

# *LAB 5 RESOURCES*

## ATTENTION TEACHERS:

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

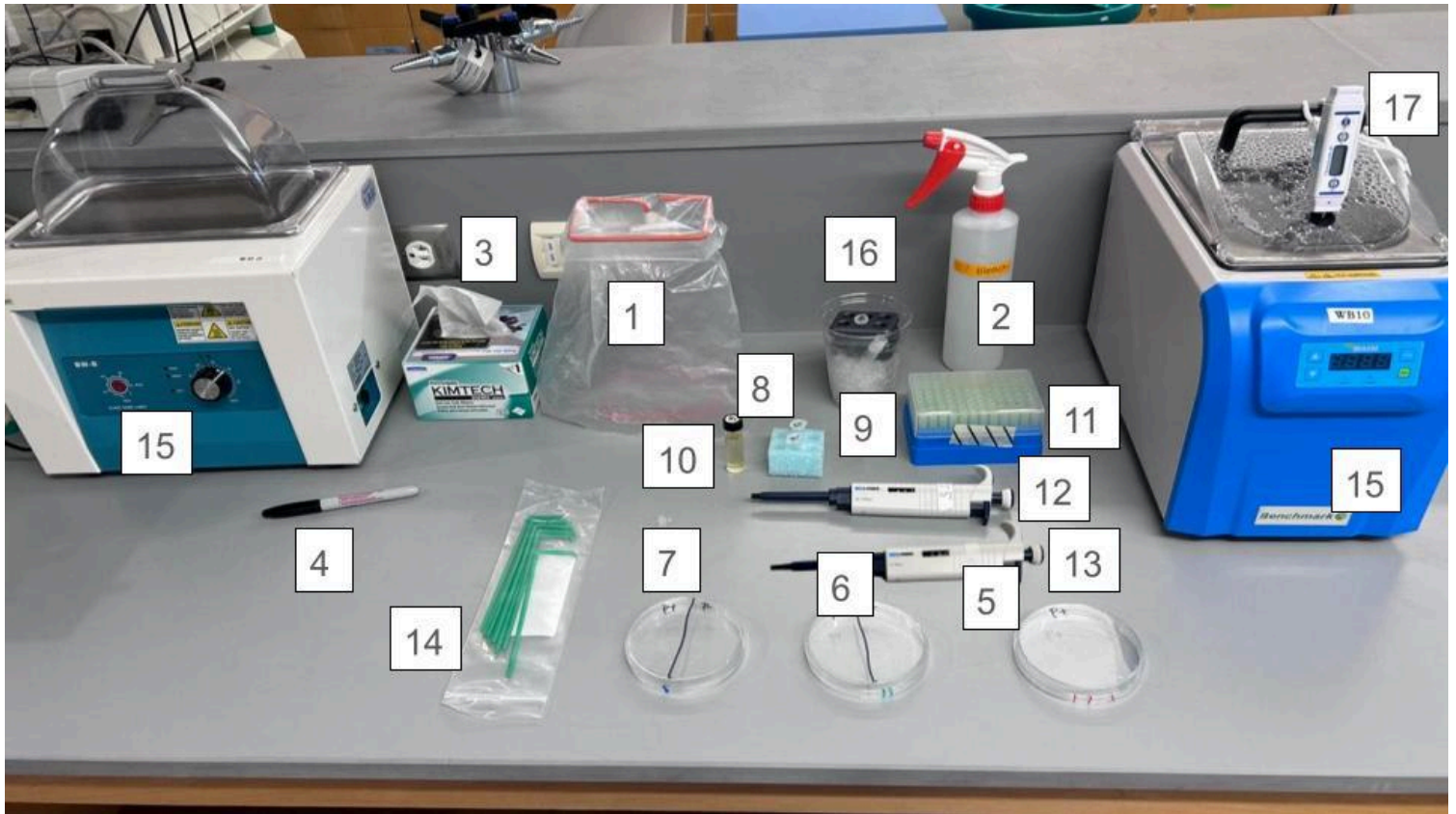
LAB 5A KIT ITEMS	LABELS	VOLUMES to aliquot
LB plates	1 blue line	---
LB/amp plates	2 green lines	---
LB/amp/ara plates	3 red lines	---
P-20 micropipette		---
P-20 pipette tips		---
P-200 micropipette		---
P-200 pipette tips		---
Biohazard waste bags		---
10% Bleach spray bottles		---
Cell spreaders/ Inoculating loops		---
Backup Rfp plate		---
<b>FREEZER BOX ITEMS</b>		
Competent cells: <b>keep frozen!</b> <b>Will Not work if not kept frozen</b>	<b>In cryotubes: Competent Cells</b>	100 $\mu$ L per group
LB Broth tubes	<b>LB</b>	350 $\mu$ L per group
p-ARA-R (5a concentration)	<b>pARA-R 5</b>	12 $\mu$ L per group

### **Notes:**

- Competent cell stock can come in either 500 $\mu$ L or 1000 $\mu$ L aliquots. Aliquot 15 minutes before class use and keep on ice in the refrigerator.
- Emergency Pause step: After adding the 150  $\mu$ L of LB broth to the tubes, samples can be refrigerated until the next day. **Refer to details in the document at the beginning of this binder.**
- After spreading cells on the petri dishes let them sit upright for 5-15 min before inverting them and putting them to incubate. Letting them sit longer will improve the chance of cells adhering to the agar.

