LAB 4 RESOURCES

ATTENTION TEACHERS:

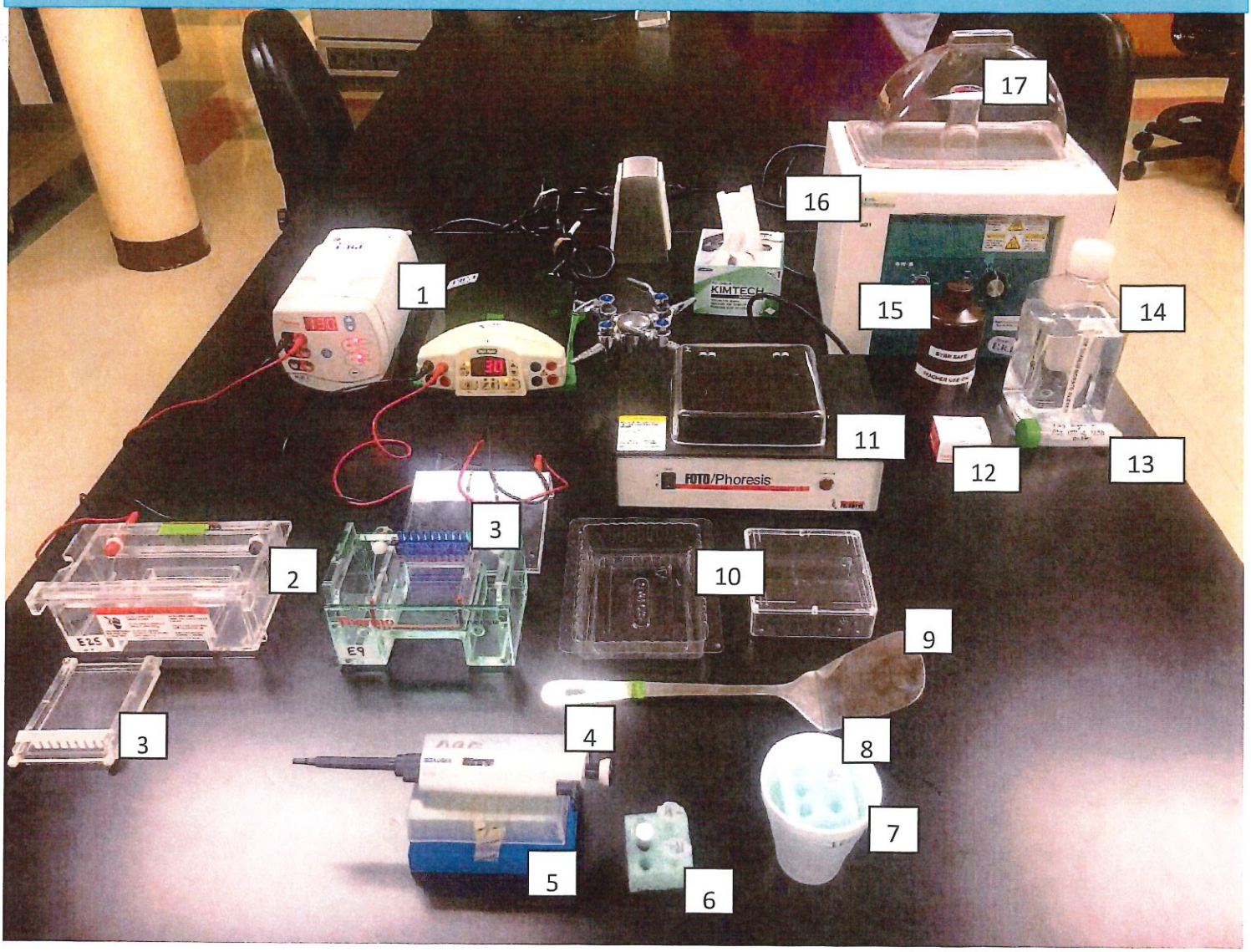
Please have your students know how to use a pipette before proceeding to do this lab!

