

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
LB plates	1 green line	--
LB/amp plates	2 blue 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		--
CaCl (keep refrigerated)	CaCl ₂	200 uL per group
FREEZER BOX ITEMS		
Competent cells	In cryotubes: Competent Cells	100 uL per group
LB Broth tubes	LB	350 uL per group
p-ARA-R (5a concentration)	pARA-R 5	10 uL per group

Notes:

*For better results have students add 100ul of CaCl to both P+ and P- tubes. This step can be done before or after adding the competent cells to each tube.

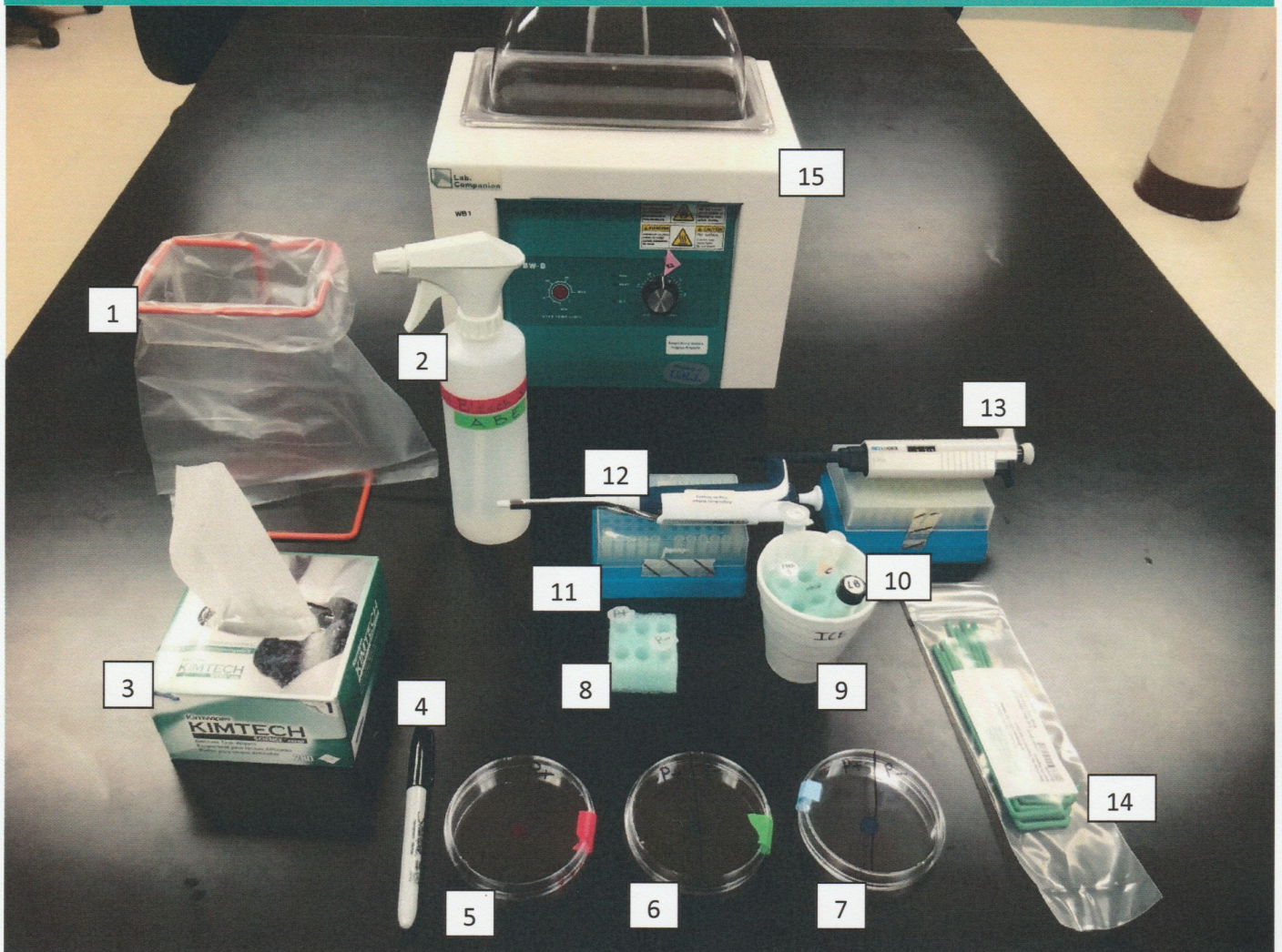
Competent cell stock can come in either 500ul or 1000ul aliquots.

