

RESEARCH METHODS IN MOLECULAR BIOLOGY - BIOL 395 FALL 2002

Instructor: Dr. Michael Temkin
124 Bewkes Hall, Office Telephone: 229-5871 Home Telephone: 386-3025

Class Tuesday and Thursday 10:10-11:40 am Valentine 103 or Bewkes 108

Meetings: Tuesday 1:15-4:15 PM Bewkes 108

Laboratory Access: If you want to spend more time working in the laboratory, a key for Bewkes 108 is available in the Science Library for after hours access.

References: **Class handouts or on Reserve in Library.**

Web Sites: it.stlawu.edu/~mtem/home.htm or <http://it.stlawu.edu/~mtem/MOLMETH.HTM> and Blackboard Site

COURSE OBJECTIVES:

1. To help you develop critical thinking skills
2. To help you learn about the use of molecular methods in biology
3. To help you become proficient in using molecular techniques
4. To help you develop and conduct research using molecular techniques

SCORING:

Exam I	50 pts.	Following Project Set I
Exam II	50 pts.	Following Project Set II and III
Final	50 pts.	Following Project Set IV and V
Lab Notebook	100 pts.	Graded after each exam
Seminar	50 pts.	
Research Proposal	75 pts.	Due December 9th
Participation	50 pts.	
TOTAL	425 pts.	

GRADES:

	Point Percentage
4 Excellent	≥90
3.5 Between Excellent and Good	85 - 89
3 Good	80 - 84
2.5 Between Good and Satisfactory	75 - 79
2 Satisfactory	70 - 74
1.5 Between Satisfactory and Lowest Passing Grade	65 - 69
1 Passing Grade	60 - 64
0 Failing Grade	< 60

MOLECULAR METHODS TO BE STUDIED DURING THE SEMESTER:

Plasmid DNA Isolation	Polyacrylamide Electrophoresis
RNA Isolation	Western Blotting
Protein Purification	Nonisotopic Detection of Biological Molecules
Measuring [Protein], [RNA], and [DNA]	Transformation Bacterial Cells
Reverse Transcription-PCR (RT-PCR)	Transfection of Mammalian Cells
Cloning	Gene Expression in Mammalian Cells
DNA Sequencing	Confocal Microscopy
Agarose Electrophoresis	Immunocytochemistry

GOAL FOR THE SEMESTER: TO OBTAIN LARGE QUANTITIES OF THE RAT BRAIN SODIUM BICARBONATE COTRANSPORTER PROTEIN

PROJECTS FOR THE SEMESTER:

I. Obtain the complete coding region of the rat brain sodium bicarbonate cotransporter gene and clone it into a mammalian expression vector

- RNA Isolation
- RT-PCR
- Agarose Gel Electrophoresis
- Cloning
- Restriction enzymes

II. Sequencing clone to determine the that it is the correct sequence and that it is inserted into the vector in the correct reading frame

- Small and large scale preparations of plasmid DNA
- Sequencing
- Rectify sequences
- Assemble sequence data

III. Transfect mammalian cell line and examine cells for gene expression

- Electroporation
- Immunocytochemistry
- Confocal microscopy

IV. Determine the presence of expressed cotransporter protein in cells and media using a western blot protocol (we will also quantify the concentration of the protein GFAP from rat brain

- Tissue extraction for Proteins
- Determining Protein Concentration
- SDS-PAGE
- Western Blot Analysis

V. Purify cotransporter protein from media and determine its concentration and purity

- Determining Protein Concentration
- SDS-PAGE

LABORATORY NOTEBOOK:

Your laboratory notebook should be an accurate and complete record of your work in lab. The type of notebook you use is your decision but must be either composition, spiral bound, or ring binder - **no folders!!!!**. **Your notebooks will be graded on organization, completeness, and readability.** For each lab you should include the following in your notebook:

- 1) a complete step by step lab procedure based on either manufacturer's instruction sheets or class handouts
- 2) notes explaining the purpose of each step or set of steps of the procedure (e.g., liquid nitrogen was used to prevent the degradation of RNA while the tissue was being ground with a motor and pestle).
- 3) notes on any special preparation for the lab (recipes for reagents, special handling requirements, etc..)
- 4) notes concerning your progress through the procedure (this may be as little as check marks as you add reagents to tubes)
- 5) description of results - using words, pictures, sequences, etc..
- 6) comments - your thoughts and analysis of the results - what do the results mean - are they what you expected? How do you know what to expect? Try to explain negative results - sometimes the reason for a failed procedure is obvious - **OOPS we forgot to add the DNA!!** Other times reasons for failure are less obvious - please do not hesitate to consult with me if you are having a problem or unsure about what to do.

Your Notebooks **MUST BE READABLE AND UNDERSTANDABLE!!!!!!**

RESEARCH PROPOSAL:

A formal research proposal states what your objectives are, how you plan to go about achieving your objectives, and why achieving your objectives is important. The Research Proposal will have a title page and 7 sections:

- 1) **Title Page** - Informative title, your name, address, and date
- 2) **Objectives** - Statement of your specific aims - what data do you plan to collect
- 3) **Introduction** - Provide background material so that someone reading your proposal will understand why you want to do the work - history and importance of the subject you want to study - broad significance
- 4) **Materials and Methods** - How are you going to conduct your research. This section must demonstrate that the design of your research plan is feasible. You must also show that you are capable of conducting your research plan. This section does not necessarily have to read like a cookbook, however it must be written with enough detail that your research plan can be evaluated. **You should also include a Data Analysis subsection in which you describe how you will analyze your data** (e.g. what statistics will you use, what computer program, etc.) **and a Time Table subsection in which you outline how long it will take you to complete each portion of your research.**
- 5) **Expected Results and Significance** - Try to think of all the possible results that may be generated from your research. Some results will seem more likely than others. You want to demonstrate to the reader of your research proposal that no matter what the results of your research are that they will be important. Therefore it is essential to show how possible results will address significant questions (e.g. significant points you raised in the Introduction). It is usually while you are

writing this section of the proposal that you realize that there are flaws in your research plan. So keep fine tuning the Materials and Method section.

- 6) **Literature Cited** - This section contains a list of all the works that you referenced in the body of the proposal. Literature citations should follow an accepted style from a major scientific journal. Try to keep to the primary literature, which does not include textbooks, magazines, and encyclopedias! **You should have at least between 20 and 30 references!**
- 7) **Budget** - We do have some money to purchase some equipment and organisms if needed. This section should be an itemized list of equipment and prices.
- 8) **Budget Justification** - If you include a budget, you must also include a justification for each item, stating the importance of each item to the research plan.

The Research Proposal will be graded on the basis of its scientific content, organization, and selected references. In addition, spelling and grammar do count.

Topic of proposal must be approved by October 9th.

SEMINAR:

A 40 minute presentation (30 min talk + 10 min question-answer period) on your research proposal. In this talk you will present background information to put your research into context, a detailed plan of your research, an analysis of possible results and their significance. Presentations need to be of professional quality - should be power point presentation making good use visual aids (overheads or slides may be used to supplement power point presentation). This presentation should not be a rambling talk about the wonders of molecular methods!