

## **PBC Day 2 Interpretation Questions and Answers**

1. Calculate the total activity for each of your samples ( DEAE/AF Load, DEAE-Total, AS-S and AS-P). Are your results consistent with your expectations? Why or why not?
  - **Answers will vary by student group depending on data. Calculations follow the example on pages 13-15, but be careful that they are using their own numbers for "total volume," "volume used in reaction," "time," and "OD420" and not those given in the example!**
  - **We would expect that AS-P would have the most total activity and AS-S the least; if they significant activity in their AS-S, it may be because AS precipitation was incomplete or their pellet was "flaky" and some remained in their AS-S when we separated supernatant from pellet. DEAE/AF-LOAD is the "desalted" AS-P, so the value of the load should not be the same order of magnitude as the AS-P, if not very close to it. (They probably did not pool every tube which had Bgal activity, so some loss will be expected here.). Finally, DEAE-Total should have the less TA than DEAE/AF-LOAD. Again, this is because loss will occur at each step of the purification (both because not every sample was saved and/or denaturation of Bgal).**
  
2. Oops! You didn't read your laboratory protocol very well, and forgot to run the PD-10 column today. Instead, you took your resuspended AS-P and loaded that sample directly onto the equilibrated DEAE column, then followed that protocol exactly.
  - a) What is the purpose of running the PD-10 column today?
    - **To desalt (remove the AS) the AS-P fraction, leaving Bgal (and other precipitated proteins) in a lower salt solution.**
  
  - b) In what fraction from **your** DEAE column (FT, 0.1M, 0.4M #1, 0.4M #2, 1.0M) will you expect to find Bgal? Explain your answer.
    - **You will likely find Bgal in the flowthrough. Under high salt conditions, the amount of anions in the solution is high. Thus, they will outcompete Bgal and other negative proteins for binding to the positively charged matrix. Thus, Bgal will not stick to the column and will simply "flowthrough."**

## PBC Day 2 Interpretation Questions and Answers (continued)

3. You have a solution containing ammonium sulfate (salt), B-galactosidase and a positively charged protein called SPA (Super Protein Andy); the pH of the solution is 7.5. Using the techniques you have learned thus far in the PBC module, design a purification scheme that will allow you to separate B-galactosidase away from the salt and SPA. Be sure to be specific about:
- What columns you'd run
  - In what order you'd run them
  - Where you'd expect to find Bgal, salt and SPA after running each column (e.g. in the flowthrough, stuck on the column until eluted with (buffer name), etc.) and why.
- **First column: To separate the salt from the two proteins (both of which are significantly larger than salt), you would run a gel filtration/size exclusion column like the PD-10 column we ran in lab. Bgal and SPA will move quickly through the column because they cannot enter the porous beads (and will likely be found in the same fraction(s)), whereas the salt will be retarded in the column because it can enter the beads.**
  - **Second column: Once you have identified the fraction(s) containing Bgal (and SPA), you would run an anion exchange column. At pH 7.5, Bgal is negatively charged, whereas SPA is positively charged. Thus, when your sample is applied to the column, SPA will be repelled by the positively charged beads, and will exit the column in the flowthrough (or perhaps the wash). Bgal will be attracted to the positively charged beads, and will stick to the column until it is eluted with increasing concentrations of NaCl. The Cl<sup>-</sup> ions will compete with Bgal for binding to the positively charged beads, and Bgal will come off the column and can be collected.**