Biology 3492: Laboratory experiments with eukarotic microbes
Spring 2007

Description:

Laboratory Experiments with Eukaryotic Microbes. An introduction to diverse molecular and cell biology techniques used in model experimental organisms to explore fundamental biological questions. Experiments will be performed using selected fungi and protozoans commonly used in major research efforts. Emphasis will be placed on choosing the appropriate organism for the question posed using the most current technologies. Prerequisites: Bio 2960 and 2970 and permission of instructor. One hour of lecture and six hours of laboratory a week. This course fulfills the laboratory requirement for the Biology major. Enrollment limited to 12.

Meeting Times:

Laboratory:	Tues/Thurs	9 am – 12 noon;	in *Rebstock 101*
Lecture:	Wed	3 pm -- 4 pm; in	*Life Sci. 118*

Instructors:

Professor Doug Chalker (coursemaster): 935-8838; dchalker@biology2.wustl.edu
Monsanto Hall 304, Hilltop

Grading:

Lab Notebooks	
Midterm evaluation	50 pts
Final evaluation	100 pts
Lab write-up 1	100 pts
Lab write-up 2 (+ oral presentation)	200 pts
(Protein localization and expression)	
Lab write-up 3 (Complete project)	
1st draft	50 pts
Final draft	150 pts
Oral presentation	100 pts
In-term exam	150 pts
Problem sets/exercises	100 pts
Total	1000 pts

General Policies:

You are expected to attend every lab and lecture session. This is a laboratory course, so hands-on experience you will gain has the major instructional value. Arrive on time so that we can complete the experiments planned. You will be working as a team this semester, so those arriving late affect everyone. If you know you have to miss a class, please inform both me and your partner. We have only one exam, so make sure that you do not miss this class period as *no make-up exam will be given*. A doctor's note stating that you were seen for an illness of sufficient severity to warrant an excuse is needed - a note simply stating that you visited the health center is insufficient. In the event of a death or serious illness in the family, certification will be needed to validate your absence. If you have a legitimate excuse for an absence, your final grade will be determined by calculating the mean of the other assignments. Unexcused, missed assignments will be given a grade of zero and may well necessitate withdrawal from the course.

Lab Reports

You will have three lab reports due this semester. Some will require drafts prior to the final report. The final report is a cumulative report of all your lab work this semester. These will take the form of a scientific paper. The due dates on the syllabus are tentative, depending upon our progress with our experiments.

Lab Notebooks

You will need to purchase a bound lab book for the semester. Keep all your notes and experimental procedures in this book. This will need to be left with Prof. Chalker at the end of the semester, but you are welcome to photocopy your notebook (I will give you access to a copier if you wish to do this).

Plagiarism

Definition (from www.Dictionary.com): n 1: a piece of writing that has been copied from someone else and is presented as being your own work 2: the act of plagiarizing; taking someone's words or ideas as if they were your own.

Plagiarism will be taken very seriously and will be reported to the dean's office for appropriate action. In writing assignments, be careful not to simply copy reference material, but use it to help you formulate and support your own thoughts and ideas. **Always give proper reference to material used**. Long sections of text taken verbatim should always be in quotations, but try to avoid using this style in most scientific writing. Make your own conclusions, don't just rely on what you read.

For those using the Credit/No Credit option, a grade of C- is required to receive credit.

Biology 3492: Laboratory experiments with eukaryotic microbes
Spring 2007 (Revised: still subject to change)

Jan 16 Computer lab (NSLC): Introduction to the Tetrahymena Genome Database (TGD)
Jan 17 Lecture 1: Scientific approaches using model systems
Jan 18 Computer lab: Predicting coding regions for analysis

Jan 23 Working with microorganisms; aseptic technique and cell counting
Jan 24 Lecture 2: DNA manipulations
Jan 25 Microscopes: making an invisible world visible; PCR amplification of candidate genes

Jan 30 Gel electrophoresis of PCR reactions; TA cloning and E. coli transformation
Jan 31 Lecture 3: RNA Processing I **(Lab report 1 due)**
Feb 1 PCR screening of E. coli transformants

Feb 6 Plasmid DNA isolation, Restriction enzyme analysis, DNA sequencing
Feb 7 Lecture 4: DNA transformation techniques
Feb 8 Fusion of coding sequence to GFP; RNA isolations

