

Bio 4024 Plant Cells and Proteins Laboratory

Spring semester, Monday 3:00-4:00; Wednesday 12:00-4:00; Friday 12:00-4:00

3 credits

Location: Monday lectures will be held in McDonnell Hall 412. Laboratory sessions will be held primarily in Rebstock Hall 126. Some lab sessions will be at the Donald Danforth Plant Science Center (DDPSC), depending on the topic.

Course coordinators:

1. Prof. Craig Pikaard, Biology Department, pikaard@biology.wustl.edu; phone: 314-935-7569;
Office: Monsanto Hall Room 505
2. Dr. Joseph Jez, DDPSC and Adjunct Assistant Professor of Biology at Wash. U.,
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Instructors:

Dr. Craig Pikaard (pikaard@biology.wustl.edu)
Dr. Joe Jez, DDPSC (jjez@danforthcenter.org)
Dr. Chris Taylor, DDPSC (ctaylor@danforthcenter.org)
Dr. Leslie Hicks, DDPSC (lhicks@danforthcenter.org)
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Teaching assistants:

Thomas Ream (tsream@artsci.wustl.edu)
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Course description:

This course focuses on methods for the engineering, manipulation and analysis of proteins. In the course, we will begin by purifying a thermostable DNA polymerase and then use it to clone, by polymerase chain reaction (PCR)-amplification the coding regions for a number of genes encoding proteins of current research interest. These proteins will be engineered for overexpression in *E. coli* and some will be fused to yellow fluorescent protein for visualization in transformed plant cells using fluorescence microscopy. Along the way, we will learn about thermal stabilities of proteins, ion-exchange and/or affinity chromatography, measurement of protein concentrations, assessment of protein purity by SDS polyacrylamide gel electrophoresis, analysis of enzyme function, protein identification by mass spectrometry and an introduction to structural analysis through X-ray crystallography. Prerequisites: Chem 252 and Bio 2960 (or equivalent). Enrollment is limited and requires the permission of Dr. Pikaard.

Grading: 50% of the grade will derive from the periodic evaluation of lab notebooks. The remaining 50% will derive from three written reports/take-home exams that will cover different aspects of the course.

Textbook: There will be no textbook for the course. Reading assignments will be handed out in class.

Laboratory notebooks:

Buy a bound (not a loose-leaf or 3-ring binder) notebook with numbered pages to serve as your graded laboratory notebook. You will also want a separate notebook for storing lecture notes and handouts; a 3-ring binder will probably be useful in this capacity.

1) Label the first page of the Laboratory notebook: Table of contents.

On this page you will list the various experiments and the page numbers where the relevant experimental procedures and data can be found. This will help you and the instructors locate information quickly.

2) Use a ball-point pen for all notes, not pencil (or ink that will run if wet).

3) Each experiment begins on a new page (on the left side of an open notebook) and gets an experiment number at the top of the page. The experiment number is composed of your initials and the dates, e.g. CP080119 would be the experiment number for Craig Pikaard on Jan 19, 2008.

-Any tubes, gels, data etc. pertaining to a given experiment will be labeled with the appropriate experiment number, even if the data are gathered on a subsequent day.

-use the left-side page for your working notes while the lab is in progress, keeping each right-side page blank. Later, you will copy (neatly) and edit (if necessary) your notes onto the right hand side page. Grading will be based on what is written on the right side.

4) Following the experiment number, the next item on the page is the experiment name - ask your instructor for what this name should be.

5) Next comes Objectives: here you state the purpose of the experiment in a sentence or two.

6) Next comes References: refer to any protocols, publications (or previous experiment numbers elsewhere in the notebook) that pertain to the experiment.

7) Next comes Procedures: here, you provide your step by step descriptions of the experiment(s). Most likely, this section will also include results as you obtain them and move forward with subsequent steps.

8) Finally comes the Summary of Results and Conclusions: did the experiment work? If so, what were the findings and what do they mean (interpretation). If not, what do you think might have gone wrong and what corrections should be tried if the experiment were repeated, etc.

