel ideas will be properly acknowledged.

Alberts and Shine, Science 66:1660, 1994.

Summary of Ethical Issues in Science

- *Responsibility Lies with the Research Community.*
  - Some by administrators, journal editors, societies, granting agencies and the government.
  - Greatest responsibility lies with the scientist conducting research.

Alberts and Shine, Science 66:1660, 1994.

Summary of Ethical Issues in Science

- *Uncertainty*

  - Past scientists learned ethics informally by working with senior scientists.

  - Today pressures to succeed are high and society has higher expectations from expenditure of research funding.

  - Pressures include: finding money, competition, teaching and administration.

  - Will formalized training on ethics of research be enough?

  - Institutions need to establish trust with the public if research is to succeed.

Alberts and Shine, Science 66:1660, 1994.

Data Control and Management

- Claims of a patentable invention (and disputes among inventors about who deserves the greater share of royalties).
- Rights to publish (authorship) and apply for grants depend on who owns the data.
- Critical - a complete set of data that identifies the investigator who collected data, when and where.

What Constitutes Research Data?

- *Intermediary products of research.*
  - Biological specimens.
  - Information recorded about biological specimens.
  - Unpublished written material based upon information describing biological specimens.
- Contrasted to the final products of research = scientific article or the patent (covered by patent law).

Biological Specimens

- Biological Organisms (Transgenic mice).
- Tissues or cells of human or animal origin or images of the same.

Information recorded about biological specimens and unpublished written material

- Written materials include laboratory notebooks, records or minutes of research meetings, grant applications, research protocols and manuscript drafts.
- Information extracted from medical records or interviews with study subjects and other survey data or time spent observing humans or animal behavior.
- Information from research which is entered into computer storage.

Integrity of Primary Data

- There is a necessity for reporting fully and accurately the results of research. Such reporting establishes data authenticity, authorship and intellectual property.
- Principle Investigators have the final responsibility for data integrity.

Responsibility of Principle Investigator

- Validity and quality of the data and manuscripts generated from their laboratories.
- Fulfilling departmental and University research and publication standards, policies and procedures.
- Orienting students, research fellows, residents and staff to those standards, policies and procedures.
- Overseeing work performed by students, research fellows, residents and staff to assure that these standards are upheld.

Protocol – carefully defined ahead of time

MØ CytoFlow

Reagents

Stimulants:
IFNγ 10Ug/ml, 3hrs
LPS (10ng/ml) + Brefeldin 10µg/ml, 2 hrs

1°:
Biotin Rat anti-mus TNFα (Pharmingen #18122D; clone # MP6-XT3)
Store at 4°C
0.5 - 2µg/ml for ELISA
Stock = 0.5mg/ml

Biotin Rabbit anti-CINC
Store at 4°C
1:200 dil'n

Isotype Cntl:
FITC Rat IgG1, Kappa (Pharmingen #6099-03)
Store at 4°C
Use at equal conc. as 1°
Stock = 1.0mg/ml
2°:
Strepavidin-RED 670 (Bibco #19543-D24; Lot # FAJB01)
Store at 4°C
1:200 dil'n