Feb 13 Plasmid DNA isolation, Restriction enzyme analysis
Feb 14 Lecture 5: Microscopy techniques
Feb 15 Tetrahymena electroporation
Homework: Selection of Transformants **arrange time with Prof. Chalker**

Feb 20 Scoring Tetrahymena electroporation; cultures for microscopy: rtPCR expression analysis
Feb 21 RNA Processing II
Feb 22 Fluorescence microscopy of GFP-MLH fusions; Protein isolations

Feb 27 rtPCR expression analysis (continued)
Feb 28 Lecture 6: Genetic Analyses
Mar 1 Gel electrophoresis of rtPCR analysis; Discussion: Scientific data presentation

Mar 6 Catch-up day: Discussion: Giving Scientific Presentations:
Mar 7 Lecture 7: Assessing protein:protein interactions **(Lab report 2 due)**
Mar 8 **Oral presentations of protein localization (Notebooks Collected and Graded)**
SPRING BREAK
Mar 20 Western-blot analysis of GFP-fusions
Mar 21 **In Term Exam**
Mar 22 Western-blot analysis of GFP-fusions

Mar 27 Creation of Antisense knockdown constructs
Mar 28 Preparation of data for Midwest Protozoology conference
Mar 29 Creation of Antisense knockdown constructs

Mar 31 Saturday Midwest Protozoology Conference McDonnell 362 Poster Presentation

Apr 3 Tetrahymena electroporation
Apr 4 Selection of Transformants
Apr 5 Analysis of Knockdown lines

Apr 10 Analysis of Knockdown lines
Apr 11 Discussion of Data
Apr 12 Analysis of Knockdown lines

Apr 17 Analysis of Knockdown lines
Apr 18 TBA **Draft of final report due**
Apr 19 Analysis of Knockdown lines

Apr 24 Wrap-up
Apr 25 Discussion of Data
Apr 26 Final Oral presentations
(Final Report due Wed May 2)

TBA= to be announced

BIO 3492: LABORATORY EXPERIMENTS WITH EUKARYOTIC MICROBES
Spring 2007

Laboratory Safety Rules

1. No eating or drinking in lab!!!!!!!!!!!!!!

2. Wear proper clothing when in lab.

3. Always wear goggles when using UV transilluminator.

4. Always wear gloves when handling ethidium bromide or other hazardous chemical

5. Keep loose hair away from bunsen burners.

6. Know where the fire blanket, the eye wash, the fire extinguisher and the first aid kit are located.

7. Know what to do if someone catches on fire (STOP, DROP and ROLL).

8. Dispose of biohazardous materials in the biohazard bags, or the glass jars provided on your benches. DO NOT put such materials in the trash.

9. Dispose ethidium bromide and other hazardous chemicals in approved containers only. DO NOT throw them in the trash, or put them down the sink.

10. Dispose of broken glass and other sharp items in boxes labeled "SHARPS".

11. If you aren't sure where to dispose of something, ask an instructor.

12. Clean up after yourself!

Laboratory Etiquette

1. Clean up after yourself! Before you leave, be sure all of your materials are put away in your cabinet and all of your samples and temperature-sensitive reagents are stored at the appropriate temperature. All samples should be clearly labeled with what they are, whose they are, and the date. Don't forget to wipe off your bench.

2. If you are using a common reagent and see that it is about to run out, inform an instructor. Also, treat enzymes and other common reagents carefully, keep things at the proper temperature, and always use a clean pipette tip to remove your aliquot. If you know you are the last one using a temperature-sensitive reagent, give it to an instructor when you are finished so that it may be put away promptly.

3. Be considerate of your lab partner - share tasks equitably. Early departures when one's lab partner is still working are seriously discouraged.

4. Use common sense and apply the "Golden Rule".

GUIDELINES FOR KEEPING A LABORATORY NOTEBOOK
BIO 3492-Spring 2007

The purpose of keeping a laboratory notebook is to: 1) help you keep track of the experiments you carry out over the course of the semester and 2) provide the instructors of the course with a means of evaluating your work in the class.

THUS, YOUR LAB NOTEBOOK MUST BE COMPLETE, LEGIBLE, LOGICAL AND GRADABLE !!!!!!

