Syllabus for Biology 498—Protein Purification Workshop, Semester II, 2012-2013
Instructor: Ed Gasque

Workshop Dates and Times: The Spring 2013 workshop is held on February 1-2-3 in TNR Room 454.
Friday 1:00 to 6:00 PM; Saturday 9:00 AM to 6:00 PM; and Sunday 9:00 AM to 6:00 PM

The purpose of this workshop is to give students experience in the theory and practice of protein purification techniques. We will be purifying recombinant Green Fluorescent Protein (GFP) from a total protein extract of genetically transformed *E. coli* using a variety of chromatographic techniques.

The workshop includes lectures on bioluminescence, GFP, protein structure, and the principles behind protein purification methods, and centers around first-hand lab experience with these techniques. Students print out a set of protocols that function as a lab manual for the workshop, using a file provided by the instructor. Students also print out background website material and a set of instructions on the use of Excel in the analysis of protein electrophoresis data. Students provide their own cloth-bound, composition-style lab notebook. The instructor provides a set of figures to accompany background lectures, as well as all laboratory supplies.

Requirements for the workshop include: (1) Full attendance and participation in all of the classroom and lab activities on Friday (1 to 6 PM), Saturday (9 AM to 6 PM), and Sunday (9 AM to 6 PM); and (2) submission of a bound laboratory notebook for evaluation. You must keep a neat, accurate and complete record of the following in your lab notebook: **(A)** *Detailed* notes based on the introductory lecture and the series of mini-lectures presented by the instructor; **(B)** any and all modifications and/or clarifications of procedures and methods, cross-referenced to the protocols handout; **(C)** all of your experimental data and observations, neatly and logically organized; **(D)** your purity and yield table, plus all of the calculations related to the data in this table, neatly presented; **(E)** *detailed* written comments on experimental results, observations, and data interpretation; **(F)** *detailed* responses to questions posed at the end of the protocols handout; and **(G)** any further information or requirements indicated verbally by the instructor during the workshop.

A grade of A will be earned for *full* attendance and participation *and* submission of a lab notebook that is *complete*, *detailed*, and *accurate*. *Since no tests or quizzes are given in this course, it is expected that you will include detailed and complete notes on all the lecture materials presented by the instructor.* **In the case of presentations related to chromatographic and electrophoretic techniques (ASP, IEC, HIC, SEC, AC, and SDS-PAGE), you *must* include *detailed* descriptions of the *animations* shown for each of these.** Grades of A- to as low as D will be earned if a student attends and participates fully, but submits a lab notebook that is *deficient* in one or more ways—*the greater the number of deficiencies, the lower the grade*. A student *cannot* pass the course without the submission of a lab notebook, and a grade of F will be given to anyone who fails to submit a lab notebook. While I do not issue blanket grades of A, typically many of those enrolled in the workshop earn grades of A or A-, since these students attend/participate fully and submit complete, accurate, and detailed lab notebooks. ***Refer to the reverse side of this sheet for further details regarding lab notebooks.***

Laboratory Notebooks: Submit by placing in the appropriate box outside the instructor's office (446 CNR) no later than 5:00 PM on Monday, February 18. You will be informed via email by the end of the day on Tuesday, February 19 of your course grade. If you earn a course grade less than an A due to one or more deficiencies in your lab notebook, you will be notified of the corrections and/or additions that are required to bring your grade up to an A. You will then be given the option of making these corrections and/or additions and submitting your revised notebook re-evaluation. **Notebooks submitted for re-evaluation are due no later than 5:00 PM, Tuesday, February 26. Re-submitted notebooks will *not* be accepted after that date.**

Biology 498, Spring 2013
Protein Purification Workshop
Reminders concerning lab notebooks

- **Include detailed and complete notes from the lectures presented during the workshop. The notes should be detailed to the point that you could study them in preparation for an exam on the material covered during the course!**

- **Include clarifications/modifications made to specific steps of specific protocols. Do not copy the detailed protocols into your notebook. Include only procedures that we changed and any clarifications that you feel are necessary.**

- **Include a neatly constructed purity and yield table, with clearly designated headings as specified in the protocols and on the board in class. Include sample calculations.**

- **Include the image of the stained SDS-PAGE gel that includes your group's samples. Do not include images of the other groups' gels. Label lanes clearly according to stage of purification. Label marker lane that you used to generate your standard curve. Write a detailed caption to accompany your gel image. Comment on how the number of protein bands pertain to how pure the GFP was after each stage of purification. Compare and contrast the information you obtain from your analysis of the stained gel with the numerical purity data in your table. Do the numerical data on GFP purity at the different stages agree with the information in your stained gel? What possible reasons might there be for any discrepancies? Address these questions in the caption. The caption to the gel image should be a detailed paragraph of sufficient length to cover these points.**

- **Present your Excel analysis and determination of GFP molecular weights in a clear and logical fashion. Include the data table and graph generated via the Excel analysis of your data. Display clearly the molecular weight of the sgGFP purified via sequential techniques ending with SEC (i.e., the single prominent band in the SEC lane), as well as molecular weight of the polyhistidine-tagged sgGFP purified by affinity chromatography (i.e., the single prominent band in the AC lane).**

- **Write clear, detailed responses to the questions at the end of the protocols. Don't forget to cite the web address for the site you visited for the last question.**

- **Submit notebooks for evaluation or re-evaluation in the box labeled "Biology 498 Lab Notebooks for Spring 2013" located outside my office door, by the specified deadline. Send me an email message just after you submit or re-submit your notebook so that I can collect it and move it into my office for safekeeping. Notebooks are returned using the same box.**