LAB MANUAL

Lab in Cell Biology & Genetics

BIO 410

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OUTLINE OF EXPERIMENTS

This course will consist of four series of multi-week exercises plus a one-month period during which student groups will execute independent projects and report back to the class. The multi-week exercises consist of the following sets:

A. Cloning and hybridization analysis of a viral DNA segment (LRB).

Lab 1: Restriction and gel analysis of lambda and plasmid DNAs. Purification of restricted DNAs and ligation.

Lab 2: Transformation of bacteria with ligation mixtures and selection for a plasmid drug-resistance marker.

Lab 3: Preparation of DNA from transformant colonies; restriction analysis.

Lab 4: Southern blotting. Preparation of probe.

Lab 5: Hybridization and detection of hybridizing fragments of the viral genome.

B. Detection of novel tissue-specific enhancers by mobilization of a *Drosophila* transposon (DET).

Lab 1: Cross transposon-bearing flies to transposase-positive flies. Familiarize with markers and general techniques.

Lab 2: Select transposase-positive flies bearing the transposon and cross to generate "jumps" to new sites.

Lab 3: Select flies containing new sites of the transposon and cross to generate new strains.

Lab 4: Dissect adult tissues and stain for enhancer-driven β-galactosidase expression.

Lab 5: Analyze stained slides for tissue-specific expression of β-galactosidase.

C. Biochemical Fractionation of Chloroplasts (BFC).

Lab 1: Isolate intact chloroplasts by density gradient centrifugation. Fractionate chloroplasts into thylakoid and stromal components.

Lab 2: Determination of protein and chlorophyll content of stromal and thylakoid fractions.

Lab 3: Analysis of stromal and thylakoid proteins by SDS-PAGE.

Lab 4: Photograph gels; discuss results.

D. Control of nitrate reductase by light and nutrient levels during leaf development (NRI).

Lab 1: Radish seeds will be planted and germination will be carried out under a variety of regimens of light and nitrate.

Lab 2: Nitrate reductase levels will be determined spectrophotometrically by both *in vitro* and *in vivo* assays.

Lab 3: Protein concentrations will be measured to determine specific activities for nitrate reductase in the *in vitro* assay.

E. Independent Projects

During April, each group of students will carry out an independent project to extend work of the four exercise series or to carry out new experimental approaches. During April, students may have access to the lab at any time by arrangement with the instructors or graduate assistants. The purpose of this component is to enable students to explore new techniques or combinations of techniques in a less structured atmosphere.

Proposals: As with most research, these projects must be planned in advance. During March, a brief proposal for the independent project must be submitted by each group, after consultation with the instructors. Proposals must be less than one-page and must include:

A. A simple statement of experimental objectives
B. A brief description of materials needed for this work
C. A brief description of the work to be performed, including a schedule

It is strongly recommended that planning begin early in March to allow ample time for discussions with the instructors and preparation of the proposal.

Oral Reports: Each group will present their April projects to the rest of the class at the regular lab meeting time during the last week of the semester. Presentations will be made in order of group number (*i.e.*, 1, then 2, ...). Each member of the group will participate in the presentation, roughly dividing the presentation equally among the group members. The presentation of each group will be graded according to the quality of the work actually carried out as well as the quality of the presentation. As usual each group member receives the grade of the group, so practice by the group is strongly recommended. Presentations should be approximately twenty minutes each and should be organized as follows:

Introduction and objectives of the work
Specific aims
Results
Conclusions
Examples of additional possible work

An overhead projector will be present, as well as chalkboard. Please contact the instructors if you need transparencies made.

Come prepared to participate in discussions of all projects.

GENERAL LABORATORY SAFETY

Principles: The laboratory is a potentially dangerous place for those who are careless or irresponsible (or those who are near such persons), irrespective of their experience or skill. Common sense combined with thoughtful use of instruments and chemicals is essential to prevent injury to yourself or to others. **The following rules apply without exception**.

1. **NO** food or drink may be brought into the lab.
   NO smoking is allowed in the building.

2. If the fire alarm is sounded, immediately exit the building using the nearest staircase. Do not use the elevator.

3. All broken glass, microscope slides, cover glasses, other glass objects or sharp metals objects must be discarded in the GLASS DISPOSAL container. **Never** place glass or sharp objects into the regular waste baskets.

4. Used solvents, fixatives, acrylamide, and other chemicals must be placed into the appropriate waste containers within and near the chemical fume hood. **Never** pour organics or any hazardous wastes down the sink.

5. Shoes must be worn in the lab at all times. Inadvertent chemical spills and glass breakage are commonplace.

6. A number of protocols must be performed in the chemical fume hood. All use and disposal of volatile organic solvents such as acetone and noxious chemicals such as 2-mercaptoethanol must be used only in the fume hood.

7. **NEVER** pipet solutions by mouth in this lab. Always use a mechanical pipetting device.

8. In case of problems, the following safety aids are present in and near the laboratory:

   - A fire extinguisher is located next to the main door of the lab
   - A first-aid kit is located next to the main door of the lab
   - An emergency shower is located in the hallway outside the main door to the lab
   - Disposable gloves and goggles are always available
   - The nearest fire alarm is located in the hallway outside the lab

9. Students must clean up their bench areas before leaving the laboratory. They must wash their own glassware, rinsing with distilled water, and leave the glassware on the glassware drain cart, inverted for drying. Always wash your hands when leaving the lab after handling microoganisms or any hazardous reagents.

10. The instructor(s) and the graduate assistant(s) supervise the lab. They are responsible for the proper and safe operation of the lab. If you have any questions about any protocol, *ask*. Your suggestions for improved safety of the lab will be appreciated.