

**Ohio University**  
**PBIO 313/693, Special Topics, 3credit hours**  
**Lab in Cell and Molecular Plant Physiology (Lab CaMPP)**  
**Summer 2007**

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Office Hours: by appointment

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**Course Overview:**

Lab CaMPP is an intensive laboratory experience in plant cell biology, molecular biology and physiology for upper division undergraduates and graduate students. This is a unique and excellent opportunity for students to gain experience in techniques routinely used in research labs. Topics include: DNA biotechnology, protein biochemistry (SDS-PAGE, Western blotting), plant cell biology (organelle fractionation), enzyme activity measurement, and plant transformation (expression of GFP)

**Lab:** MTWThF 8 am- 6 pm

**Lab manual:** provided

**Class Format:** The class will consist of 5 laboratory exercises in plant cell and molecular biology and plant physiology. Lectures will be integrated throughout the lab exercises as needed to supplement and enhance the laboratory component.

**Grading:** Your final grade (1000 points) will be determined by adding points from take home assignments (mostly dealing with the interpretation of the data generated during the labs), and your performance in labs. The class has 5 laboratory exercises and each lab is 200 points that includes 100 points for the assignment and 100 points for bench work. Your final grade will be determined by adding points as follows:

A+, A, A-	100-90%
B+, B, B-	89-75%
C+, C, C-	74-65%)
D+, D, D-	64-60%)
F	<60%

**Policies on Attendance:** You are expected to attend all lab sessions. Missed work will not be excused without adequate proof of legitimate absence as per University policy.

**Academic dishonesty:** Any evidence of cheating, including falsification or misrepresentation of data or plagiarism will result in failure of the course.

# Lab CaMPP content

## Cell Biology experiments:

### **Lab 1: Cell growth (Monday):**

1-Growth curve and cell titer estimation by absorbance at 660nm and by plating..

### **Lab 2: Transient expression of GFP in tobacco leaves**

1-Inoculation of tobacco leaves (Monday)

2-After 5 days take pictures and discussion (Friday)

### **Lab 4: Cell fractionation (Wednesday)**

1-Organelle fractionation

2-Marker enzyme assays to assess organelle distribution

3-Collecting the data and analysis

## Molecular Biology experiments:

### **Lab 3: Recombinant DNA technology (Tuesday-Friday)**

1-Genomic DNA extraction, gel electrophoresis

2-PCR-based cloning and bacterial transformation

3-Southern blotting, colony lifting, probe preparation

4-Making the probes, hybridization, and detection

5-Extraction of plasmid DNA and diagnostic digests using restriction enzymes

6-Analysis of the orientation of the gene in the plasmid

## Protein Biochemistry:

### **Lab 5: SDS-PAGE and western blotting (Thursday-Friday)**

1-Preparation of gel and samples

2-Running the gel and transfer to membrane (western blotting)

3-Processing the gel and membranes

4-Analysis of the bands and creating standard curve

# Block Plan

Time	Monday	Tuesday	Wednesday	Thursday	Friday
8-9	Introduction	Lab 3 – gDNA extract, PCR	Lab 4 – cell fraction	Lab 5 – -Running protein gel  -Protein transfer to membranes	Lab 3– -Plasmid extraction
9-10	Lab 1 – Cell growth		Lab 1 – (finish)		Lab 5 – -Diagnostic digests using RE
10-11			Lab 5 – Western (Finish)		
11-12					
12-1					
1-2	Lab 1 – (cont.) Cell growth	Lab 3 – gel, TA clone PCR product, transform <i>E.coli.</i>	Lab 4 – cell fraction	Lab 5 – -Gel staining	Lab 2 –GFP in tobacco leaves (cont.)
2-3	Lab 2 – GFP in tobacco leaves		Lab 3 – -Colony lift -Probe preparation	Lab 3 – -Washes and detection	-Final discussion
3-4					
4-5					
5-6					
6-7					