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
Shaker		--
Inoculating loops		--
Arabinose (for flask)	**ARA**	--
Sterile LB/amp flask		--
Columns		--
Lysis buffer	**LYS**	150uL per group
Elution buffer	**EB**	--
Binding buffer	**BB**	--
Column Equilibration buffer	**CEB**	--
Wash buffer	**WB**	--
20 % ethanol		--
Backup RFP cell broth	**EC (*E.coli* culture)**	1mL per column

Notes:

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. 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.

Lysing cells: Optimal lysing can be achieved if you are able to do multiple free/thaw/steps. After freezing, place cell in 37^0C (you can use the water bath) or room temperature if you do not have access to 37^0C. 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.

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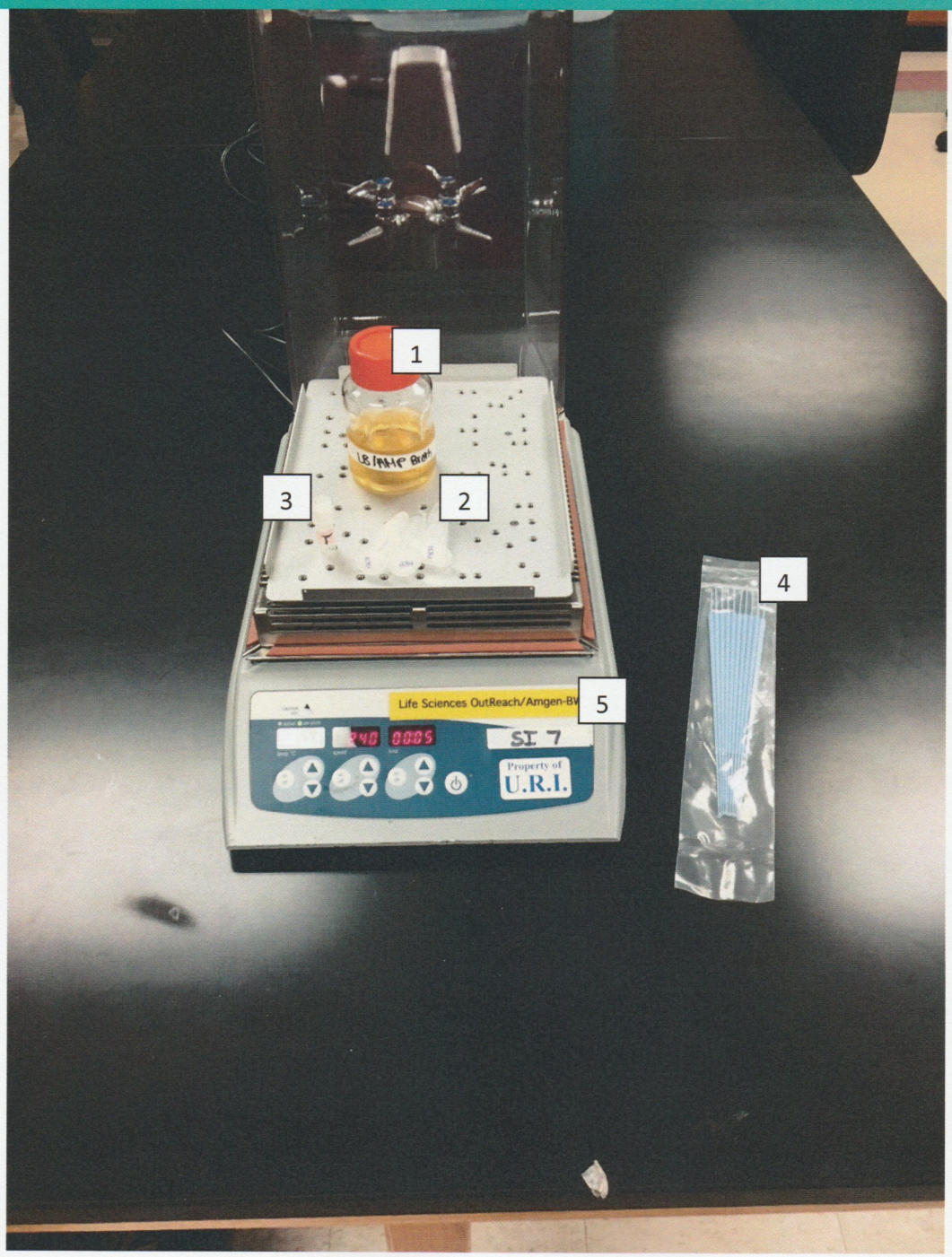
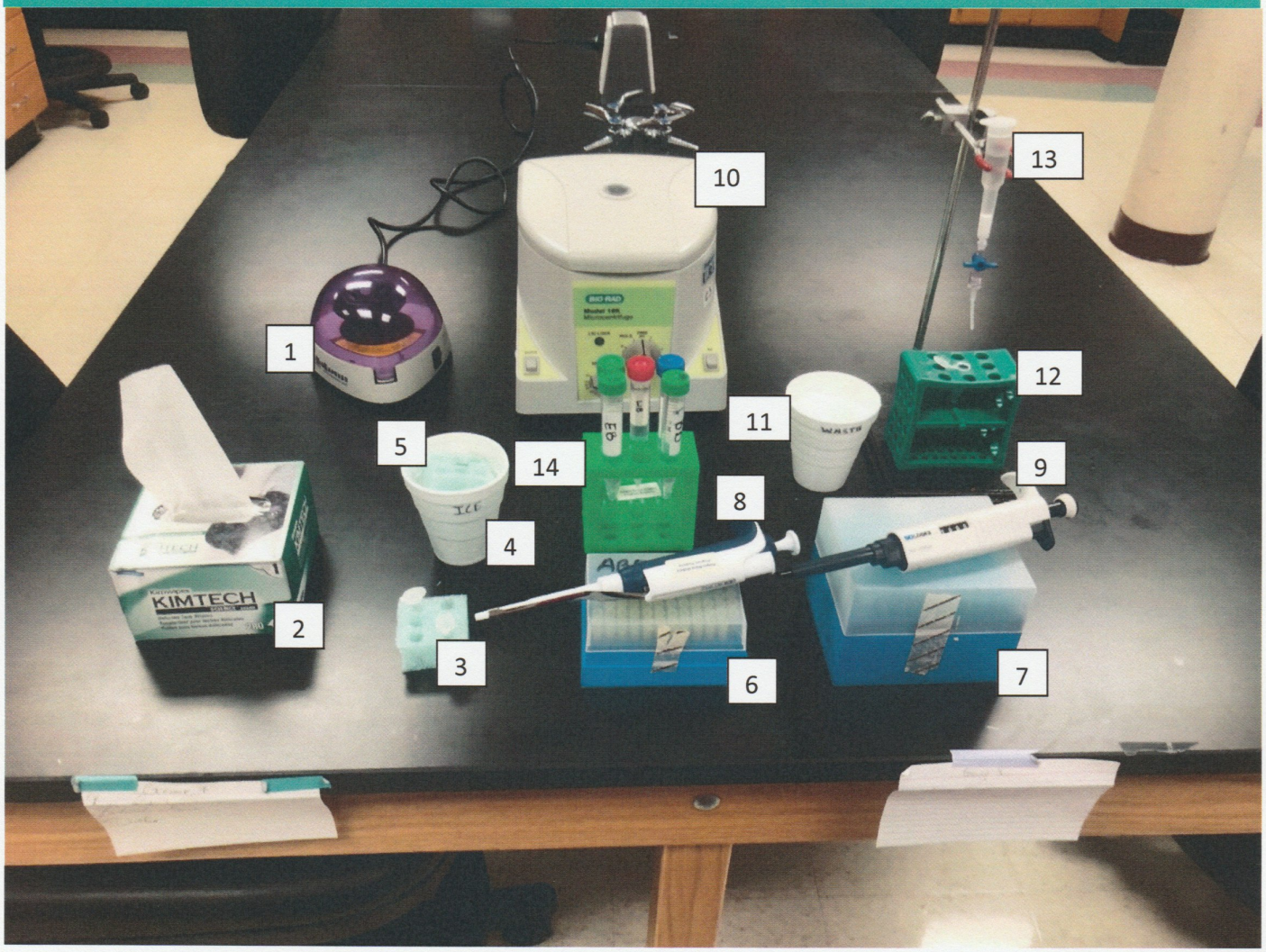


