Post lab analysis for Part A:
Osmotic shock extraction of alkaline phosphatase from E. coli

1. What is the molecular weight of dimeric and monomeric AP?

   Monomeric: ~47 kDa = 47 g/mol
   Dimeric: ~94 kDa = 94 g/mol
   http://websites.umich.edu/~protein/AP/D2O/AP-structure.html

2. What is being tested in the PNP activity assay? What does this assay tell you about yield of AP?

   The PNP activity assay is testing whether (and/or how much) PNPP is dephosphorylated by AP to PNP. PNP is yellow above pH 7.5 (as it is in this case) so you can qualitatively observe the activity of AP by the speed and intensity of which the sample turns yellow.

3. Estimate the concentration of total protein based on your nano-drop absorbance measurement. What assumptions do you make this estimation?

   Stage 1:
   A280 = 21.126 mg/ml
   260/280 = 1.94
   Cannot use Beer's law because many species of protein are in solution. There is not a fixed extinction coefficient for all proteins.
   Assume that most all/proteins have absorbance activity at or around 280 nm?

Post lab analysis, Part B.
Heat Denaturation and Ammonium Sulfate Precipitation of Alkaline Phosphatase

1. What happens when most proteins are heated? What about when they are then rapidly cooled? How are these properties used to purify heat-stable proteins? Why do denatured proteins pellet when centrifuged?

   Most protein denature when rapidly heated. The rapid cooling is to slow digestion of protein (by proteases) we want to keep. Dimeric AP is a heat stable protein while most contaminating proteins are not, so this is a viable purification step for this protein. When protein is denatured, hydrophobic regions are no longer hidden, thus it is less soluble, thus it can be precipitated easier from solution.

2. How is protein solubility generally affected by solution ionic strength? What is happening when Ammonium Sulfate is added to your protein suspension? Why?

   Generally, at low ionic strengths, increasing the solution ionic strength increases the solubility of a protein. As ionic strength increases, protein solubility again decreases (bell shaped curve). Ammonium sulfate dissolves into its ions when put into solution. Its positive and negative charges interact with proteins in solution and prevent the protein from interacting with water. Thus, the protein ammonium sulfate complex can be precipitated more easily.

3. What is dialysis? Why do you need to know the molecular weight cutoff for the tubing?

   Dialysis can help remove small compounds from solution. It works via concentration gradient diffusion. Protein sample and a buffer solution are placed on opposite sides of a semi-permeable membrane. The buffer solution is then changed for a solution of much lower concentration. This causes small molecules from the sample to move down their concentration gradient into the buffer solution. The process can be repeated as many times as one desires. You need to know the molecular weight cutoff for tubing so you don't diffuse and lose the sample of interest.

4. Estimate the concentration of total protein in your stage II and III extracts and compare to Stage I. How does the activity assay compare for stages 1-3 extracts? Describe how these changes are expected/unexpected based on the purification schemes.

   Stage 2:
   A280 = 19.024 mg/ml
   260/280 = 2.18

   Stage 3:
   A280 = 2.483 mg/ml
   260/280 = 1.91

Post lab analysis, Part D. (no questions, write notes)
Purification of Alkaline Phosphatase to Stage V via Anion Exchange Column

Best fraction appeared to be #2. This is probably because the first fraction contained some left-over wash buffer.

Stage 4 skipped

Stage 5 extract has 10 total fractions. Fractions 1-3 analyzed in AP2.