After spreading cells on the petri dishes let them sit upright for about 15min 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

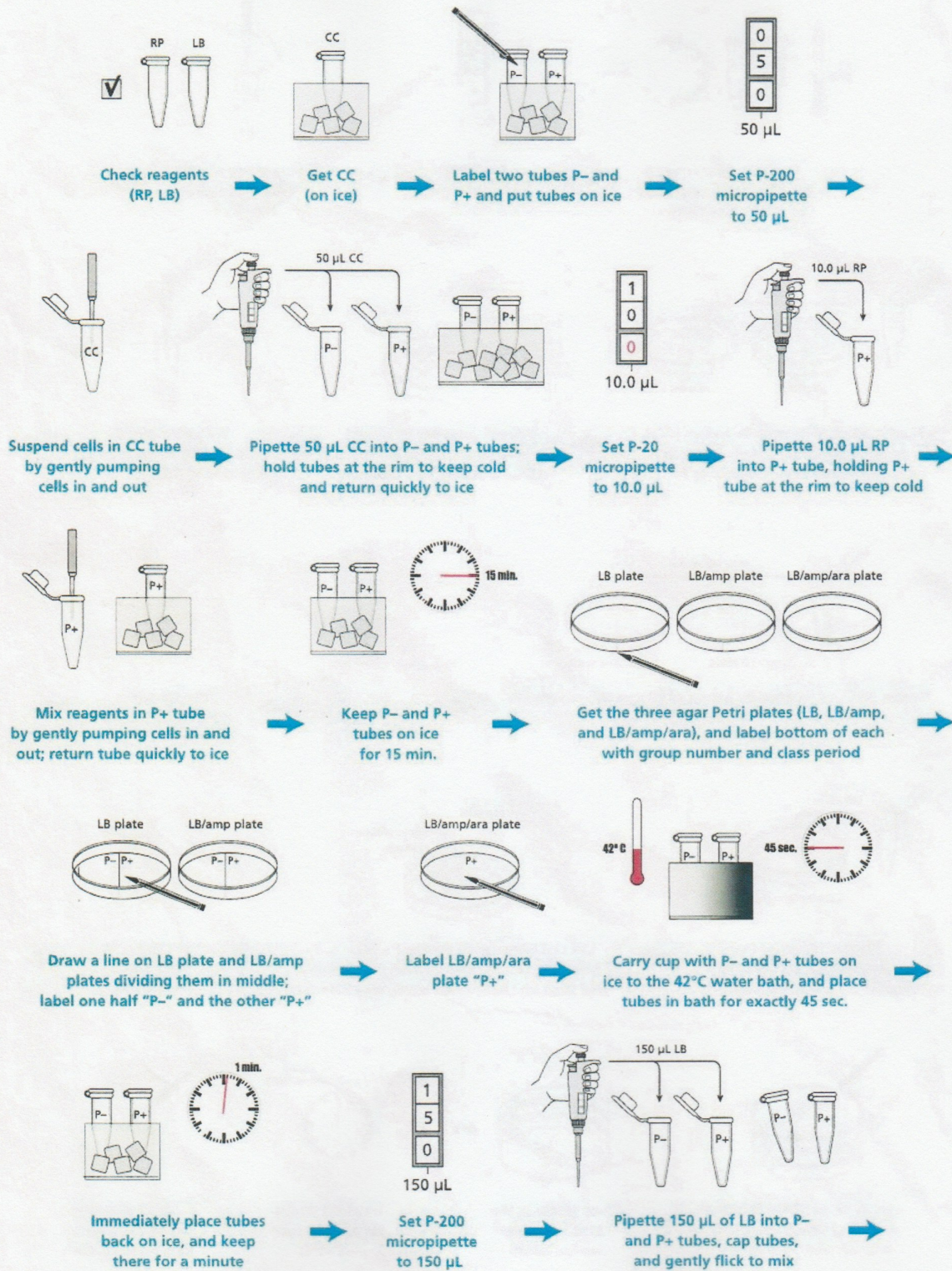
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Lab 5

