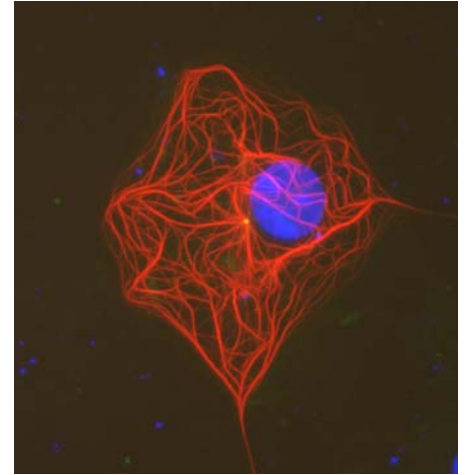


BIOL 447

Laboratory in Cell Biology

This is an in-depth lab course on the current and most utilized techniques in the study of cells. A weekly lecture covering the theory of these techniques accompanies a weekly lab session that is limited to 15 students. Students will also be introduced to several cell biology careers that do not require advanced degrees.

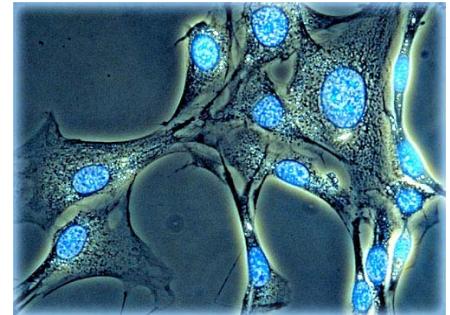


Tissues

Plant cell tissue culture, Handling explants, Organ regeneration from single cells, Preparing plant protoplasts, Producing transgenic plant tissue, Animal cell culture, Adherent cell manipulations, *in situ* gene expression, cell transformation

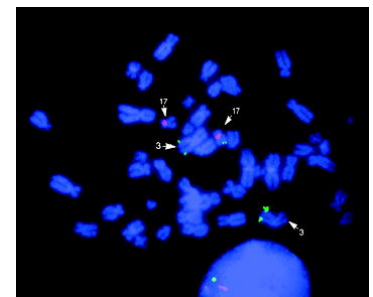
Cells

Electron microscopy, Tissue fixations and dehydrations, Tissue sectioning for light microscopies, Staining slides, Brightfield and fluorescence Microscopies, Determining membrane potential, Cell homogenizations



Subcellular

Isolation of organelles, Staining polytene chromosomes, Heterologous expression of reporters in frog oocytes, *In situ* hybridization, Chromosome painting



Molecules

Protein determination, SDS PAGE, Immunoblot analysis, Tissue printing for enzymatic activity, Chromatographies, Use of prokaryotic and insect cells for heterologous expression of eukaryotic proteins for antigen production, Preparation of rabbit polyclonal antibodies

Biol. 447 Laboratory in Cell Biology Spring 2010

Instructor: Dr. Alan Jones, ALAN_JONES@UNC.EDU

Teaching Assistant: Erin Friedman efriedman@unc.edu and assorted guests

Lecture Wed. 9-10 AM, room 139 Wilson Hall

Jan. 13	No lecture
Jan. 20	Introduction, Clinical Lab Science, Guest lecturer
Jan. 27	Biosafety, Protein expression, antibody production
Feb. 3	Transformation technologies
Feb. 10	Small G proteins and cancer
Feb. 17	Brightfield/fluorescence microscopy
Feb. 24	Brightfield/fluorescence microscopy continued
Mar. 3	Midterm exam (through microscopies)
Mar. 6-14	No lecture, Spring Break
Mar. 17	Drosophila embryo development
Mar. 24	Guest speaker on Genetic Counseling
Mar. 31	Guest speaker on cytogenetics
Apr. 7	Programmed Cell Death for Beginners
Apr. 14	Electrophoresis and immunotechniques
Apr. 21	TBA
Apr. 28	Final exam (from Mar 17)

Lab Exercise Thurs 1-5PM or later, room 130 Wilson Hall

- Jan. 14 Informatics, Mining databases, **MEET IN DAVIS LIBRARY ROOM 246**
- Jan. 21 Protein expression for antibody production
- Jan. 28 Plant tissue culture, plant cell transformation, visualizing tissue-specific gene expression, fix plant tissues. This is a multiple-day lab over 1 week
- Feb. 4 Scientific Method- start research projects
- Feb. 11 Animal tissue culture, mammalian cell transfection, fix mouse embryos
- Feb. 18 Brightfield/fluorescence microscopies, **requires coming in Feb 16th to transfect cells**
- Feb. 25 Polytene chromosomes- Tissue into paraffin
- Mar. 4 Electron microscopy, EM project due Mar 18th
- Mar. 6-14 **No lab, Spring Break**
- Mar. 18 In situ hybridization, Note: This is a two-day lab, **requires coming in Mar 16th and 17th for 2- 4 hours**
- Mar. 25 “Death by Design” primer for programmed cell death, tissue sectioning
- Apr. 1 TUNEL, tissue staining
- Apr. 8 Electrophoresis
- Apr. 15 Immunological techniques
- Apr. 22 Chromosome painting- Note: This is a two-day lab, requires **beginning on Wednesday Apr. 21st**
- Apr. 29 **Project presentation**

Schedule subject to change

Grading

1. Midterm 25%
 2. Final 25%
 3. Performance 50%
 - a. Participation, Unexcused absences result in a failing grade- no exceptions. Coming to class/lab late results in a C for the total performance category, two or more equals an F, regardless of the other aspects of 'Performance' Moreover, if you are late to 2 lectures and/or labs, the highest possible final grade is C- regardless of your exam grades.
 - b. Lab book, EM project report, other reports, weekly assignments, final project, your NY Times –style article on a UNC faculty member
 - c. **curiosity, enthusiasm, teamwork , and attitude**
- If you receive a D on both midterm and final exam, your final grade cannot be higher than a D. **No make-up exams.**

Your manual is a collection of protocols used for this class (some are being tried out this year). You will only use a selected set this year but I thought you would like to have the entire collection. Some protocols continue for several exercises.