LAB 4A KIT ITEMS	LABELS	VOLUMES to aliquot
P-20 micropipette		--
P-20 pipette tips		--
Agarose		Weigh out 1.44g agarose and add 180mL of 1X SB
Loading dye Solution #2)	**5X LD or Sol 2**	20 µL(need only 4uL per group)
Microcentrifuge tubes		---
Electrophoresis chambers		--
UV Transilluminator		--
Light box/ amber filter		--
Gel trays/combs		--
Spatula		--
*Sybr Safe (vial and bottle)	**Sybr Safe**	Add 15ul to 180ml of melted agarose
FREEZER BOX ITEMS		
Marker/ 1kb ladder	**DNA Mark**	10 µL

Notes: Let gel run for approximately 30-40 minutes, make sure to check on the gel after 30 minutes to make sure it doesn't run off. Diluting 20x SB Buffer to 1x SB buffer---- Diluting 20x SB Buffer to 1x SB buffer---- Mix 9mLs of 20x SB Buffer with 171 mLs of deionized water. You can find this in the ABE Teacher Guide (2015) on page OV-30 or pg 30 in 2019 guide.
Also refer to picture guide on Gel Making. Lab 4A: Diluting 20x SB Buffer to 1x SB buffer----.
Pre-stain gel Method: Spin the SYBR safe DNA gel stain tube and then mix with your pipette before aliquoting it into melted agarose solution. Pipet 15µL of the SYBR safe stain (from the vial) into 180mL of melted agarose just before pouring your gel. Gently, swirl the melted agarose to mix the SYBR safe. Please put return all unused stock of SYBR safe (amber tube). We will need the stock tube for the next kit cycle. Make sure to always keep the SYBR safe away from light.

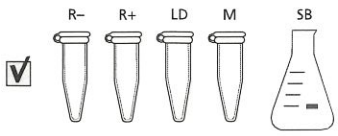
Post Stain Method. Teacher must prepare if needed. **NOT PROVIDED**
Diluting 20x SB Buffer to 1x SB buffer---- Mix 25mLs of 20x SB Buffer with 475 mLs of deionized water and then add 50µL of SyberSafe. Keep away from light. Solution 2 is the same reagent as LD.
P-20, P-200, and P-1000 pipettes may contain locks on them: Please **UNLOCK** the pipette when

Lab 4A

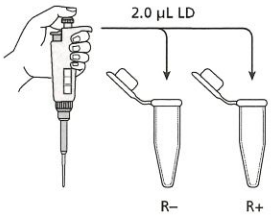


1. Electrophoresis power packs (both types that may be provided in kits are being displayed)
2. Electrophoresis chambers (both types that may be provided in kits are being displayed)
3. Trays and combs (both types that may be provided in kits are being displayed)
4. P2-20 micropipette
5. P20-200 micropipette tips
6. Green float tube holder- should be used to hold tubes labeled: **R+, R-,** and 5x loading dye (**LD**); it is the same as Sol. 2.
7. Ice cup
8. Tubes that should be on ice: **1Kb Ladder**
9. Spatula
10. Gel trays (both types that may be provided in kits are being displayed)
11. UV trans illuminator
12. SybrSafe tube
13. 1.44g of agarose tube
14. 20x SB buffer
15. SybrSafe Post Stain Bottle
16. Water bath
17. Hot agarose in a flask should be placed in water bath to cool down (make sure to set water bath to **55-60°**)

Laboratory 4A Flowchart

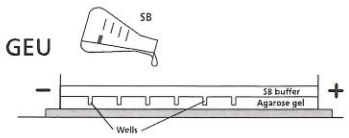


Check reagents
(R–, R+, LD, M in rack, 1x SB flask)



Pipette 2.0 µL LD into the R– and R+ tubes

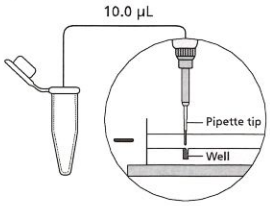
Centrifuge the two tubes



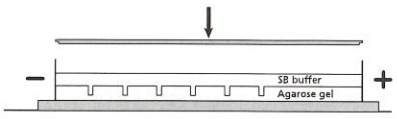
Review gel electrophoresis unit (GEU)

Cover gel in GEU with 1x SB

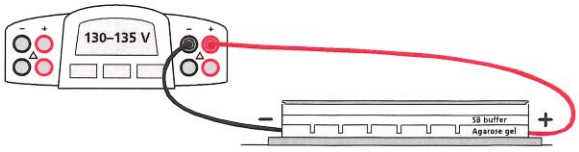
Draw location of each well, and label which solution is loaded in each in notebook