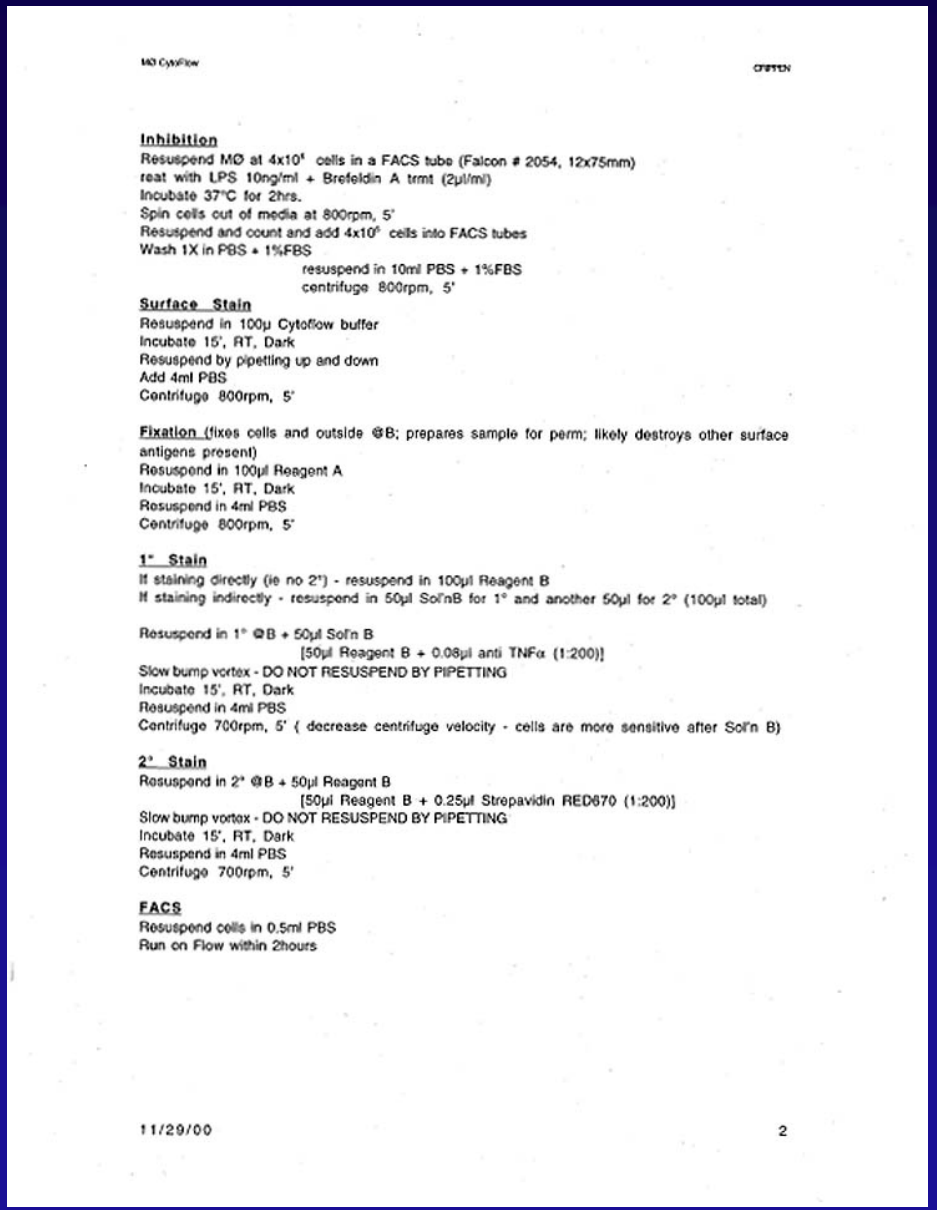
Brefeldin A (Epicentre Technologies #B905MG; $C_{16}H_{24}O_4$, MW=280.37)
Store at 4°C, crystalline
Dissolve in EtOH or MetOH
Stock = 5mg/ml = 5µg/µl
Dose = 10µg/ml = 2µl Brefeldin at 5mg/ml
Store at -20°C, reconstituted
A fungus metabolite specifically and reversibly blocks protein transport from the endoplasmic reticulum to the golgi apparatus.

Fix & Perm (CalTag #GAS-004)
Store at 4°C
Reagent A fixes cells while reagent B permeabilizes cells giving antibodies access to intracellular structures while leaving the cells morphological scatter characteristics intact.

PROTOCOL:

Take peritoneal MØ and remove and squish spleen (3 mice)
Mix spleen and perit MØ and let settle RT, 3'
Remove supernate and spin 800rpm, 10'
Resuspend pellet in DMEM + FCS and add to 100mm dish (2 dishes)
incubate 1hr, 37°C
treat w/ IFNγ (10U/ml) and incubate 3hr, 37°C
Scrape MØ's from plate and wash 3X in 1ml DMEM (800rpm, 10')

Protocol – carefully defined ahead of time



MØ CytoFlow

Inhibition
Resuspend MØ at 4x10⁶ cells in a FACS tube (Falcon # 2054, 12x75mm)
reat with LPS 10ng/ml + Brefeldin A trmt (2µl/ml)
Incubate 37°C for 2hrs.
Spin cells out of media at 800rpm, 5'
Resuspend and count and add 4x10⁶ cells into FACS tubes
Wash 1X in PBS + 1%FBS
resuspend in 10ml PBS + 1%FBS
centrifuge 800rpm, 5'