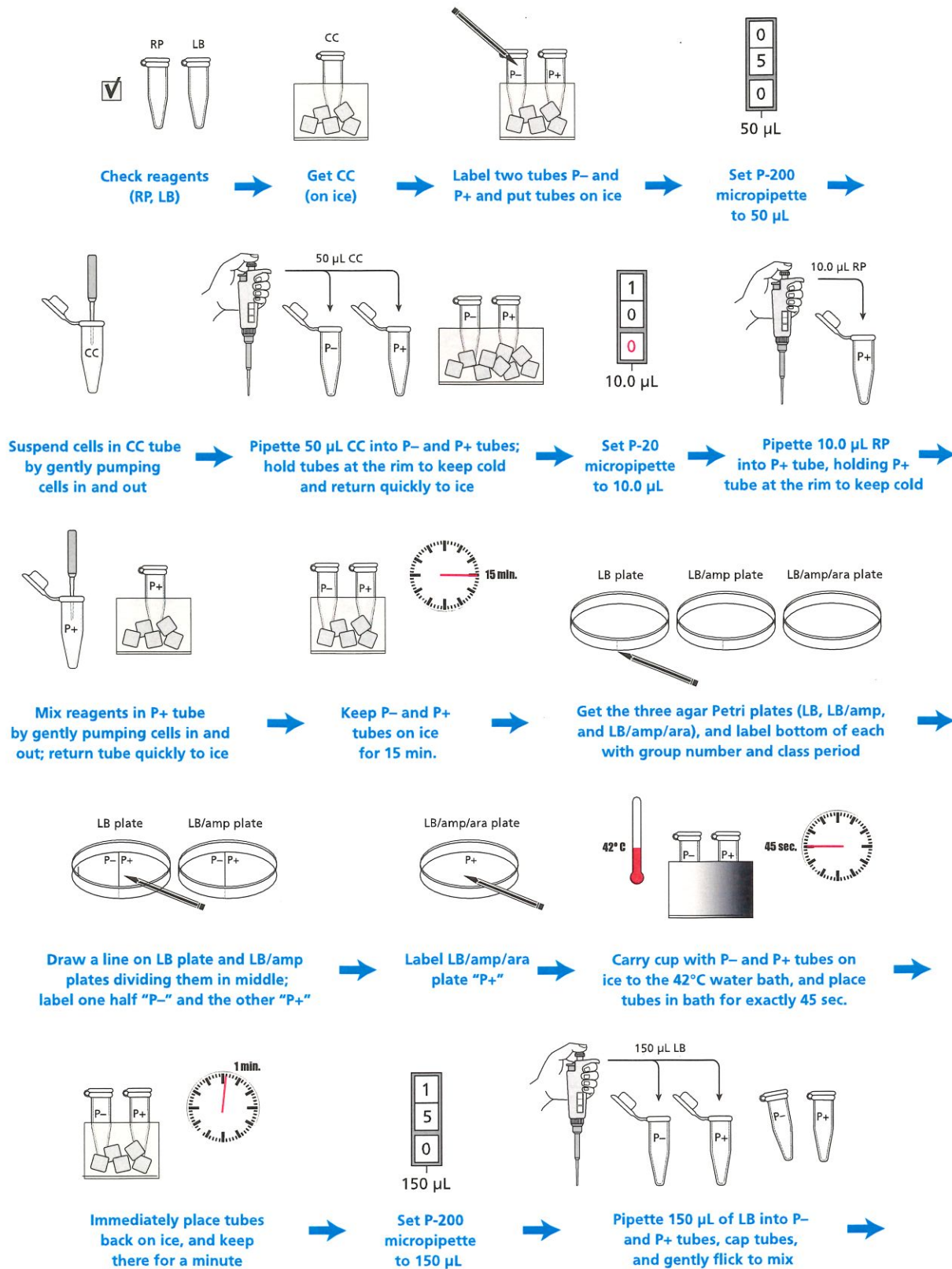
**P-20, P-200, and P-1000 pipettes may contain locks on them:** Please **UNLOCK** the pipette when adjusting the measurement