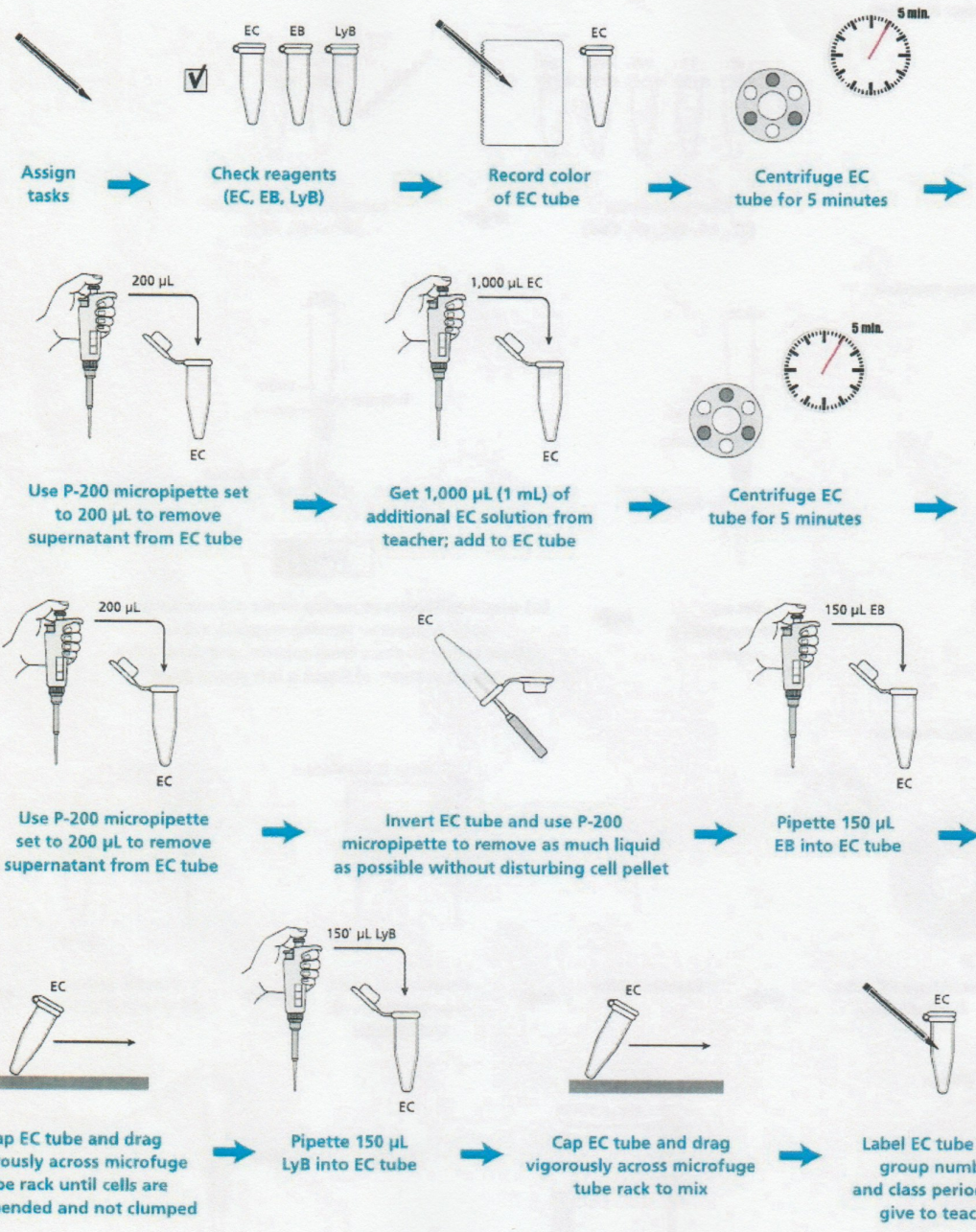
1. 100 mL sterile Lb broth
2. 3 tubes of arabinose
3. 1mL tube of **Transform** cells
4. Inoculating loops
5. Shaker/ incubator