Surface Stain
Resuspend in 100µ Cytoflow buffer
Incubate 15', RT, Dark
Resuspend by pipetting up and down
Add 4ml PBS
Centrifuge 800rpm, 5'

Fixation (fixes cells and outside @B; prepares sample for perm; likely destroys other surface antigens present)
Resuspend in 100µl Reagent A
Incubate 15', RT, Dark
Resuspend in 4ml PBS
Centrifuge 800rpm, 5'

1° Stain
If staining directly (ie no 2°) - resuspend in 100µl Reagent B
If staining indirectly - resuspend in 50µl Sol'nB for 1° and another 50µl for 2° (100µl total)

Resuspend in 1° @B + 50µl Sol'n B
[50µl Reagent B + 0.08µl anti TNFα (1:200)]
Slow bump vortex - DO NOT RESUSPEND BY PIPETTING
Incubate 15', RT, Dark
Resuspend in 4ml PBS
Centrifuge 700rpm, 5' (decrease centrifuge velocity - cells are more sensitive after Sol'n B)

2° Stain
Resuspend in 2° @B + 50µl Reagent B
[50µl Reagent B + 0.25µl Strepavidin RED670 (1:200)]
Slow bump vortex - DO NOT RESUSPEND BY PIPETTING
Incubate 15', RT, Dark
Resuspend in 4ml PBS
Centrifuge 700rpm, 5'

FACS
Resuspend cells in 0.5ml PBS
Run on Flow within 2hours

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Methods Protocol

Paraformaldehyde (PFO) Fix: crosslinks proteins

Stock Sol'n - 10% PFO
- Make up sol'n in a hood
- 1g PFO
- 10g dH_2O
- heat to almost boiling (80°C)
- add 2 drops 10N NaOH to get PFO into sol'n
- let cool

Working Sol'n - 1% PFO
- 1ml 10% PFO
- 9ml PBS
- pH to 7.2 with pH paper (PFO will wreck your pH electrode)

Wash cells 3X in PBS (ph7.2)
Add 1% PFO
Incubate 10', RT
Wash cells 3X in PBS

Formaldehyde Fix:

Dilute 37% formaldehyde 1:10 in PBS
add 3.7% formaldehyde
Incubate 10', RT
Wash cells 3X in PBS

To permeablize membranes after PFO or Formaldehyde fixation -

Put samples into a glass dish
Add ice cold 100% Acetone
Incubate 3-5min, -20°C
remove from acetone and let air dry

Methanol (MeOH) Fix: permeablizes membranes

Wash cells 3X in PBS (ph7.2)
Add cold MeOH (-20°C)
Incubate 10', -20°C
Wash cells 3X in PBS to rehydrate

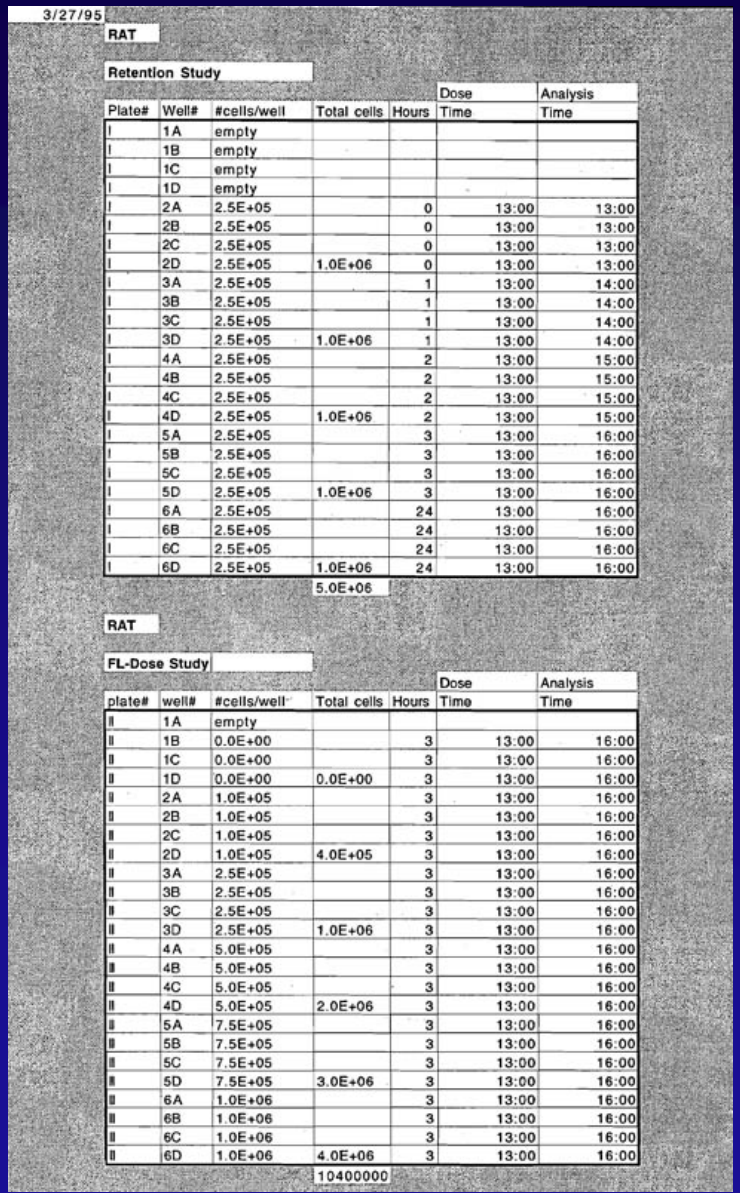
Storage:

a) Store cells at 4°C in PBS + 0.1% NaN_3

b) Mount slides with commercial mount (eg. Accumount; Permount; etc.)

c) Mount FL labeled slides in antibleach mounting sol'n -> 9:1 Glycerol:PBS + 3% *n*-propylgallate
seal edges with nail polish

Set up Sheet



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RAT

Retention Study

Plate#	Well#	#cells/well	Total cells	Hours	Dose Time	Analysis Time
I	1A	empty				
I	1B	empty				
I	1C	empty				
I	1D	empty				
I	2A	2.5E+05		0	13:00	13:00
I	2B	2.5E+05		0	13:00	13:00
I	2C	2.5E+05		0	13:00	13:00
I	2D	2.5E+05	1.0E+06	0	13:00	13:00
I	3A	2.5E+05		1	13:00	14:00
I	3B	2.5E+05		1	13:00	14:00
I	3C	2.5E+05		1	13:00	14:00
I	3D	2.5E+05	1.0E+06	1	13:00	14:00
I	4A	2.5E+05		2	13:00	15:00
I	4B	2.5E+05		2	13:00	15:00
I	4C	2.5E+05		2	13:00	15:00
I	4D	2.5E+05	1.0E+06	2	13:00	15:00
I	5A	2.5E+05		3	13:00	16:00
I	5B	2.5E+05		3	13:00	16:00
I	5C	2.5E+05		3	13:00	16:00
I	5D	2.5E+05	1.0E+06	3	13:00	16:00
I	6A	2.5E+05		24	13:00	16:00
I	6B	2.5E+05		24	13:00	16:00
I	6C	2.5E+05		24	13:00	16:00
I	6D	2.5E+05	1.0E+06	24	13:00	16:00
			5.0E+06			

RAT

FL-Dose Study

plate#	well#	#cells/well	Total cells	Hours	Dose Time	Analysis Time
II	1A	empty				
II	1B	0.0E+00		3	13:00	16:00
II	1C	0.0E+00		3	13:00	16:00
II	1D	0.0E+00	0.0E+00	3	13:00	16:00
II	2A	1.0E+05		3	13:00	16:00
II	2B	1.0E+05		3	13:00	16:00
II	2C	1.0E+05		3	13:00	16:00
II	2D	1.0E+05	4.0E+05	3	13:00	16:00
II	3A	2.5E+05		3	13:00	16:00
II	3B	2.5E+05		3	13:00	16:00
II	3C	2.5E+05		3	13:00	16:00
II	3D	2.5E+05	1.0E+06	3	13:00	16:00
II	4A	5.0E+05		3	13:00	16:00
II	4B	5.0E+05		3	13:00	16:00
II	4C	5.0E+05		3	13:00	16:00
II	4D	5.0E+05	2.0E+06	3	13:00	16:00
II	5A	7.5E+05		3	13:00	16:00
II	5B	7.5E+05		3	13:00	16:00
II	5C	7.5E+05		3	13:00	16:00
II	5D	7.5E+05	3.0E+06	3	13:00	16:00
II	6A	1.0E+06		3	13:00	16:00
II	6B	1.0E+06		3	13:00	16:00
II	6C	1.0E+06		3	13:00	16:00
II	6D	1.0E+06	4.0E+06	3	13:00	16:00
			10400000			