Pipette 10.0 µL each of DNA ladder (M), R–, and R+ into designated wells in GEU

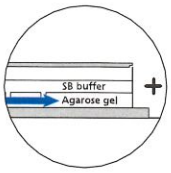


Place cover on GEU



Connect leads to power supply

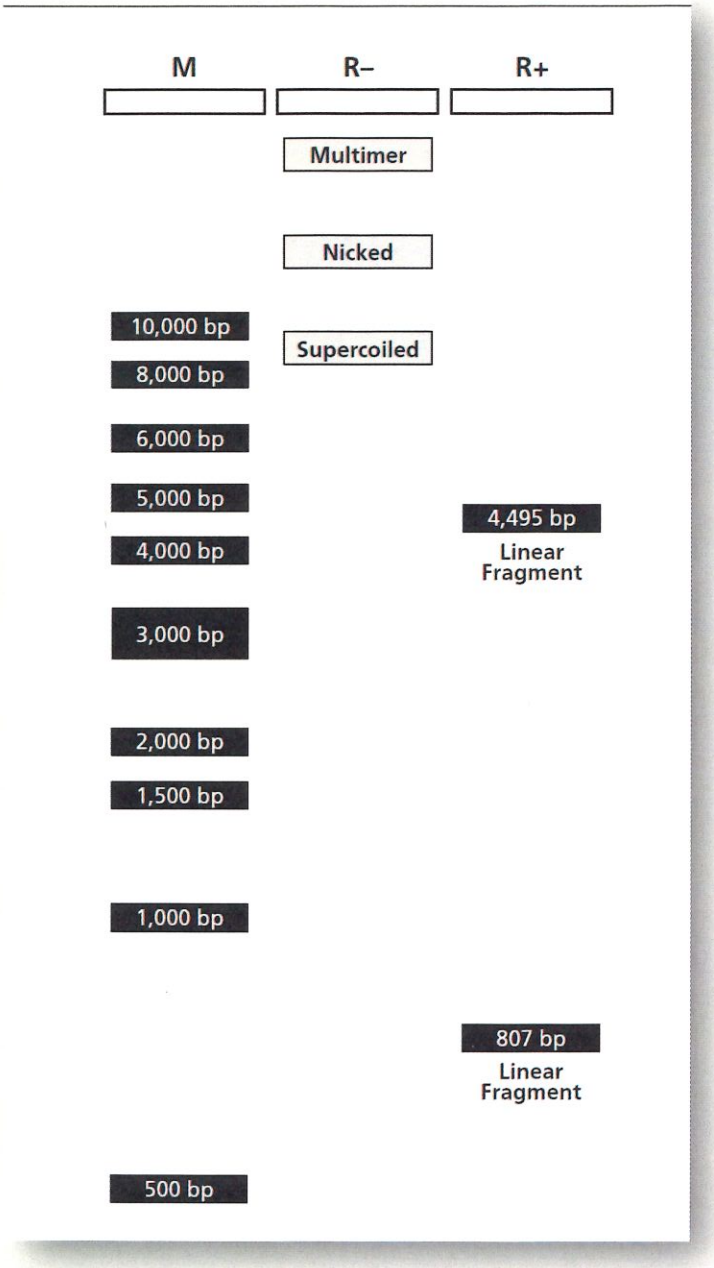
Turn on power supply and set voltage to 130–135 V



Check to see if the purple loading dye is moving toward the positive (red) electrode

