

LAB 6 RESOURCES

ATTENTION TEACHERS:

Please have your students know how to use a pipette before proceeding to do this lab!

LAB 6 KIT ITEMS	LABELS	VOLUMES to aliquot
Shaker		---
Inoculating loops		---
Arabinose (for flask)	ARA	--
Sterile LB/amp flask		---
Columns		---
Lysis buffer	LYS	160 μ L per group
Elution buffer	EB	---
Binding buffer	BB	---
Column Equilibration buffer	CEB	---
Wash buffer	WB	---
20 % ethanol		---
Backup RFP cell broth	EC (<i>E.coli</i> culture) Pink broth in Orange cap bottle.	2 (2@ 1mL aliquots) mL per column

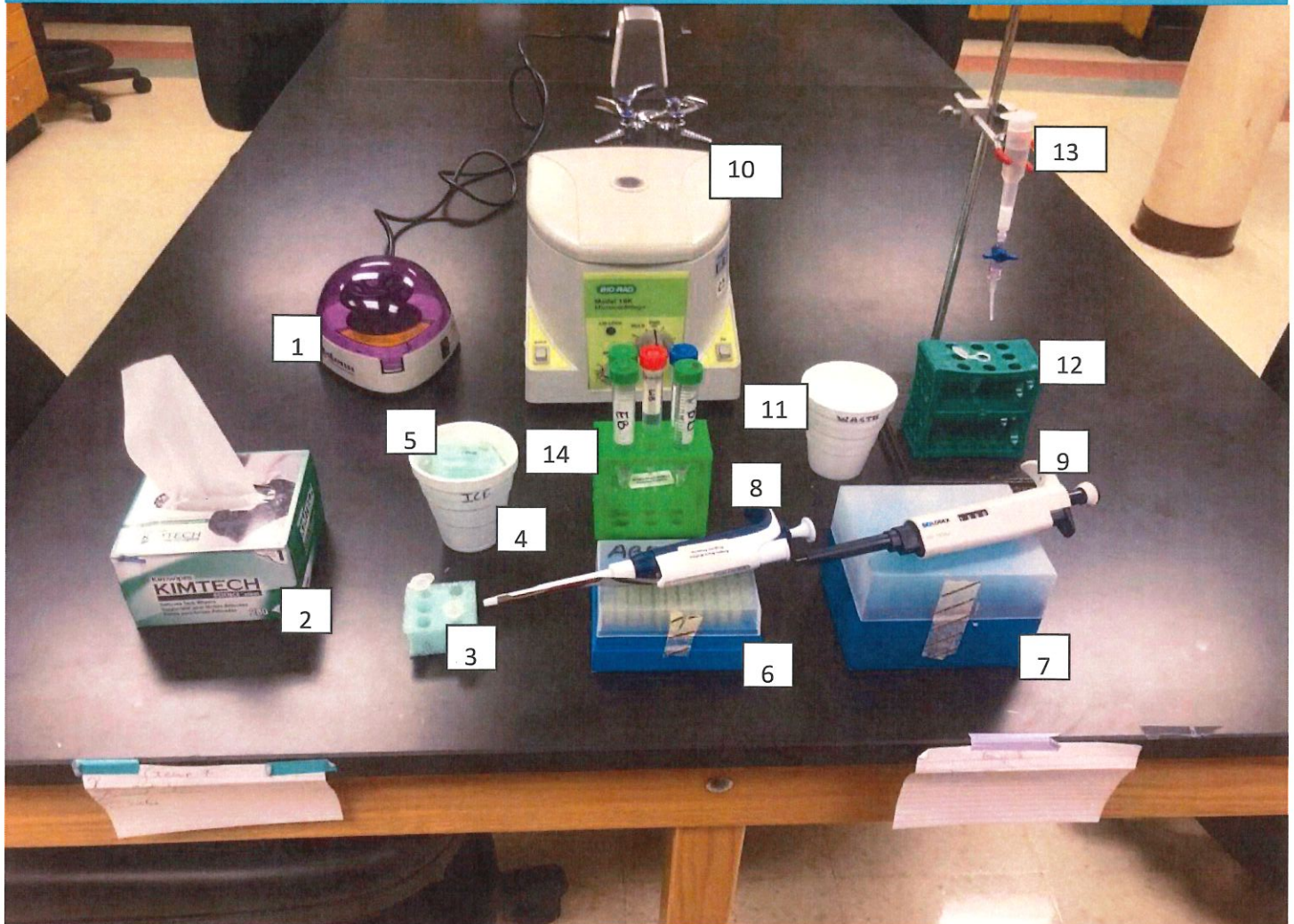
Notes:

Only if you request this step other wise use the Pink "Transformed " Broth provided to perform 6A!
Transformed LB broth: Start your lab 6 culture 4-5 days BEFORE you will need it. This will leave enough time for me to grow a backup culture if yours does not grow. It can be stored in the refrigerator until the lab. Inoculate the LB amp broth with vial of transformed cells when you get to school in the morning. After several hours of shaking (This can be anywhere from 2-4 hours) and when the broth starts to turn cloudy but not TOO cloudy), add the arabinose (1 full tube) and continue shaking overnight. Add another vial of arabinose before you leave. If your culture is not bright pink the next morning, add the other tube of arabinose and let it continue to shake through the next day.

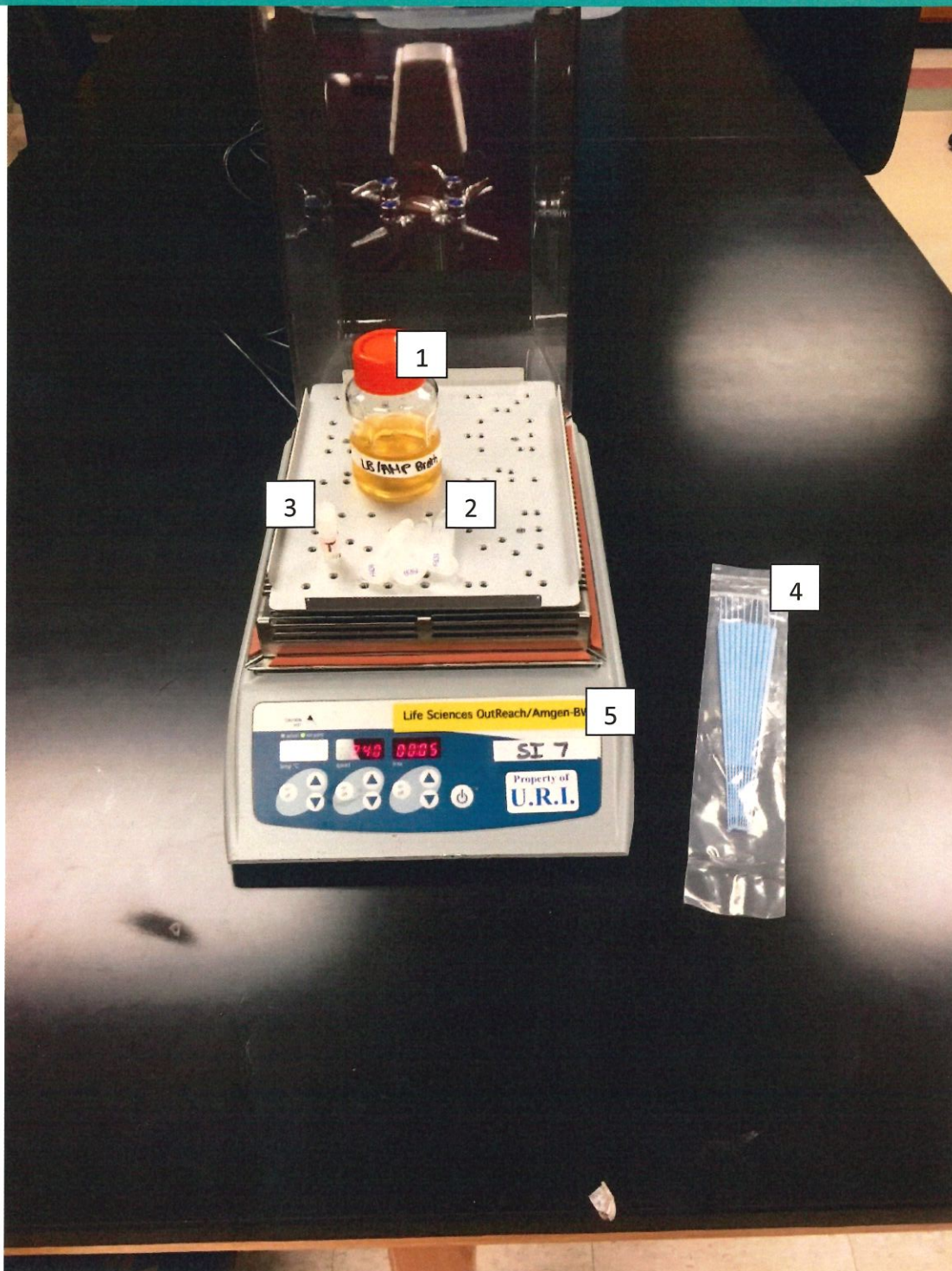
6A: Lysing cells: Optimal lysing can be achieved if you are able to do multiple freeze/thaw/steps. After freezing, place cell in 37 $^{\circ}$ C (you can use the water bath) or room temperature if you do not have access to 37 $^{\circ}$ C. If you have access to a vortex or use the plastic micro centrifuge tube rack provided, mix cells after thawing. Freeze again. This repeat freeze/thaw will help lyse the cells.

