

## STUDY GUIDE - Molecular Biology Midterm Fall 2009

MIDTERM EXAM is Oct 5-7 in class.

Review session: Sunday Oct 4 at 4 PM in the lab. If it is too crowded we will move and I'll write the room number on the board. Please come *with QUESTIONS* (don't expect me to repeat the first half of the semester). If you cannot make the review and you have questions, let me know and we will try to set up a time to meet.

This exam will cover both theory and practice of the techniques we have performed this semester. You will not have to demonstrate techniques such as loading a gel. It will not include anything on 16s rDNA. M, T, W sections have all covered the same topics, but perhaps not in the same order or depth, so it is *important* that each student understand what we covered.

Lab #1, safety handout: Proper use and care of the micropipettors. Choosing the correct micropipettor. Determining the volume setting. How to draw up a sample. How to properly use the microfuge and other lab equipment. Understand how to work safely in the lab and how to handle problems such as broken glass or an alcohol fire.

Use of bacteria in the Molecular Biology lab: How to streak plates (with a plastic loop!). Understand how antibiotic resistance is achieved. Be able to explain our plating results with antibiotic resistant bacteria. Aseptic techniques and safe handling (importance of sterilized broths, agar, loops, pipette tips etc). Proper disposal of recombinant bacteria. Importance of using bacteria non-pathogenic to humans. Why are bacteria the workhorses of a modern molecular biology lab?

Bacterial culture techniques (*E. coli* growth curve): What growth phase is best for making competent cells or for isolating plasmid DNA? What techniques use broth cultures vs. individual colonies? When do we streak a plate and when do we spread?

Lab #2, cloning experiment: Understand how a gene can be cloned into a plasmid vector. Understand our cloning scheme. What bands to expect from restriction enzyme digestion of the starting plasmid pGEM and the successful plg recombinant.

**Understand all steps;** restriction enzyme cutting, heat inactivation, ligation, making cells competent, transformation, recovery, spreading plates.

A) Cutting the starting DNA, gel electrophoresis, and ligation.

- Care and use of restriction enzymes. Math problems on diluting buffers or DNA. Know how to set up a restriction digest using stock DNA and reagents. Know what a Unit of enzyme activity is, and how much enzyme to use in a digest. What is a palindromic DNA sequence? You should know how to determine the size of a plasmid or a gene after it is cut with RE. Know the difference between "sticky" and "blunt" ends.
- You should understand a plasmid map and the useful information it contains. What are selectable markers? Why are antibiotics most commonly used as selectable markers? What are the key features necessary for a plasmid to be useful to a molecular biologist? Be able to identify the important features, such as the drug<sup>R</sup> gene, polylinker or MCS, and ori.
- Be able to design your own cloning scheme if given the starting plasmid and gene. Remember that our cloning scheme was unusual because we cut the starting DNA with different enzymes than the enzymes used to identify the positive clones. One result is the possibility of re-forming the starting plasmid pGEM. How do we know that this was a problem in the cloning scheme?

- I was able to prevent most of the pGEM plasmid from religating by using the enzyme alkaline phosphatase (either the bovine or shrimp enzyme). Look up this enzyme and its mode of action. Be able to explain how the addition of alkaline phosphatase increased our chances of obtaining a positive clone.
- It is important to have a complete digest before cloning (why?) *and* to look at the gel to verify this point before you set up a ligation! We cheated here because of time constraints. Why must the restriction digests be heated before the ligations are assembled? How does ligase enzyme work? Why does the ligase buffer contain ATP?
- Be able to design a common cloning scheme, where you have only the gene and the vector. The gene is often obtained by amplifying it from its location on the chromosome using PCR, or by cutting a large amount of DNA (such as another plasmid) to release the gene and isolation only the gene from an agarose gel.
- Review the principles of gel electrophoresis. Know the concepts of how this technique can be used to separate DNA. Remember that movement in the gel is logarithmic, the smaller pieces move faster and the larger pieces move slower than if it was an arithmetic relationship. Know the importance of loading dye, ladders, how a gel is made (agarose and buffer), how to calculate the amount of agarose needed in a gel. How does ethidium bromide work? What is the difference between tracking/loading dye and ethidium bromide?

#### B) Making *E. coli* competent and plasmid transformation

Review how cells become "competent" and the importance of cold temperature and calcium ion. How do we get a plasmid into bacteria? What is thought to happen during the heat shock? Why do the cells require a recovery period before plating on the antibiotic-containing plates? Go over the different control transformations that we performed - competent cells alone, pGEM (the starting vector) alone. What purpose did each one serve? Know how to sterilely spread bacteria onto agar plates. What were the control plates we spread during this experiment? What would we expect to see on each of these plates? Why?

Some researchers do additional control transformations, such as a sample of the digested pGEM or a sample of the gene to be inserted. What would be the expected transformation results in each case? Suppose the cut pGEM transformation resulted in several colonies. What might be an explanation? Discuss with your lab mates and come up with an explanation.

#### C) Minipreparation of plasmid DNA

What growth stage of bacteria is used for the miniprep? Understand what each step in the mini prep procedure accomplishes. What is in the "white goop" that we create during the miniprep? Why do we have a timed step after alcohol addition but before centrifugation? Why did we do one wash of our DNA with alcohol?

#### D) Interpreting gels

- This is an acquired skill in Molecular Biology. Uncut (mini-) versus cut (mini+) DNA: Be sure you can interpret gel pictures, including banding patterns of circular plasmid DNA. You should know what conformations to expect in uncut plasmid DNA; super coiled, maybe linear, nicked circle. What RNA looks like on a gel (the "RNA cloud"); which fragments should be produced by digestion of our starting plasmids; what a partial digest might look like.

- What features of running miniprep DNA on a gel are usually absent in the more pure large-scale isolation procedures, such as DNA degradation visible in the lanes or chromosomal DNA in the wells? Interpreting pLIG DNA (pIg) is the most challenging, but you should be able to identify which fragments originated from starting plasmid or gene. In other words, how do we know if we have a positive clone?

#### E) Lab Math

Review unit conversions. For example, how many pmol make up 0.022 nmol? How would you express 330 mL in uL or in L? Know how to do the type of calculations you did in the homework. Remember you must show how you would actually *make the solution*; don't forget to bring solutions *up to volume*. Also, we add 1x buffer (not water) to the agarose powder. Know how to make a buffer containing two or more stock solutions. Know how to set up a restriction digest.

#### F) Controls

Finally, as we discussed in class, it is important that all scientists use the proper controls in their experiments. For Molecular Biologists, this is critical because most of the time *we cannot see* what we are studying!

- Understand the use of controls in the procedures we have done so far this semester. Include the proper controls in your exam answers, or you will lose points. Be able to suggest a proper positive or negative control. Be able to suggest possible errors in the experiment that can be detected by the controls (such as an inactive antibiotic resulting in growth on a negative control plate). Remember that "contamination" is a very *vague* and not acceptable answer.
- The Virtual Lab Book reading on Controls in Molecular Biology discusses controls for transformation and running gels, so this will be a good study guide. Chances are very good that you will have a cloning problem of some type. However, it will not be pIg. Don't spend a lot of time memorizing that cloning exercise. Concentrate on the main ideas that you can apply to a new cloning problem.

Remember the controls!