Teacher will explain final steps

LAB 4A DNA LADDER DIAGRAM



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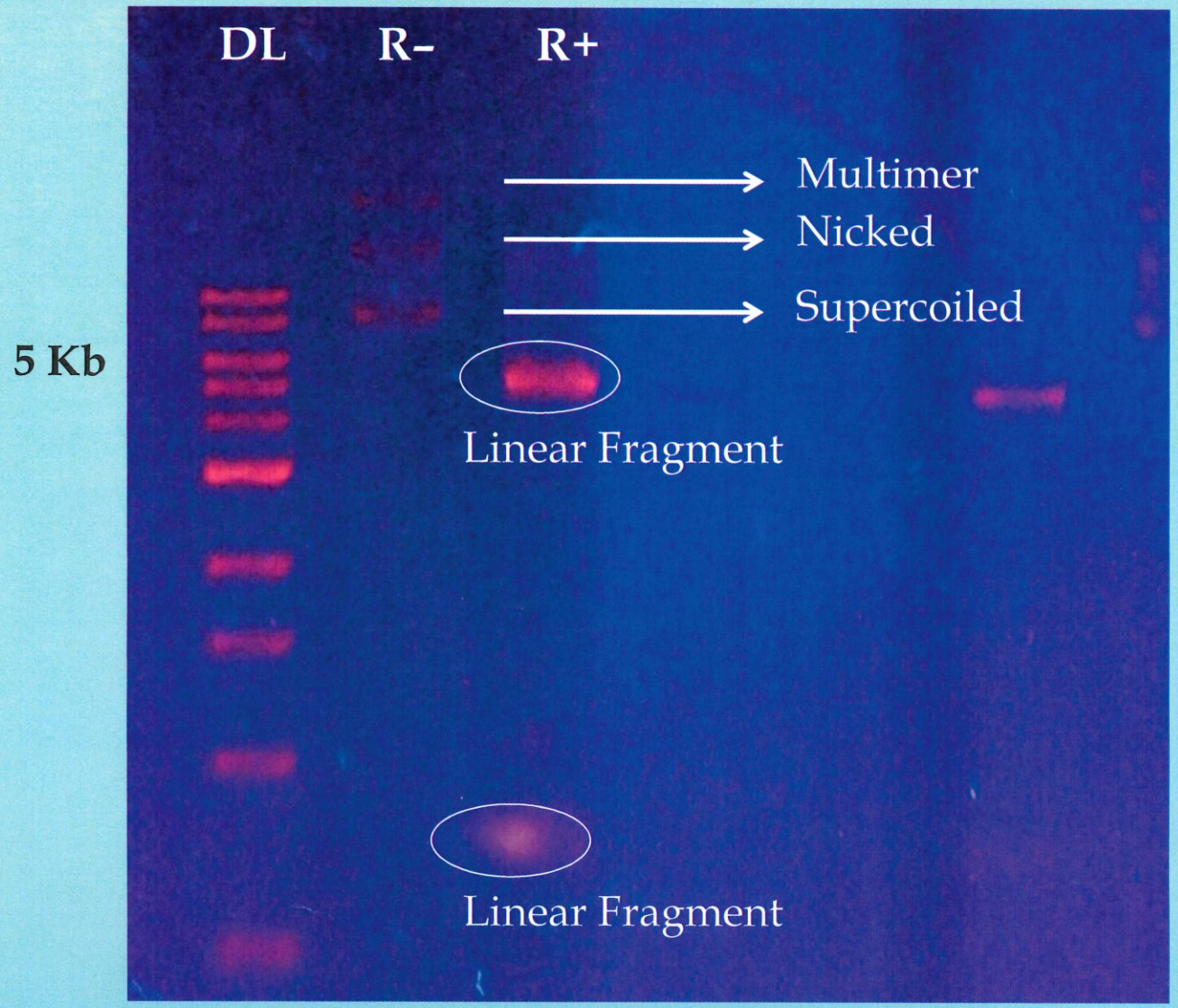
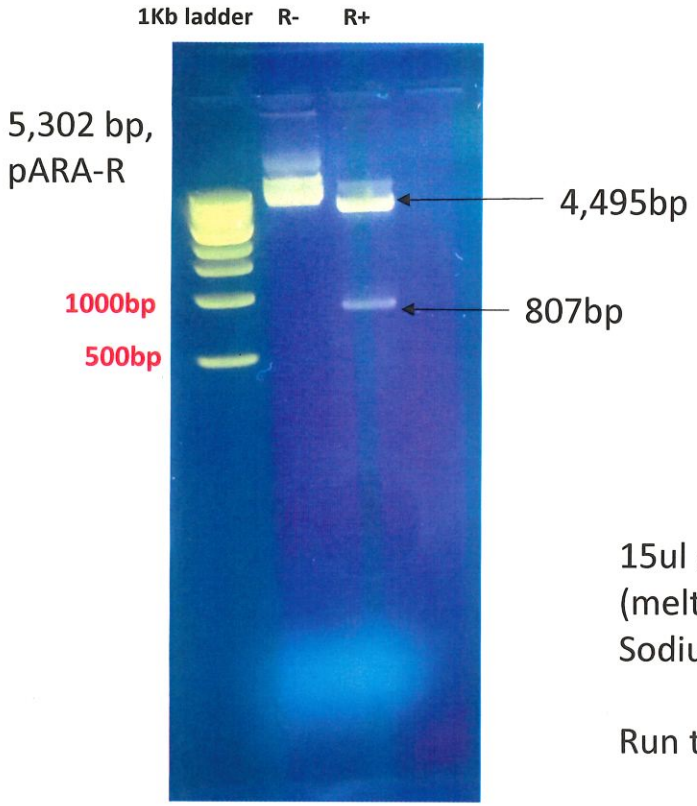
Lab 4A Gel Results





15ul of sybrsafe to 150ml of (melted) 8% agarose gel in 1X Sodium Borate Buffer

Run time: 40 minutes

LAB 5 RESOURCES

ATTENTION TEACHERS:

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

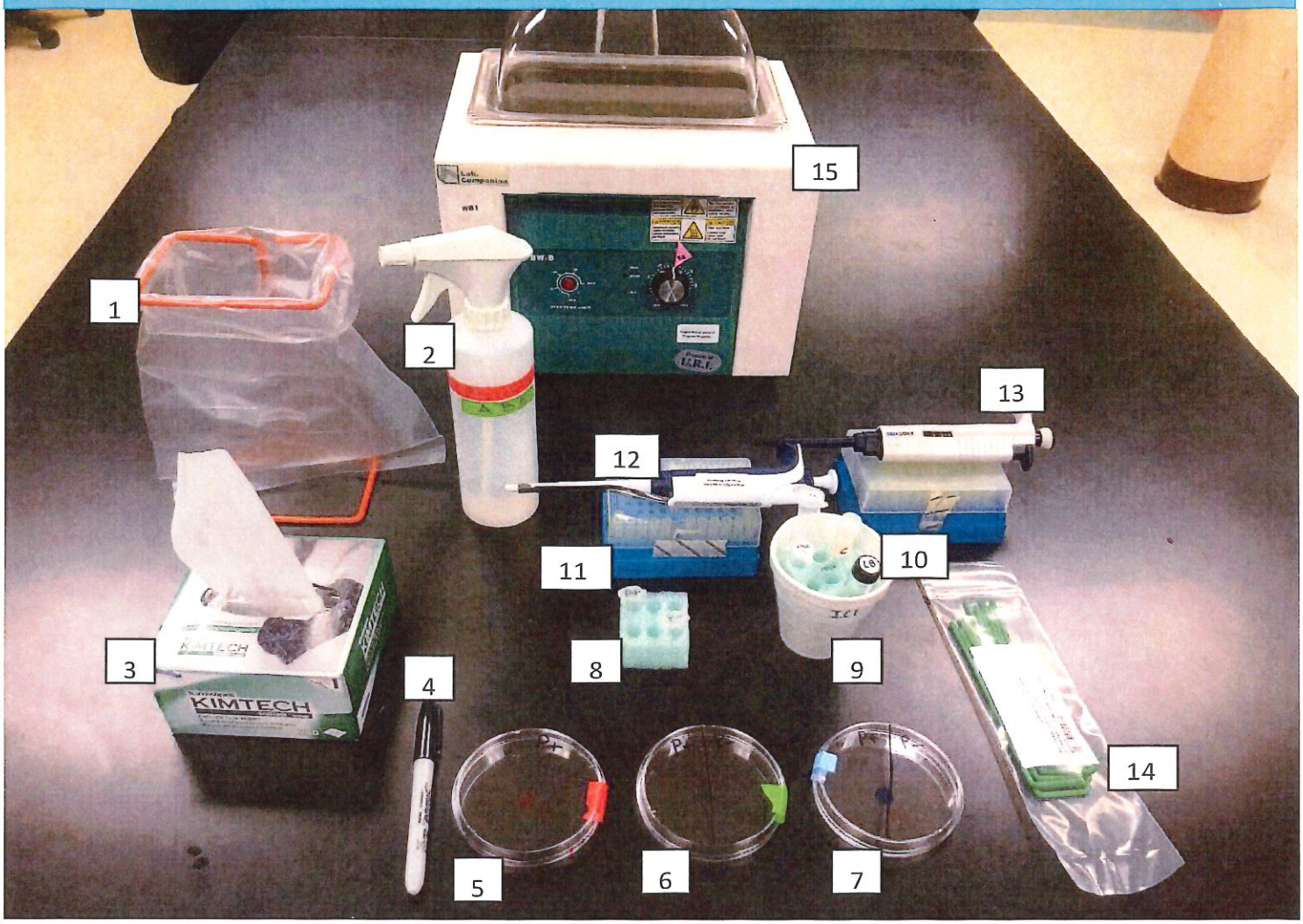
LAB 5A KIT ITEMS	LABELS	VOLUMES to aliquot
LB plates	**1 blue line**	---
LB/amp plates	**2green lines**	---
LB/amp/ara plates	**3 red lines**	---
P-20 micropipette		---
P-20 pipette tips		---
P-200 micropipette		---
P-200 pipette tips		---
Biohazard waste bags		---
10% Bleach spray bottles		---
Cell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

Notes:

- Competent cell stock can come in either 500µLor 1000µL aliquots. Aliquot 15 minutes before class use and keep on ice in the refrigerator.
- Emergency Pause step: After adding the 150 µL 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.

