

# Nickel Affinity Purification Worksheet

Name \_\_\_\_\_

1. Briefly describe how you made the genetically modified bacteria used in this lab.
2. What is the difference between column and batch purification?
3. On the first day of the protocol, you picked a pink colony to grow overnight. Why did you avoid picking a white colony? If only transformed bacteria should have grown on the plate, why were there white colonies on your amp ara plate?
4. What is the purpose of lysing the cells? How will this be accomplished? Name at least two techniques that will be used.
5. Why are nickel beads used during the protein purification? Briefly describe the role they play in this procedure.

6. Professor Farnsworth is purifying a 6-His-tagged RFP. While the nickel beads have turned pink, he is surprised that the cell lysate is also still pink following the incubation period with the nickel beads. Offer a hypothesis to him to explain these results.
  
7. What is the purpose of the PBS buffer used in step 13 of the third day of the lab? What could happen if you skipped this step?
  
8. Briefly describe how the elution buffer works.
  
9. Instead of purifying 6-His-tagged RFP from *E. coli*, Professor Wernstrom wants his graduate student to purify the red fluorescent protein directly from cells of sea anemone he caught in the wild to speed up the process by avoiding all the genetic recombination steps in *E. coli*. Why or why not is this a good idea if he still wants his student to use nickel affinity chromatography?
  
10. Professor Farnsworth's student Amy decides to run a gel to compare the crude protein lysate to the effluent. She stains the gel to visualize all proteins. What purpose does this gel serve? Describe what you believe the gel will show.