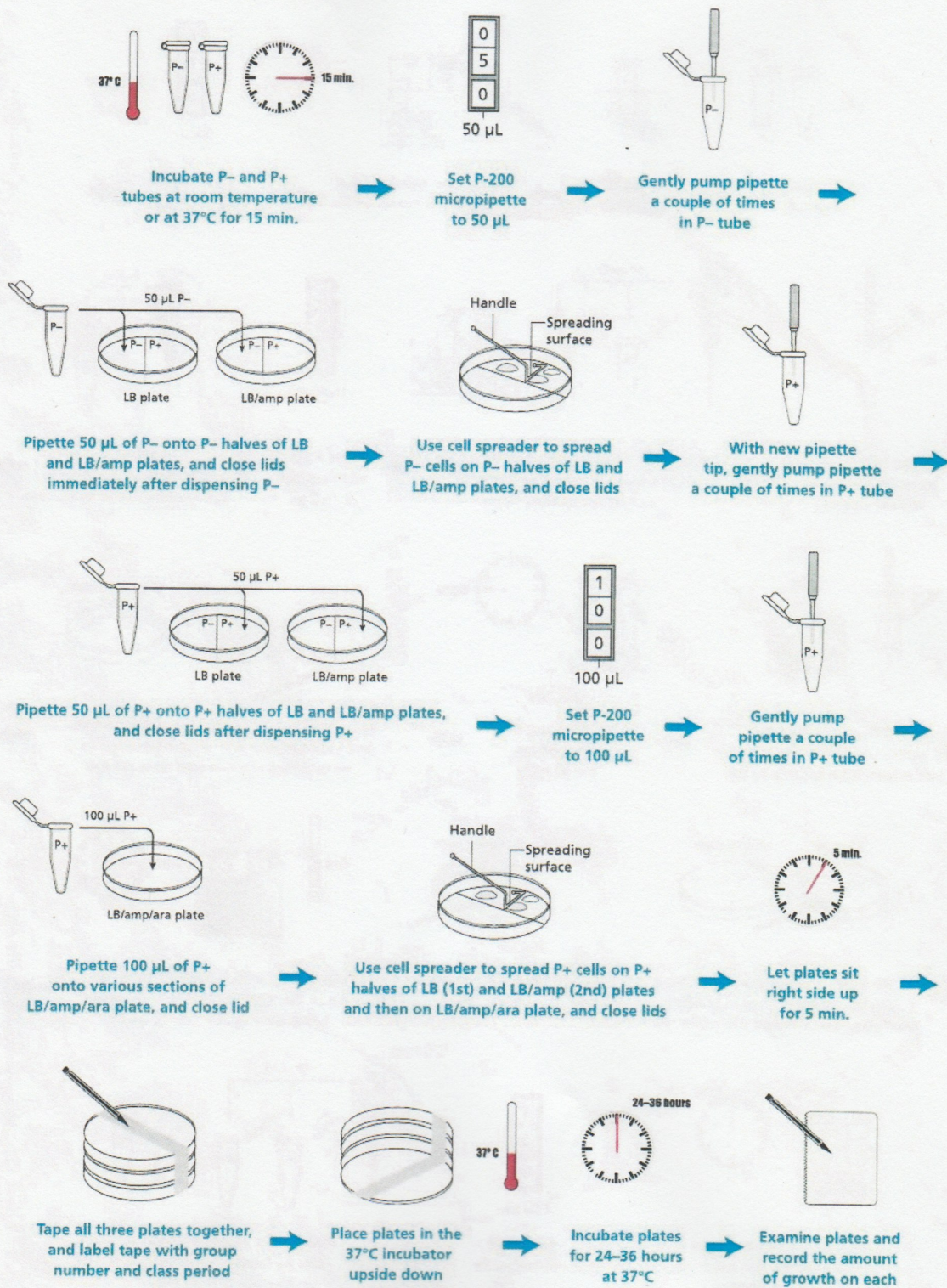


1. Biohazard bag and waste stand
2. 10% bleach bottle
3. Kimwipes
4. Sharpie marker
5. LB/AMP/ARA plate (marked by **3 red stripes**)
6. LB/AMP plate (marked by **2 green stripes**)
7. LB plate (marked by **1 blue stripe**)
8. Green float: used to hold tubes marked **P+ & P-**
9. Ice cup
10. **LB glass vial, competent cells, pARA 5a and Calcium chloride (CaCl)** should all 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 specific experiment)

