

Molecular Genetics Lab (Fall 2006)
BIO 443/BIO 543

Professor: Lizabeth A. Allison, Millington 308, X12232, laalli@wm.edu

Class time & place: Section 1 – Wed 1:00-3:50 pm; Section 2 – Thurs 1:00-3:50 pm
Millington 19

Objectives: The primary objective of this course is to provide a hands-on introduction to molecular biological methods. Molecular biology, in particular recombinant DNA research, has transformed research in the biological and medical sciences. Molecular biology techniques currently influence all aspects of biological and genetics research, have far-reaching applications in clinical diagnosis, and have led to important developments in agriculture and biotechnology. The lab will begin with an introduction to basic lab techniques and bacterial culture techniques. You will subsequently learn how to purify plasmid DNA and genomic DNA, and then use these preparations in experiments involving techniques such as restriction enzyme digestion, agarose gel electrophoresis, preparation of probe DNA, Southern transfer and chemiluminescent detection, mammalian cell transfection, analysis of green fluorescent protein expression by fluorescence microscopy, basic cloning techniques, and polymerase chain reaction (PCR).

Office hours: Scheduled hours: Mondays 1:00-2:00 pm, Thursdays 4:15-5:15 pm. Plus, “open door” policy for my office and research lab. Appointments are recommended, if you have more than just a quick question.

Grading: The final grade will be based on assessment of your lab notebook. Lab notebooks will be handed in half way through the course to provide informal feedback, and then handed in at the end of the course for grading.

Lab Manual: Handouts containing background information and lab protocols will be provided for each lab.

LAB SCHEDULE

Sept. 6 or 7 – Lab 1: Basic Lab Techniques

1. Introduction to the course.
2. Lab safety.
3. Basic sterile techniques: Measurements, micropipetting, and sterile techniques.
4. Pour LB agar plates containing ampicillin for use in Lab 2.

Sept. 13 or 14 – Lab 2: Transformation with Recombinant DNA

1. Discussion of the classic procedure for preparing competent bacterial cells.
2. Transformation of prepared competent DH5 α *E. coli* cells with recombinant plasmid DNA (for use in Lab 3)

Sept. 20 or 21 – Lab 3: Plasmid Purification

1. Analyze culture plates from Lab 2.
2. Purification of plasmid DNA from prepared DH5 α *E. coli* overnight cultures (from culture plates from Lab 2).

Sept. 27 or 28 – Lab 4: Gel Electrophoresis & Spectrophotometry

1. Agarose gel electrophoresis of samples of purified plasmid DNA (from Lab 3).
2. Quantify yield and assess purity of plasmid DNA by spectrophotometry.

Oct. 4 or 5 – Lab 5: Genomic DNA Purification

1. Discussion of the procedure for transfection of mammalian (mouse) cultured cells with recombinant plasmid DNA.
2. Extraction of genomic DNA from prepared transfected and untransfected mouse cells (transfected with plasmid DNA prepared in Lab 3).

Oct. 11 or 12 – Lab 6: Random Primed DNA Labeling & Restriction Digests

1. Nonradioactive probe preparation for use in Lab 7: Random primed DNA labeling with digoxigenin (DIG)-dUTP (plasmid DNA prepared in Lab 3).
2. Quantify yield and assess purity of genomic DNA (prepared in Lab 4) by spectrophotometry.
3. Set up restriction digests of genomic DNA and plasmid DNA (for use in Lab 7).

HAND IN LAB NOTEBOOKS FOR INFORMAL FEEDBACK BY FRIDAY, OCT. 13, 5:00 pm

Oct. 18 or 19 – Lab 7: Southern Transfer

1. Run agarose gels of genomic DNA and plasmid DNA from Labs 3-6 and set up Southern transfer.
2. Quantify yield of DIG-labelled probe made in Lab 6.

Oct. 25 or 26 – Lab 8: Southern Blot – Chemiluminescent Detection

Chemiluminescent detection of Southern blots (hybridization with probe will be carried out for you prior to the lab).

Nov. 1 or 2 – Lab 9: Basic Cloning Techniques

1. Analyze results from Southern blots.
2. Cloning: Ligation of λ DNA restriction fragments into the plasmid pUC18.

Nov. 8 or 9 – Lab 10: Green Fluorescent Protein Expression in Transfected Cells

1. Transformations from ligations (Lab 9). Plate on selective LB/Amp/X-gal agar.
2. Analysis of green fluorescent protein expression in transfected mouse cells: Fixation of transfected cells and slide preparation.

Nov. 15 or 16 – Lab 11: Polymerase Chain Reaction

1. Analyze slides from Lab 10 by fluorescence microscopy.
2. Analyze culture plates of ligations from Labs 9-10.
3. Detection of an *Alu* insertion polymorphism by polymerase chain reaction: isolate cheek cell DNA and amplify by PCR

THANKSGIVING BREAK (Nov. 22-26)

Nov. 29 or 30 – Lab 12: *Alu* Insertion Polymorphism Data Analysis

1. Gel electrophoresis of PCR products from Lab 11.
2. Analyze results.

Dec. 6 or 7 – Lab 13

Final analysis of all experiments.

TURN IN LAB NOTEBOOKS FOR ASSESSMENT BY THE END OF LAB.