Monocyte/ Macrophage Culture

RAW 264.7
Mouse Monocyte Macrophage ATCC TIB-71 Batch# F10547

Date ___-___-___
#Flasks ____ x T25 ____ x T75
Media: DMEM + 10%FCS + 1%Pen/Strep
Condition ____

Vials thawed => Date ____
#Flasks Split ________ Split ________ Pass# ____ Refill ____ = #Flasks ____
#Flasks Frozen ________ => #vials ________

mRNA #Flasks ____ x T25 ____ x T75
Stimulant ________ conc ________ Start Time ________ Harvest ____

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Date ___-___-___
#Flasks ____ x T25 ____ x T75
Media: DMEM + 10%FCS + 1%Pen/Strep
Condition ____

Vials thawed => Date ____
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Stimulant ________ conc ________ Start Time ________ Harvest ____

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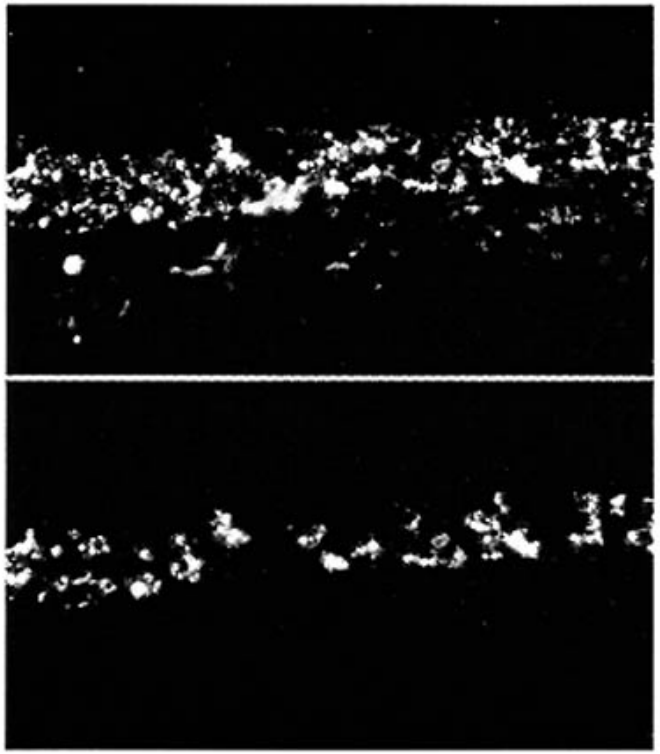
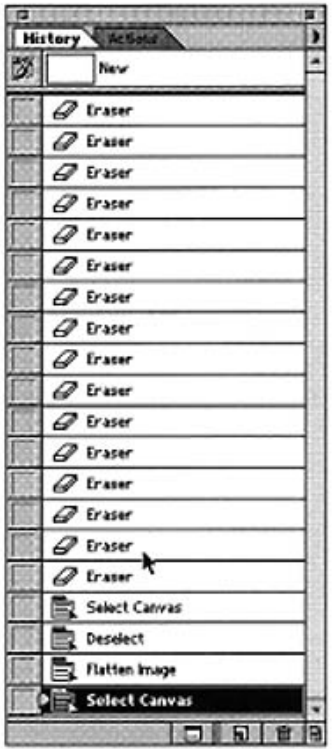
Digital Alteration of Images



Actions on Original Image

Actions on "Final" Image



Actions on "Red" Image

Journal of Cell Biology Guidelines on Image acquisition and manipulation

- 1. Make and model of microscope
  2. Type, magnification, and numerical aperture of the objective lenses
  3. Temperature
  4. Imaging medium
  5. Fluorochromes
  6. Camera make and model
  7. Acquisition software
  8. Any subsequent software used for image processing, with details about types of operations involved (e.g., type of deconvolution, 3D reconstructions, surface or volume rendering, gamma adjustments, etc.).
- No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.