## Lab 5

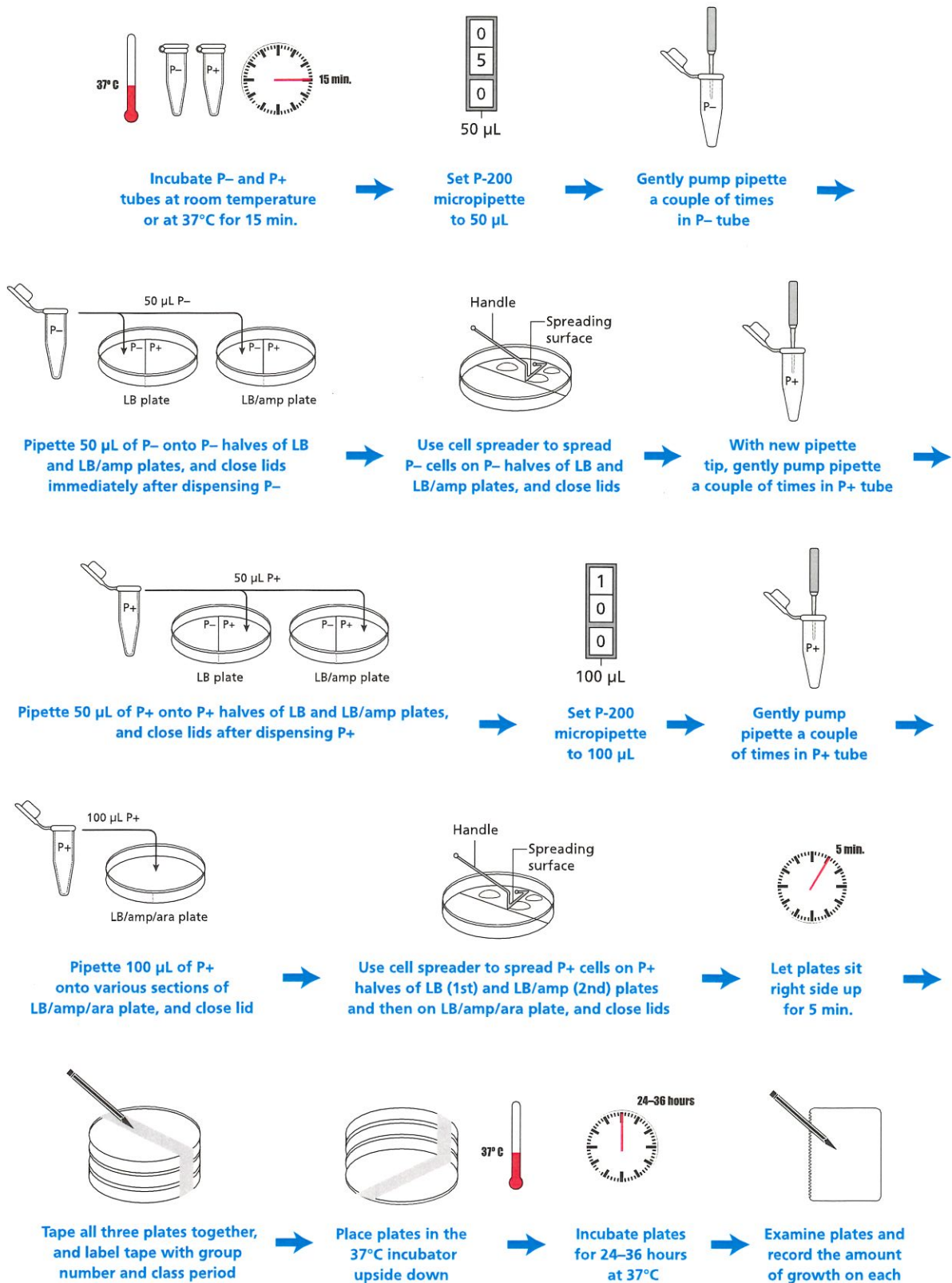


1. Biohazard bag and waste stand
2. 10% bleach bottle
3. Kimwipes
4. Sharpie marker
5. LB/AMP/ARA plate (marked by **3 red** stripes)
6. LB/AMP plate (marked by **2 green** stripes)
7. LB plate (marked by **1 blue** stripe)
8. Green float: used to hold tubes marked **P+ & P-**
9. Ice cup
10. **LB glass vial, competent cells (CC), pARA 5a should be on ice.**
11. P20-200 pipette tips
12. P20-200 pipette
13. P2-20 pipette
14. Spreaders
15. Water bath (should be set at 42°C for this experiment) (both types that may be provided in kits are being displayed)
16. Small gray cool rack (freeze before using)
17. Thermometer