Remember, you must provide enough information so that anybody who has experience with molecular biology can read it and figure out what you have done, and what your results were.

Of course there is more than one way to keep a lab notebook. I prefer (and will require for this class) bound notebooks that are kept chronologically as a "diary" with each experiment or large goal given a number (usually Roman Numeral). The main reason for this is related to patents and patent disputes in court. These are the types of notebooks kept in industry. In the biotech industry, at the end of a work day scientist are often required to get their notebook signed or initialized by other members of the lab below their last entry. This helps protect accusations in court that they later went back and added information to their experiments.

Do not try to record your work several days after the fact! This only leads to disaster. Notes kept on paper towels also leads to the loss of vital information. Write down what you are going to do before you do it. Include base recipes and master mixes as well as subsequent steps as you proceed through the protocol. As you perform each, check them off. This way you can go back and see if you forgot to do something (we all forget sometimes). Then tape in any figures or pictures (such as gel pictures) that represent your results. Write your conclusions under the results. Talk with others about your results. If someone gives you a good idea or a unique interpretation on your data, write it in your notebook giving the appropriate acknowledgements. Science should not be done in a vacuum!

All lab write-ups must include the following:

1. **DATE** the experiment was started. You also must date any subsequent entries made for each experiment.

2. Experiment number and a brief title describing the experiment.

3. Purpose of the experiment - i.e. "restriction mapping to determine if we have obtained our sub-clone".

4. Procedure: If you follow a standard protocol, such as a plasmid prep or running an agarose gel, you do not need to describe this in detail, unless you prefer to rewrite it. Simply state that the experiment was carried out as described in protocol XX. However, when assembling reactions, such as restriction digests or PCR reactions, it's a good idea to write them out again in your notebook, even if specific instructions were given in the protocol. You should record incubation

times and temperatures, where in a protocol you stopped on any given day if you could not complete an experiment, and note any mistakes, strange observations, etc. You must also record any deviations from the standard protocol that you take, whether the changes were intentional or not.

5. Observations and Results: Record your data, include photographs of gels, **clearly dated and with all lanes labeled**, original or photocopies of X-ray films, etc.

6. Conclusions: This is written after you have thought about the results and comes at the end of every experiment. It should explain [in words]: what results were expected, what results you obtained, what observations you made that might influence your interpretations or were unusual, interesting, etc., what you will do next or what you need to fix. How would you change the experiment if you repeated it (even if it worked!), GIVE OPINIONS about what you did, what happened, and what it means. This might be as simple as " the Eco R1 digest was complete because I have bands of 9 KB and 750 bp, as expected. I will now gel purify the 750 bp fragment (next page) and use it in my next step to ligate to pUASp that is EcoR1 cut – see previous page for prep of vector." BUT every small part of each project should have this!!!!

7. **Table of Contents**. As you accumulate experiments, make a table of contents and mark particularly important steps/successes/changes in plan with a post-it or tab. Make data summaries that are compilations of a series of experiments frequently.

Your notebook is extremely valuable, as it is the only record of how you spent your time and energy, AND what results you obtained! You should take the time to make it as approachable by others as possible.

Grading: Lab notebooks will be graded during the semester as indicated on the lecture schedule. They will be graded on the following criteria:

1. A clearly stated purpose.

2. A comprehensible procedure, such that the grader could walk into lab and repeat your experiment exactly the way you did it.

3. A complete record of the results obtained.

4. Thoughtful conclusions.

5. Legibility: This does not mean it has to be a work of art, just readable. It is better and more time efficient to keep your notebook as you go, and occasionally cross things out, than it is to try to rewrite everything on your own time. If you tend to write small, or very lightly, please use an ink pen.

Rough Notes vs. Neat notes:

Some people like to go back a rewrite their notebooks in an organized, legible manner. Though these may look better than those that are written up as the experiment progresses, they do not serve the purpose of a lab notebook. The risk with this method is that you will miss important information during the "rewriting". It is easy to tell when books are kept this way and though they may look better, they will not be graded more favorably. In fact, if they look like they are being written after the fact, this will negatively affect the grade. If you insist on keeping this type of book, then use the left page for your rough notes and recipes. The right page can be left blank and filled in with more legible notes later on.

If you have any questions regarding how lab notebooks should be kept, feel free to ask Prof. Chalker during the lab sessions.