P-20, P-200, and P-1000 pipettes may contain locks on them: Please **UNLOCK** the pipette when adjusting the measurement

Lab 6



1. Mini centrifuge
2. Kimwipes
3. Greet float tube holder: holds tubes throughout the experiment
4. Ice cup
5. Tube labeled **LYB**; the Lysis buffer tube should be on ice
6. P20-200 pipette tips
7. P100-1000 pipette tips
8. P20-200 pipette
9. P100-1000 pipette
10. Large centrifuge
11. Waste cup
12. Microfuge tube rack holder: holds the **RFP** tube the collects sample
13. Column
14. Microfuge tube rack that holds: elution buffer (**EB**), binding buffer (**BB**), wash buffer (**WB**), column equilibration buffer (**CEB**), and **20% ethanol**



1. 100 mL sterile Lb broth
2. 3 tubes of arabinose
3. 1mL tube of **Transform** cells
4. Inoculating loops
5. Shaker/ incubator

Grow Bacteria for Protein Purification

To be arranged with the RI program site.

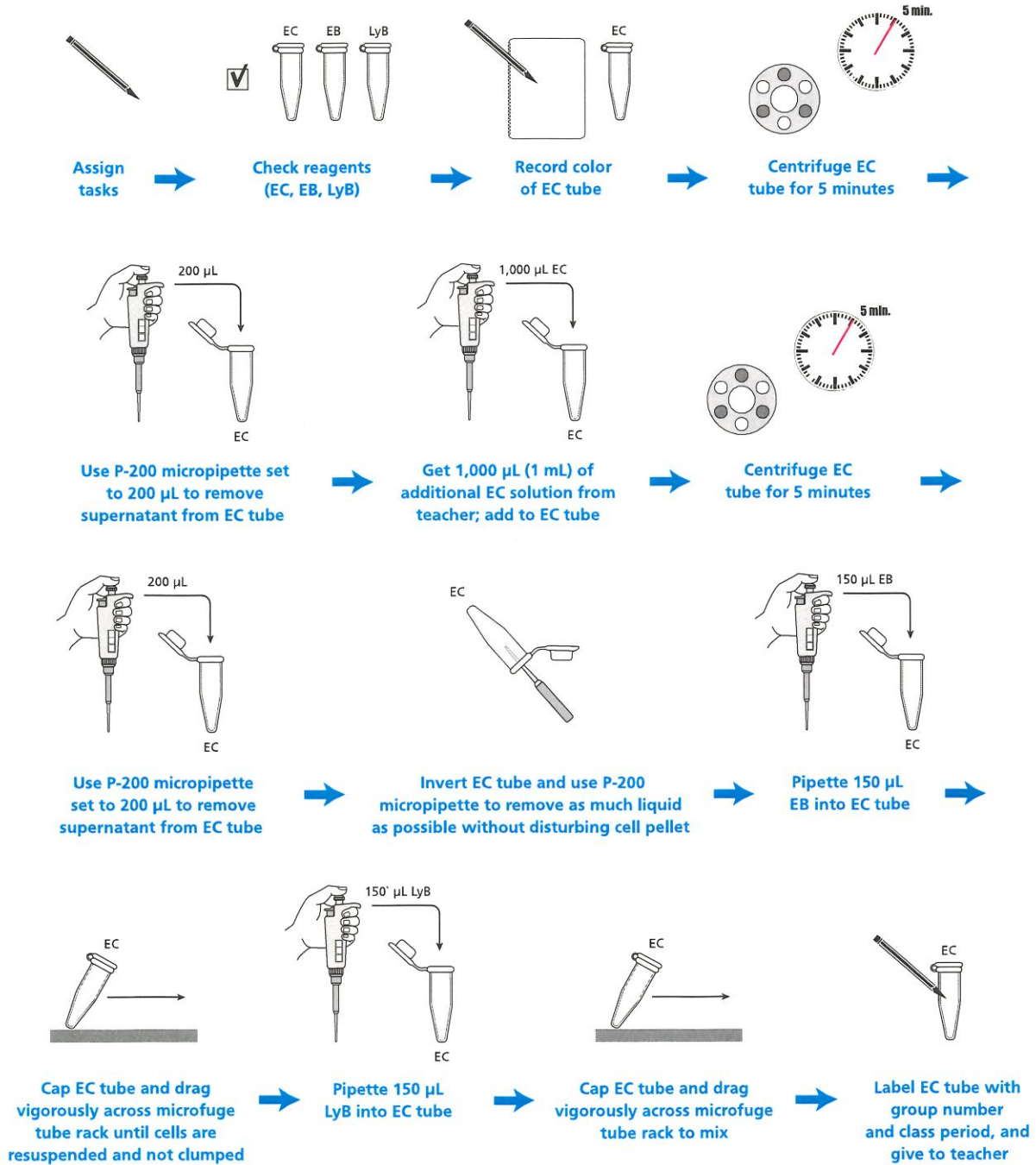
A couple days before Laboratory 6, prepare a suspension culture of bacteria that have been transformed with the pARA-R (provided in your kit). (typically marked with a T or "transf cells")

Materials:

- 1000ul Pipette
- Transformed cells (provided in kit)
- Sterile flask containing LB/amp broth
- Shaker
- 3 tubes of sterile arabinose
 1. **Start your lab 6 culture 4-5 days BEFORE you will need it. It can be stored in the refrigerator until the lab.**
 2. **Inoculate the LB amp broth with a vial of transformed cells when you get to school in the morning.**
 3. **After several hours of shaking (This can be anywhere from 2-3 hours) and when the broth starts to turn cloudy but not TOO cloudy), add the arabinose (1 full tube).**
 4. **Continue shaking overnight. We have enclosed an additional tube of Arabinose; you can add before you go home for the night.**
 5. **If your culture is not bright pink the next morning, add the other tube of arabinose and let it continue to shake through the next day. Compare the suspension culture:**
 6. Optimal