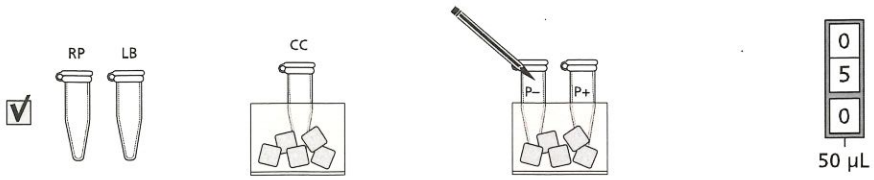
P-20, P-200, and P-1000 pipettes may contain locks on them: Please **UNLOCK** the pipette when adjusting the measurement

Lab 5

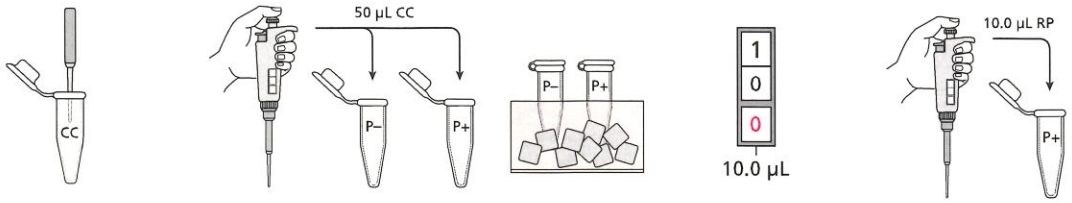


1. Biohazard bag and waste stand
2. 10% bleach bottle
3. Kimwipes
4. Sharpie marker
5. LB/AMP/ARA plate (marked by **3 red** stripes)
6. LB/AMP plate (marked by **2 green** stripes)
7. LB plate (marked by **1 blue** stripe)
8. Green float: used to hold tubes marked **P+ & P-**
9. Ice cup
10. **LB glass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

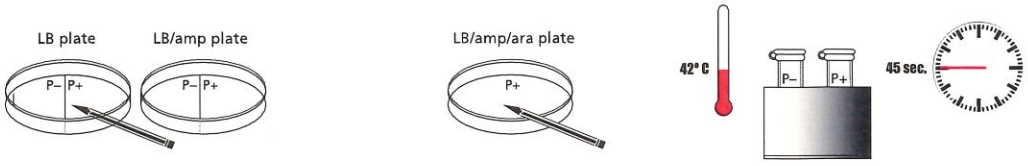


Check reagents (RP, LB) → Get CC (on ice) → Label two tubes P– and P+ and put tubes on ice → Set P-200 micropipette to 50 µL →

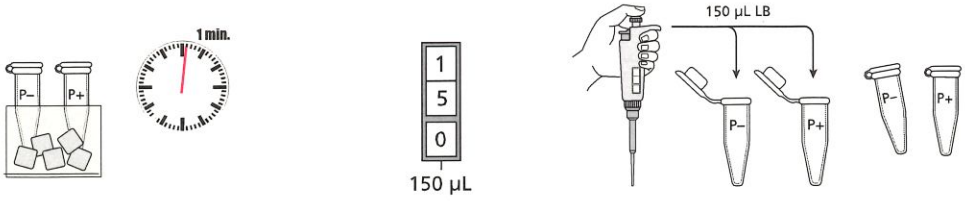


Suspend cells in CC tube by gently pumping cells in and out → Pipette 50 µL CC into P– and P+ tubes; hold tubes at the rim to keep cold and return quickly to ice → Set P-20 micropipette to 10.0 µL → Pipette 10.0 µL RP into P+ tube, holding P+ tube at the rim to keep cold →

Mix reagents in P+ tube by gently pumping cells in and out; return tube quickly to ice → Keep P– and P+ tubes on ice for 15 min. → Get the three agar Petri plates (LB, LB/amp, and LB/amp/ara), and label bottom of each with group number and class period →

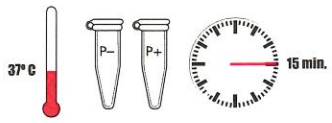


Draw a line on LB plate and LB/amp plates dividing them in middle; label one half "P–" and the other "P+" → Label LB/amp/ara plate "P+" → Carry cup with P– and P+ tubes on ice to the 42°C water bath, and place tubes in bath for exactly 45 sec. →

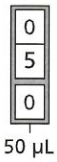


Immediately place tubes back on ice, and keep there for a minute → Set P-200 micropipette to 150 µL → Pipette 150 µL of LB into P– and P+ tubes, cap tubes, and gently flick to mix →

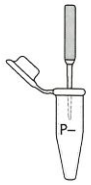
Laboratory 5A Flowchart (Continued)



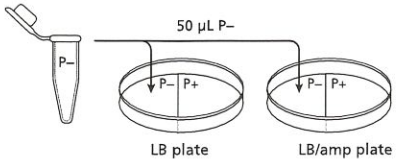
Incubate P– and P+ tubes at room temperature or at 37°C for 15 min.