## Laboratory 5A Flowchart



## Laboratory 5A Flowchart (Continued)

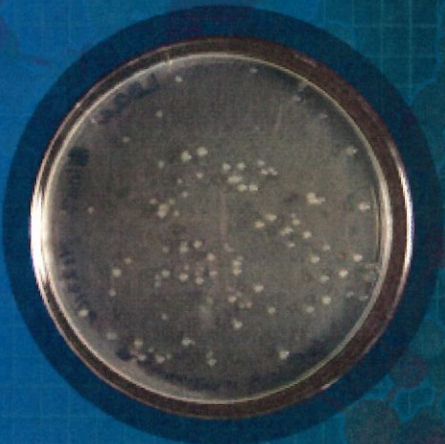


**AMGEN** growth of transformed bacteria on various plates

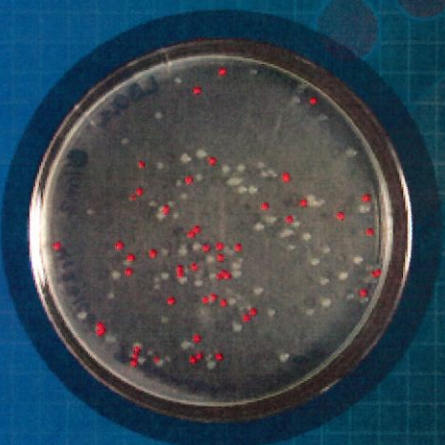
P+ plates



LB



LB/amp



LB/amp/ara

P- plates



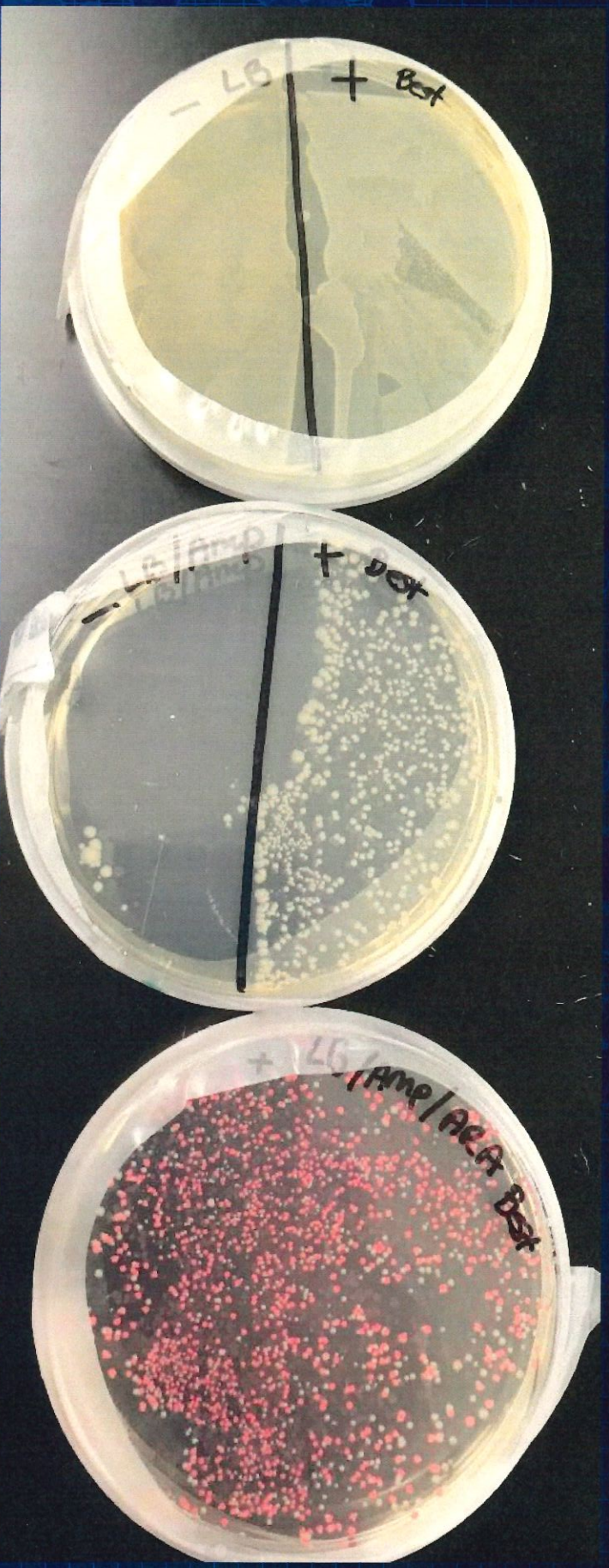
LB



LB/amp

**AMGEN** preparing an overnight culture of *E. Coli* for RFP expression

Colony isolation and culture



LB

LB/amp

LB/amp/ara