Journal of Cell Biology Guidelines on Image acquisition and manipulation

Choosing the correct application: The problem with PowerPoint

- Adobe **Photoshop** is an image-processing application. The only way to properly ensure the integrity of the resolution of your original image is to open it in Photoshop at the size and resolution at which it was acquired.
- Microsoft **PowerPoint** , however, is presentation software. That is, it is meant to create figures that will be projected on a screen, not printed. You may be able to obtain a nice printout of your PowerPoint files, but that is because the printer driver is creating resolution to place on the paper.

Journal of Cell Biology Guidelines on Image acquisition and manipulation

- **RGB:** A 6 X 6 inch image at 300 dpi should be about a 10 Mb TIFF file.

- **Grayscale:** A 6 X 6 inch image at 300 dpi should be about a 3 Mb TIFF file.

Jessica Banks Case

- Jessica Banks has just earned her Ph.D. and wants to take her laboratory notebooks when she leaves for her new job (tenure-track position in a mid-sized western liberal arts college).
- Her major professor, Brian Hayward, objects. He exclaims "You can't take those notebooks away – they belong to the lab!"
- Banks is confused. "But I did the work, and I wanted to follow up on it. I can't do that without the notebooks."

Jessica Banks Case

- Professor Hayward is adamant. “I’m sorry, but you should understand this. This lab is a joint enterprise, and all the work you did was funded by money I brought in with my grants. The notebooks don’t belong to you, nor to me; they belong to the lab, and the work will be continued in this lab. I’ve already talked to one of the new students about working on those projects this fall.”
- Banks, seeing her plans fall apart around her, protests, but Hayward is implacable. After a few minutes, she stalks away, without the notebooks.

Data Ownership

- Methodology and experimental notebooks and related data and records (including computer files) are the property of the University of California and the PI of the laboratory/funding source.
- Should remain in the laboratory. Copies may be taken away from the University with approval from the PI and the University.
- If an investigator resigns from the University, arrangements should be made to transfer ownership of original materials.

Graduate Student/Post doc versus PI/Major Professor

- PI owns the data even if grad student/post doc generated it in the PI's laboratory.
- If grad student/post doc leaves the University, their lab notebook and all original data stay with the PI.
- PI determines when is the appropriate time to publish the data.
- Laboratory culture is critical determinant.

Maintenance of Data Notebooks and Records

- Gold standard = Bound notebook with consecutively numbered pages with permanent, sewn binding.

Minimum Information for Data Notebook

- Title of the study.
- PI’s name.
- Dates the study starts and ends.
- Associated Investigator’s names.
- Hypothesis or study goals.
- Biological materials used.
- Special reagents, antibodies and probes used.
- Study specific treatment groups and projected number of subjects (animal protocol).
- Statistical methodology used.
- Time line showing sequence of events.
- Raw data and special procedures.
- Brief experiment and study conclusions.

Keeping Experimental Notebooks

- Each entry should be stand alone permitting others to replicate work.
- Experiments are logged in chronological sequence and dated plus initials.
- Should be kept only in ink with no erasures or whited-out changes. Mistakes should have a single line through ink in a different color with corrected data beneath with a brief explanation.

Keeping a Master Log

- Multi-laboratory studies with several investigators, the PI should keep a master log to catalog the experiments.
- Titles of the studies done, investigator's names and inclusive dates of the experiments.
- Computer files should be have multiple backups with at least one off site.

Storage of Data

- All data notebooks and related data and records should be stored in the department or laboratory for 5 years after the date when funding for the study ends.

Research Data and the Freedom of Information Act

- Data that can be requested must be related to findings that are used by the federal government in developing an agency action.
- “Data” excluded include – preliminary analyses, plans for future research and communications with colleagues.
- Also excluded are human subject identification data and protection of intellectual property data.

California Public Records Act

- Designed to give the public access to information “records” in possession of public agencies.
- "Records" include all communications related to public business "regardless of physical form or characteristics, including any writing, picture, sound, or symbol, whether paper,…, magnetic or other media." Electronic records are included, but software may be exempt.

California Public Records Act

- Access is immediate and allowed at all times during business hours. Staff need not disrupt operations to allow immediate access, but a decision whether to grant access must be prompt.
- An agency has 10 days to decide if copies will be provided.
- The agency must justify the withholding of any record by demonstrating that the record is exempt or that the public interest in confidentiality outweighs the public interest in disclosure.
- Copy costs are limited to "statutory fees" set by the Legislature (not by local ordinance) or the "direct cost of duplication", usually 10 to 25 cents per page. Charges for search, review or deletion are not allowed.