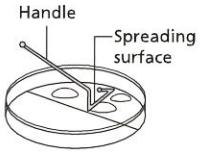
Set P-200 micropipette to 50 µL



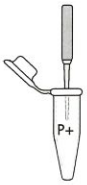
Gently pump pipette a couple of times in P– tube



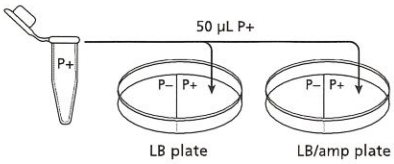
Pipette 50 µL of P– onto P– halves of LB and LB/amp plates, and close lids immediately after dispensing P–



Use cell spreader to spread P– cells on P– halves of LB and LB/amp plates, and close lids

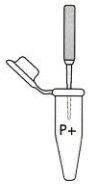


With new pipette tip, gently pump pipette a couple of times in P+ tube

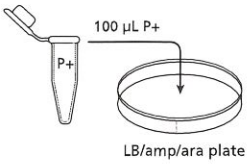


Pipette 50 µL of P+ onto P+ halves of LB and LB/amp plates, and close lids after dispensing P+

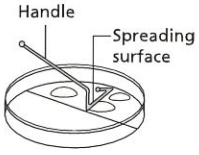
Set P-200 micropipette to 100 µL



Gently pump pipette a couple of times in P+ tube



Pipette 100 µL of P+ onto various sections of LB/amp/ara plate, and close lid



Use cell spreader to spread P+ cells on P+ halves of LB (1st) and LB/amp (2nd) plates and then on LB/amp/ara plate, and close lids

Let plates sit right side up for 5 min.

Tape all three plates together, and label tape with group number and class period

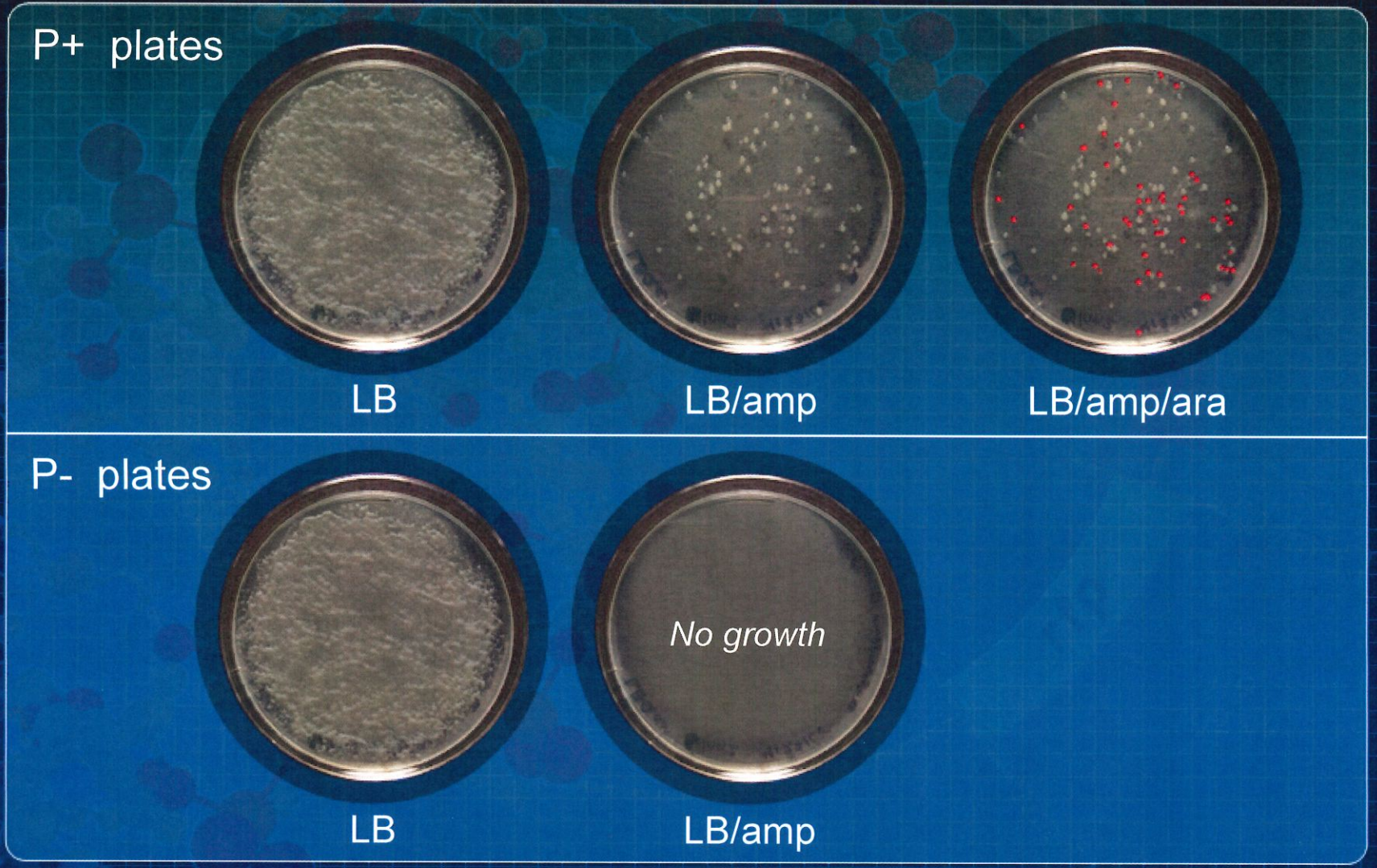
Place plates in the 37°C incubator upside down