Lab 6



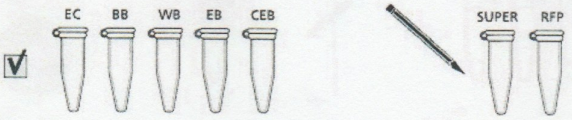
1. Mini centrifuge
2. Kimwipes
3. Greet float tube holder: holds tubes throughout the experiment
4. Ice cup
5. Tube labeled **LYS**; the Lysis buffer tube should be on ice
6. P20-200 pipette tips
7. P100-1000 pipette tips
8. P20-200 pipette
9. P100-1000 pipette
10. Large centrifuge
11. Waste cup
12. 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**

Laboratory 6, Part A Flowchart



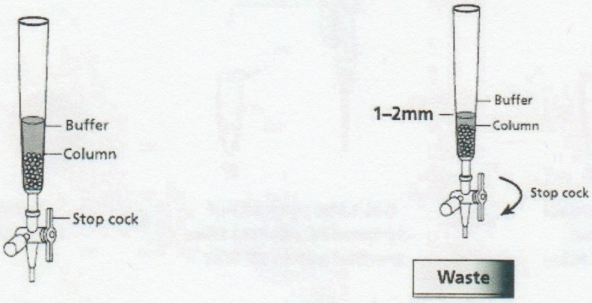
Laboratory 6, Part B Flowchart

One group member:



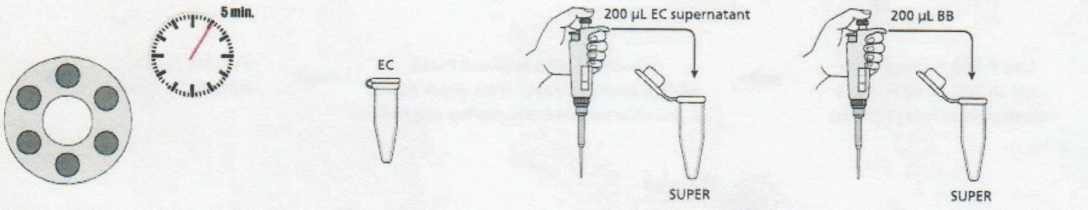
Check reagents (EC, BB, WB, EB, CEB) → Label microfuge tubes (SUPER, RFP)

One group member:



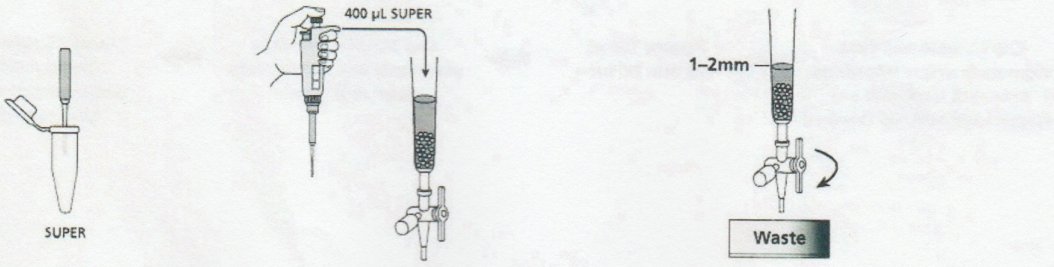
Set up chromatography column → Set waste collection container under column stopcock, open column by turning stopcock valve, allow buffer to drain from column, and close valve when 1–2 mm of liquid is left above resin

One group member:



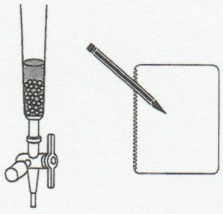
Centrifuge EC tube for 5 minutes → Examine tube → Pipette 200 µL EC supernatant into SUPER tube → Pipette 200 µL BB into SUPER tube →

Whole group:

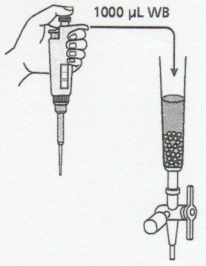


Mix reagents in SUPER tube by gently pumping solution in and out → Pipette 400 µL SUPER down side of chromatography column → Set waste collection container under stopcock, open valve, allow buffer to drain, and close valve when 1–2 mm of liquid is left above resin →

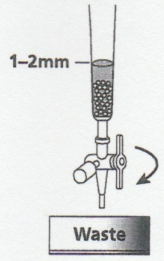
Laboratory 6, Part B Flowchart (Continued)



