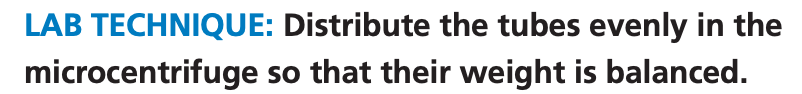
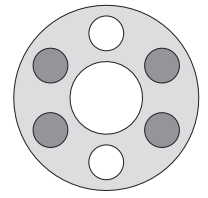
**Lab 6B: Separating the RFP Using HIC Column Chromatography**

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| **LAB TECHNIQUE NOTES: All appropriate safety precautions and attire required for a science laboratory should be used. Please refer to your teacher’s instructions. Use *aseptic technique* when working with E. coli bacteria. Discard all tubes and tips from this lab into the WASTE containers indicated by your teacher.** |

Place your EC tube in the high speed microcentrifuge and spin down for 5 minutes.

Label two microfuge tubes “SUPER” and “RFP” and with your group and period ID..

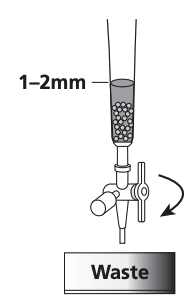
3. Prepare the column:

Place the small waste container under the stopcock

Open the stopcock by turning it clockwise and allow the liquid to drain.

Once there is only 1-2mm of liquid above the resin bed, close the stopcock.

NOTE: Never allow the column resin to dry out completely.



4. Remove your tube from the microcentrifuge being careful not to disturb the cell pellet at the bottom. Record the color of the pellet and supernatant.

5. Using the p1000 micropipette, transfer 200µL of the supernatant to the SUPER tube. Do not disturb the pellet.

\*START HERE\* if your teacher had you skip Lab 6A and start with supernatant. Label a tube “RFP”

6. Using a new tip, add 200µL of Binding Buffer [BB] to the SUPER and gently mix by pumping up and down. Discard the tip.

7. Change the volume to 400µL and using a new tip, carefully dispense the solution down the side of the column without disturbing the column resin bed.

8. Open the stopcock and allow the column to drain until there is only 1-2mm of liquid above the resin bed. Close the stopcock. Observe the column resin and where the RFP is located within it.

9. Change the volume to 1000µL and using a new tip, carefully dispense 1mL of Wash Buffer [WB] down the side of the column without disturbing the column resin bed.

10. Open the stopcock and allow the column to drain until there is only 1-2mm of liquid above the resin bed. Close the stopcock. Observe the column resin and where the RFP is located within it.

11. Using a new tip, carefully dispense 1000µL of Elution Buffer [EB] down the side of the column without disturbing the resin bed. Do this TWICE so that 2mL of EB are added to the column.

12. Open the tube labeled “RFP”. Open the stopcock and allow the liquid to drain into the waste container. Once the pink RFP begins to drain out of the column, place the “RFP” tube under the stopcock and collect the RFP. Close the stopcock when there is only 1-2mm of liquid above the resin bed.

13. Using a new tip, carefully add 1000µL of Column Equilibrium Buffer [CEB] TWICE to the column without disturbing the resin bed.

14. Compare your RFP tube to the other groups. Is there a difference in color intensity? Place your tube on the transilluminator. Try putting the tube into a beaker of boiling water. What happens? Record your observations.

15. Dispose all tubes and tips in the WASTE container and disinfect your lab bench.