Incubate plates for 24–36 hours at 37°C

Examine plates and record the amount of growth on each

AMGEN

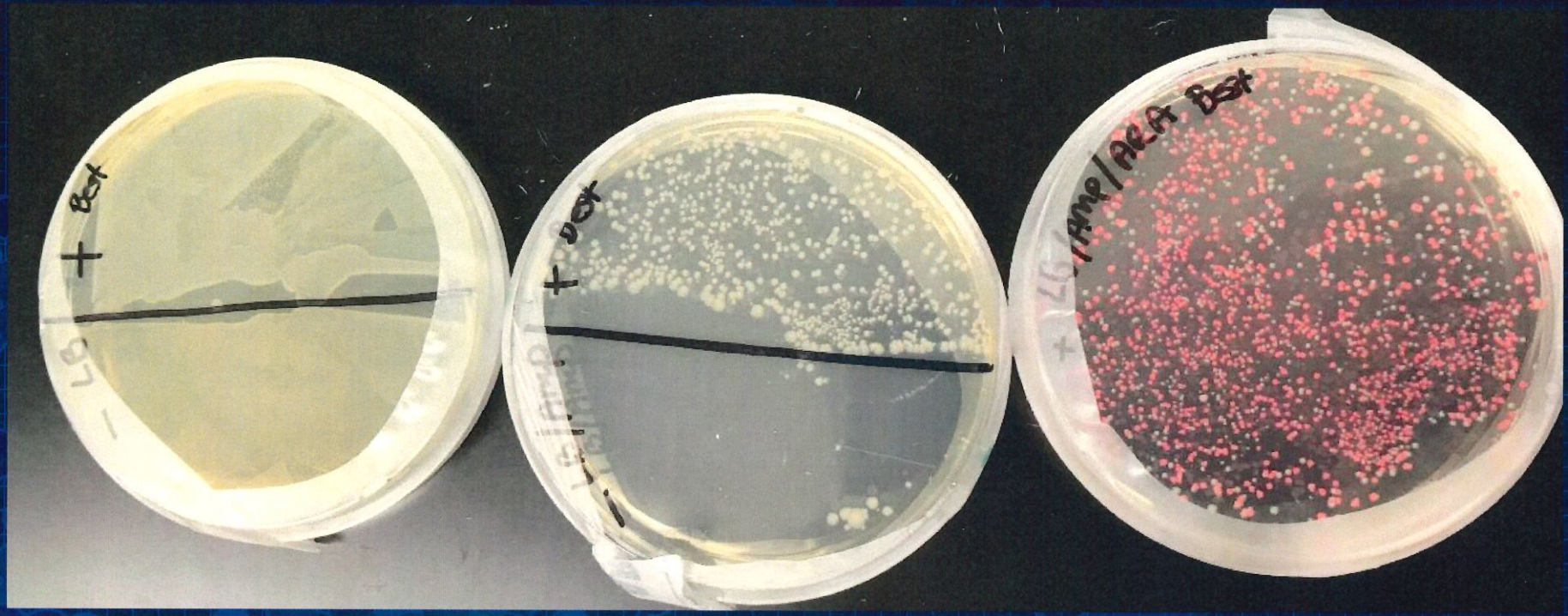
growth of transformed bacteria on various plates



AMGEN

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

Colony isolation and culture



LB LB/amp LB/amp/ara