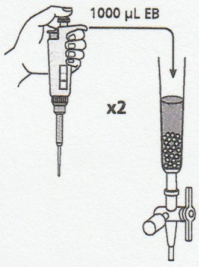
Locate RFP and record observations in notebook



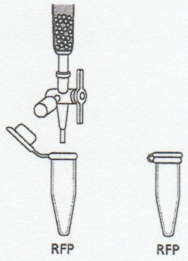
Pipette 1,000 µL WB down side of chromatography column



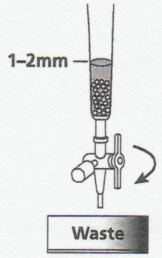
Set waste collection container under stopcock, open valve, allow buffer to drain, and close valve when 1–2 mm of liquid is left above resin



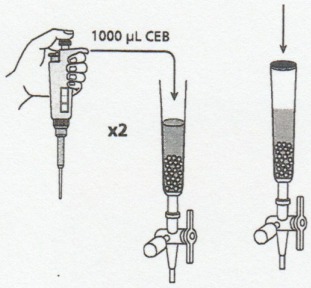
Twice more, pipette 1,000 µL EB down side of chromatography column



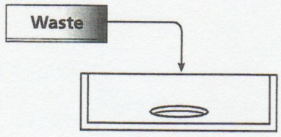
Set RFP tube under stopcock, open valve, allow only red part of eluate to drain, close valve, and cap tube tightly



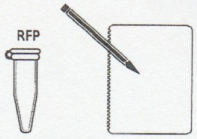
Set waste collection container under stopcock, open valve, allow buffer to drain, and close valve when 1–2 mm of liquid is left above resin



Two times, pipette 1,000 µL of CEB down side of chromatography column, and cap column tightly for next class



Pour contents of waste collection container down sink drain



Compare color intensity of RFP tube with other groups' tubes, and record observations in notebook

Grow Bacteria for Protein Purification

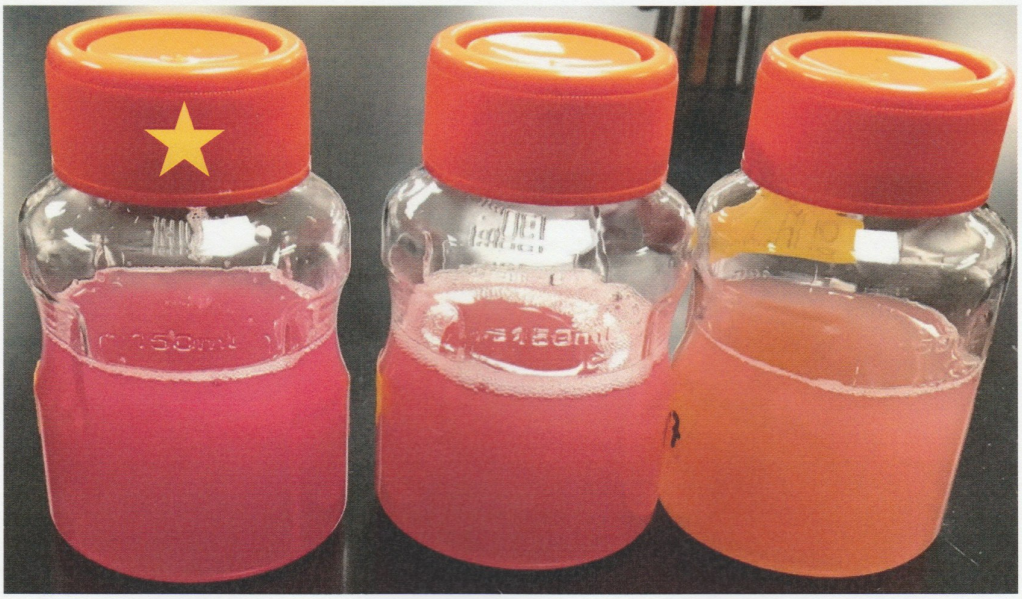
A couple days before Laboratory 6, prepare a suspension culture of bacteria that have been transformed with the pARA-R (provided in your kit).

Materials:

1. 1000ul Pipette
2. Transformed cells (provided in kit)
3. Sterile flask containing LB/amp broth
4. Shaker
5. **3** 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 ¼ 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.
7. Repeat Step 6.



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Lab 6 Results