Laboratory 5A Flowchart

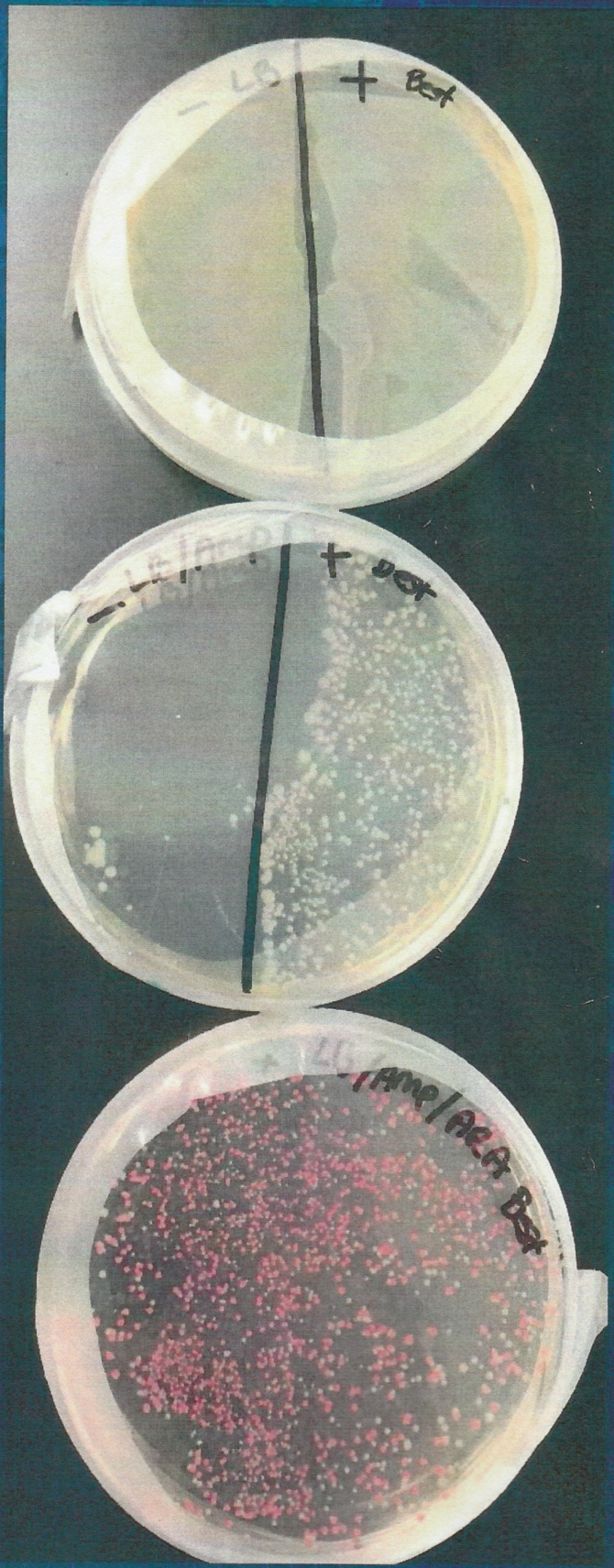


Laboratory 5A Flowchart (Continued)



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

Colony isolation and culture



LB

LB/amp

LB/amp/ara

AMGEN

growth of transformed bacteria on various plates

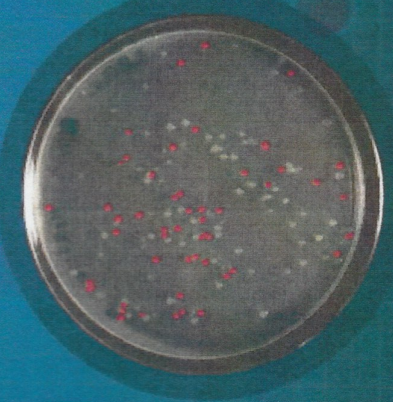
P+ plates



LB



LB/amp



LB/amp/ara

P- plates



LB



No growth

LB/amp