Quick Guide to Protocols used this Semester (subject to change).

Jan 21st

Heterologous expression of a eukaryotic gene in *E. coli* for antibody production- polyHis version

Jan 28th

Sterilizing Arabidopsis Seeds

Visualizing Gene Expression Using GUS in situ

Acetocarmine staining of lateral root primordia

Preparing Permanent Impressions of Surface Cells Using Non-evasive Dental Impression Vinyl Polysiloxane.

Feb. 11th

Cell counting

FuGene Transfection of NIH 3T3 mouse fibroblasts

Culturing Adherent Mammalian Cells

Mouse Embryo Fixation

Feb. 18th

Brightfield and Fluorescence Microscopies

Feb 25th

Staining Polytene Chromosomes from *Drosophila* Salivary Glands

Metaphase Chromosomes in Root Tip Squashes

Mar 4th

Onion Root Tip Squashes

Staining Polytene Chromosomes from *Drosophila* Salivary Glands

Mar 4th

Visualizing Negatively Stained Microtubules by Electron Microscopy

Mar 16th - 18th

In situ hybridization of mRNA in *Drosophila* oocytes

Apr. 1st

TUNEL staining Cells Undergoing Programmed Cell Death

Staining Animal tissues for Brightfield Microscopy

Apr. 8th and Apr. 15th

SDS PAGE and Western Blots

Developing a Western Blot

Whole-mount Antibody staining

Apr 21st - 22nd

Chromosome Painting", Fluorescent In Situ Hybridization (FISH)

Exercise questions and things to do

I suggest that you read these beforehand so that you can ask questions like these in the lab when you have the chance. Otherwise, you're on your own to get the answers!

(due Jan 22nd)

1. Why are tags added to recombinant proteins?
2. What kind of tags are used?
3. What kinds of expression systems are used and what are the advantages and disadvantages of each?
4. If a protein is not made as a soluble form in *E. coli*, what can be done to remedy this problem?
5. What is the lac promoter and how does IPTG activate heterologous gene expression?
6. What is a baculovirus and how is it used for heterologous expression of recombinant protein?
7. Read the review on ras at the end of this packet.

(due Jan 29th)

1. Describe the configuration of a sterile hood and the proper placement of hands and objects relative to the "clean" samples. Draw it.
2. Describe the structure of a gene and how recombinant forms of it can be used to measure gene expression. Include ways to visualize gene expression. How can the same method be modified to look at steady state levels of a protein?
3. What are the small G proteins and what do they do? How are they involved in cancer?
4. Describe how adherent animal cells are maintained in culture, including the passaging.
5. Describe the purpose and means for fixing tissues.
6. Explain some of the regulations that must be followed by certified animal care facilities.

(due Feb 26th)

1. Compare and contrast brightfield and transmission electron microscopy. Draw side-by-side the light/electron paths through the respective scopes.
2. Give examples of different types of fluorescent microscopy fluorophores and how they are used.
3. Compare and contrast fluorescent and scanning electron microscopy. Draw side-by-side the light/electron paths through the respective scopes.
4. Define numerical aperture and explain what this is and how the optics set the NA for imaging.
5. Describe in words and pictures how to properly adjust for Koehler illumination.

(due April 2nd)

1. What is in situ hybridization detecting? If we do not do in situ hybridization in lab, then look up the answer on your own.
2. Describe Drosophilla embryo development in 200 words or less, include the concept of developmental boundaries and how they can be established.
3. What is TUNEL detecting and how is this related to programmed cell death?
4. What is Rita Levi-Montalcini's twin sister's first name?
5. Give 5 examples in normal development where programmed cell death is required.
6. What is the exact role of programmed cell death in forming our immune system.

(due Apr 16th)

1. Why are SDS and β ME used in SDS PAGE? What do they do? How is PAGE altered if one or both are not used?
2. Compare and contrast SDS PAGE with size exclusion chromatography.
3. Describe in detail how one performs 2-dimensional PAGE. What is the difference in resolution between 1- and 2-dimensional PAGE?
4. What is tandem mass spec? How does it work? How can it be used to sequence proteins?
5. How are monoclonal antibodies made? Include in your description, the use of selection drugs.

(due Apr 23rd)

1. What is the difference between a gene probe and a paint?
2. How can translocations be detected using FISH?
3. What gives polytene chromosomes their appearance? What cell type has polytene chromosomes and why would it need to?
4. Explain the difference between a band-pass and a cut-off filter? How is excitation of a fluorophore and its emission be detected along the same optical path?
5. Read the article "Suicide for beginners" at the end of this packet.