Syllabus:

- Mon 1/14 Course overview, grading, keeping notebooks; introductory lecture on protein synthesis, biochemical properties and analysis of proteins (Pikaard)
- Wed 1/16 Begin Pfu polymerase purification; harvest E. coli cells, make extract, heat denature and clarify homogenate by centrifugation
- Fri 1/18 Ion exchange chromatography (SP FF). Save aliquots of column fractions for SDS-PAGE and protein concentration assay; dialyze fractions; set-up PCR reactions for overnight run.
- Mon 1/21 Martin Luther King Holiday; no class
- Wed 1/23 Analyze PCR reactions by agarose gel electrophoresis. Run SDS-PAGE gel of column fractions; stain and destain. Compare resolved proteins with PCR results. Perform protein concentration assay.
- Fri 1/25 Make cDNA from RNA. Set up PCR reactions to clone cDNAs and genomic clones of target proteins (RNA polymerase RPB7 and RPB9 subunit variants; others)
- Mon 1/28 Lecture: Gateway recombinational cloning (Pikaard/Ream)
- Wed 1/30 Analyze PCR products by agarose gel electrophoresis. Purify amplified bands of correct size, clone into Gateway pENTR vector using topoisomerase-mediated capture. Transform pENTR reactions into TOP10 cells. (TA can plate cells, if necessary)
- Fri 2/1 Miniprep plasmids to verify positive clones identified by TAs using colony PCR. Restriction enzyme digests and DNA sequencing reactions to verify clones.
*Lab notebooks due
- Mon 2/4 Lecture: Protein Purification & Expression (Jez)
- Wed 2/6 Analyze sequence data. Set-up recombination reactions to transfer cloned inserts into pDEST17 for overexpression of 6x His-tagged proteins in E. coli BL21 cells. Also mobilize into pEarleyGate plant YFP fusion vector.
- Fri 2/8 Verification of clones by restriction digestion (TAs will inoculate cultures for minipreps)
- Mon 2/11 Lecture: Protein Analysis (Jez)
- Wed 2/13 Analyze cultures set-up by TAs for protein overexpression in E. coli. (TA: Induce, grow, & pellet) Resuspend, chemical lysis, spin to obtain soluble and insoluble (e.g inclusion body) fractions; nickel-affinity column for soluble fraction. Prepare samples for SDS-PAGE
- Fri 2/15 SDS-PAGE analysis of protein samples. Set up lysozyme crystallization experiment.
*Lab notebooks due
- Mon 2/18 Lecture: DDPSC Safety lecture
- Wed 2/20 Mass spectrometry (MS) lecture (Hicks); Isolate bands from gels, trypsin digestion
- Fri 2/22 Protein identification via MS analysis of tryptic peptide fragments
- Mon 2/25 Lecture: Plant transformation (Taylor)
- Wed 2/27 Transform Yfp-fusion constructs into Agrobacterium by electroporation
- Fri 2/29 Inoculate stem sections *Report 1 (covering 1/14-2/22) Due
- Mon 3/3 Lecture: Microscopy (Berg)
- Wed 3/5 Nicotiana benthamiana leaf infiltration. TAs will also infiltrate leaves on Tuesday.

Fri 3/7 Examine infiltrated leaves by fluorescence microscopy; transfer last weeks inoculated tissue to perlite
 *Lab notebooks due
 Mon 3/10 Spring break
 Wed 3/12 Spring break
 Fri 3/14 Spring break

 Mon 3/17 Lecture: X-ray crystallography (Jez)
 Wed 3/19 Crystallography lab
 Fri 3/21 Crystallography lab

 Mon 3/24 Lecture: Enzyme Kinetics (Jez)
 Wed 3/26 Kinetics lab
 Fri 3/28 Kinetics lab
 *Lab notebooks due

 Mon 3/31 Lecture:
 Wed 4/2 Microscopy; composite plants
 Fri 4/4 Microscopy; composite plants
 *Report 2 (covering 3/17-3/28) Due

 Mon 4/7 Lecture: antibodies and immunological techniques
 Wed 4/9 Western blotting- run gel and electroblot
 Fri 4/11 Western blotting- Filter prep, blocking and primary antibody incubation

 Mon 4/14 Lecture
 Wed 4/16 Western blotting- Secondary antibody; washing; develop
 Fri 4/18 TBA

 Mon 4/21 TBA
 Wed 4/23 TBA
 Fri 4/25 *Lab notebooks due
 *Report 3 (covering 2/25-3/7 & 3/31-4/25) Due