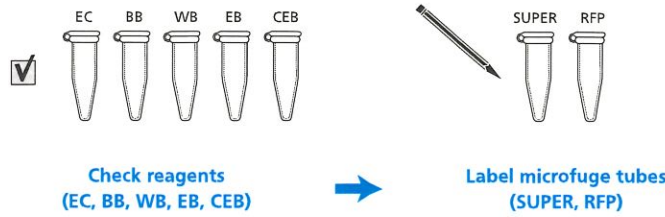


Laboratory 6, Part A Flowchart

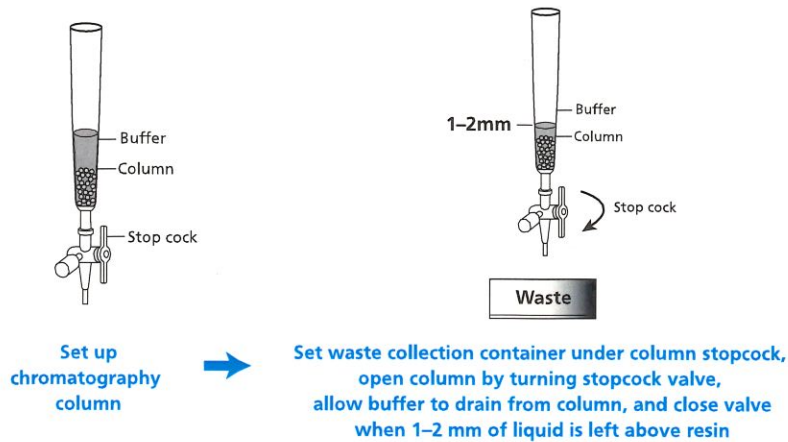


Laboratory 6, Part B Flowchart

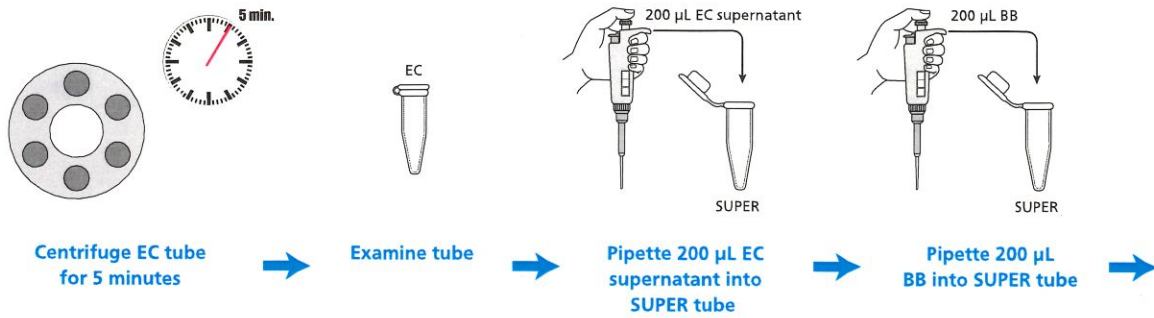
One group member:



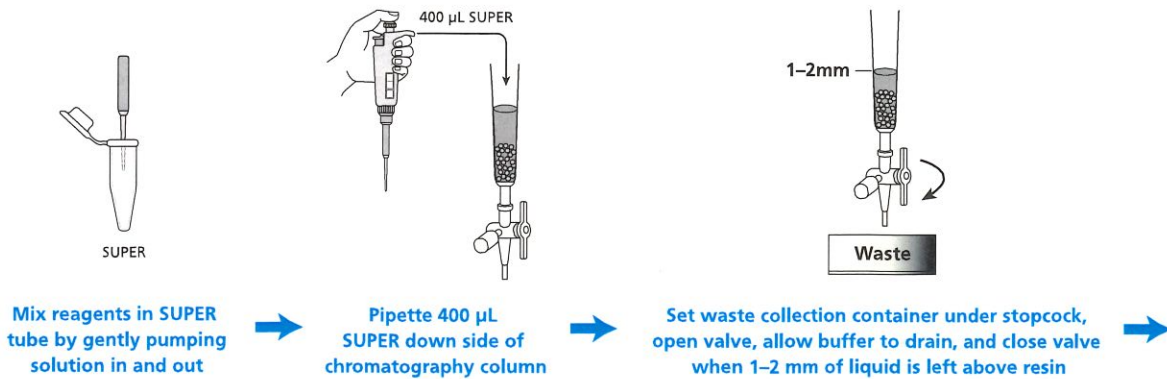
One group member:



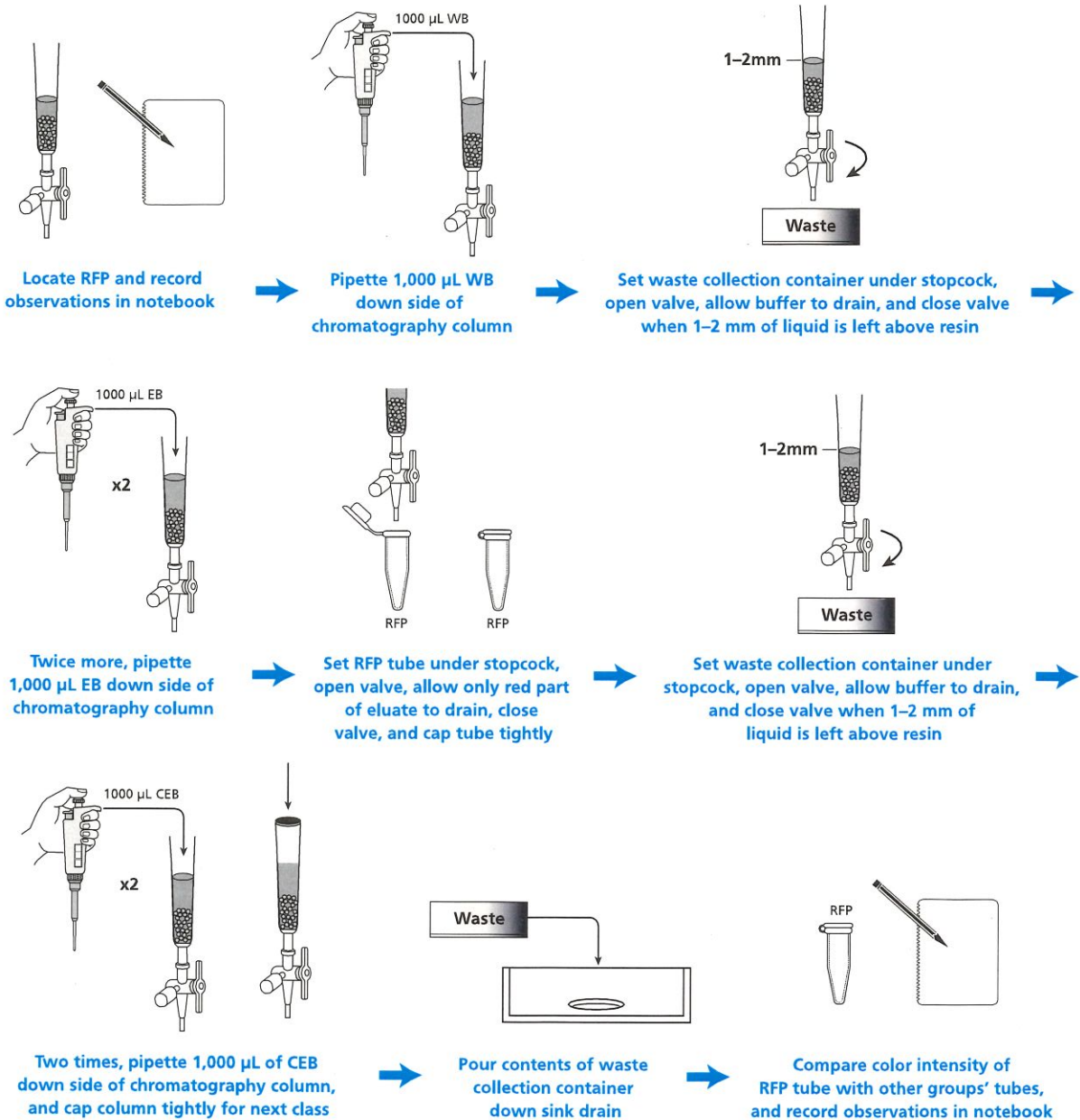
One group member:



Whole group:



Laboratory 6, Part B Flowchart (Continued)



Grow Bacteria for Protein Purification

A couple days before Laboratory 6, prepare a suspension culture of bacteria that have been transformed with the pARA-R (provided in your kit).

Materials:

1. 1000ul Pipette
2. Transformed cells (provided in kit)
3. Sterile flask containing LB/amp broth
4. Shaker
5. 2 tubes of sterile arabinose

Prepare the suspension culture:

1. Using the pipette, aseptically transfer transformed cells into the sterile flask containing LB/amp broth.
2. Replace the cap, make sure to loosen the cap $\frac{1}{4}$ of a turn.
3. Shake and incubate the flask (at 37°C) for four to five hours. The LB/amp broth should become cloudy, indicating the cells are growing.
4. Add one tube of sterile arabinose to the flask.
5. Continue to shake overnight.
6. Check flask in the morning if solution has not turned pink add the other tube of arabinose and shake 4-5 more hours.



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Lab 6 Results

