

Chem 405 Biochemistry Lab I Experiment 5 -Purification Tutorial

Introduction: For the next five weeks, you will be working on purifying your fusion MGH protein. One of the colonies from your transformation was selected and grown up in a broth that included IPTG, a chemical to induce the bacteria to express (produce) the protein on the plasmid (MGH). These cultures were centrifuged down and the cells lysed (using both sonication and enzyme membrane degradation) by your instructor). YOU will be responsible for making the buffers (from stock concentrations) planning the experiments (using the information, protocols and teg videos provided) save your samples, prepare them for the next chromatography and analyze at each step of the way. Below are the steps that you will carry out this week in preparation for next week when YOU will be starting your purifications. To start you off, in class, your instructor will show you how to use the equipment (column adaptors, columns, fraction collector, dialysis buffers...). Outside of class, you will watch the teg files to help remind you how to do this, and EACH of you will work through a tutorial to walk you through the methods necessary for your purification. There will be a simple worksheet for you to fill out when doing the tutorial (see below).

References

Stratagy of Purification Web Based Tutorial (go to the lab website for the live link)
<http://home.btconnect.com/agbooth/archive/swingPP/Protlab.html>

Procedure - You will follow the instructions below and answer the questions. The questions will be due at the next laboratory. You are to do this tutorial independently. Use either the computers in the biochem lab or on your own (following the instructions on the starting page).

Steps:

- 1) Start by reading the instructions on the home page. There is a link at the very top of the page "go to the first exercise" this is optional if you seem to get stuck.
- 2) Otherwise, start by clicking on the "click here" to start the applet in a new window".
- 3) Under that start file menu, select start from beginning. Select default_mixture. Then chose a number 1-20 to pick your protein. Do not do protein #10. Note the info for your protin, it will help you in your purification.

Basics for Chromatography: Based on the tutorial, you will choose two different chromatographic medias (techniques) and plan how to prepare your sample and run the proteins through the column. With each step of the purification, you will need to remember the following general flow of activity

1. Design Chromatography
2. Prepare column and buffers
3. Load and run the chromatography
4. Analyze the fractions for MGH and total protein.
5. Pool fractions (save portion)
6. Prepare pooled samples for next chromatography.

To best prepare for this, you are assigned to go through a virtual purification tutorial. This tutorial will take from 3 to 4 hours depending on the number of interruptions and pacing of your study.

Next week we will work on the mechanics of using the equipment and start your purification from lysate

- 4) At this point you will get a sort of blank screen. The help menu and read scenario and getting started menu choices. Then choose the Strategy option under the help menu. There are a few questions based on this in the questions below. If you need additional help, there are links to each of the techniques as well as sections in your textbook to help your choices along the way. Again, under the help menu, choose the costs to review how much time it will take to do the steps.
- 5) Now you may start by picking a separation method. Again, if you are not certain which method to start with, read the index option in the help menu to read about each step. You can also use the info button when using a separation technique.
- 6) After running through a separation method, then analyze your results. To do this, assay the sample for enzyme activity. If the activity curves are too high (off scale) dilute the sample in the fraction window.
- 7) Use the Electrophoresis menu to conduct gels and blots. Choose several fractions in the middle of your peak as well as away from your activity peak to assess the effectiveness of the purification. Then look at the immunoblot to determine which band is yours. **DO NOT ASSUME THAT THE BIGGEST BAND IS YOURS.** The biggest band could easily be a contaminant!
- 8) Pool your fractions. Use the activity and electrophoresis (not the western blot) to determine which fractions should be pooled. This is a step where you will be forced to choose between high yield or purity. Protein purification typically has only a few percent yield as compared to a yield usually found in an organic chemistry lab.
- 9) Run the sample through another method if needed.
- 10) Print out the final result once you reach purity. If you are fired due to inefficient work, then start over. You must have a finished progress report for the successful completion of the assignment.
- 11) Finish the page of questions found below. The assignment is due next week in class and **MUST** be typed.

Turn **typed** at the next lab meeting (40 points)

Name _____ Lab Section (Tuesday or Thursday)

Turn in the print-out (or record the final purification report if you can't get it to print) and included that with your answers.

Answer Each of the Following Questions:

- 1) What are the basic steps in purification. Briefly describe each step. THIS IS NOT FROM MY CLASS NOTES!!!!
- 2) How can you determine where the enzyme is?
- 3) What is specific activity and how do you calculate enzyme enrichment?
- 4) How will you know which band on the gel is yours?
- 5) What steps did you take in your purification AND why?
- 6) What is hydrophobic interaction chromatography?
- 7) What would happen if you use a steep ion gradient (salt gradient) versus a shallow gradient (using low salt as an ending point in the gradient)? Explain in terms of purification and the concentration of samples eluting from the column.
- 8) How can an antibody be used in affinity chromatography? How does one choose which elution method when using an affinity chromatography?
- 9) What happens if you take a wide cut (pool all possible fractions with activity) when pooling your samples vs taking the fractions that represent 70% of the peak of enzyme activity?
- 10) Did this program help prepare you to understand the purification processes you are about to undertake? Do you have a little more confidence in planning your purification?