

# *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 $\mu$ L( 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
<b>FREEZER BOX ITEMS</b>		
Marker/ 1kb ladder	DNA Mark	10 $\mu$ L

**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----.

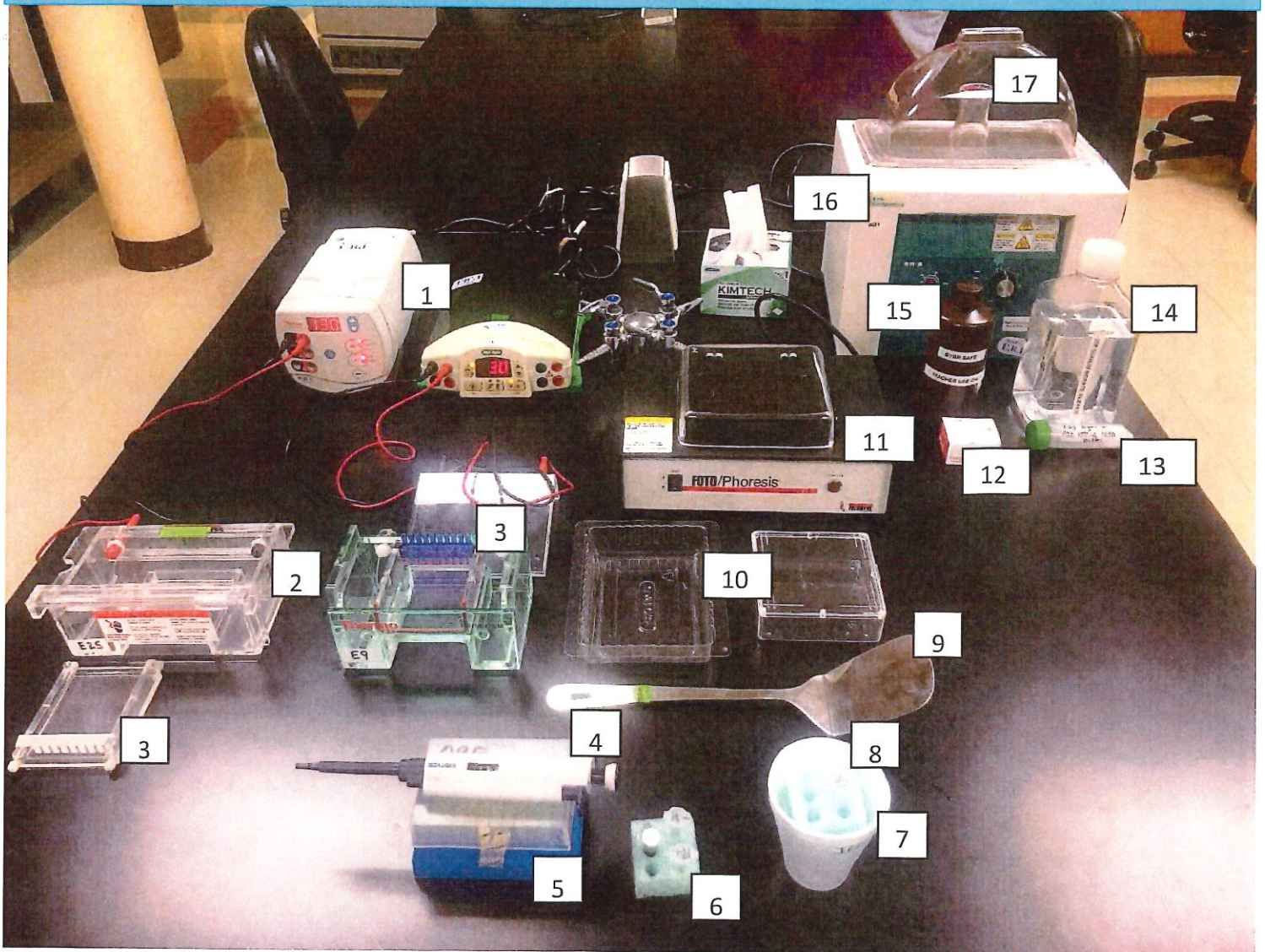
**Pre-stain gel Method:** Spin the SYBR safe DNA gel stain tube and then mix with your pipette before aliquoting it into melted agarose solution. Pipet 15 $\mu$ L of the SYBR safe stain (from the vial) into 180mL of melted agarose just before pouring your gel. Gently, swirl the melted agarose to mix 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 50 $\mu$ L of SyberSafe. Keep away from light. Solution 2 is the same reagent as LD.

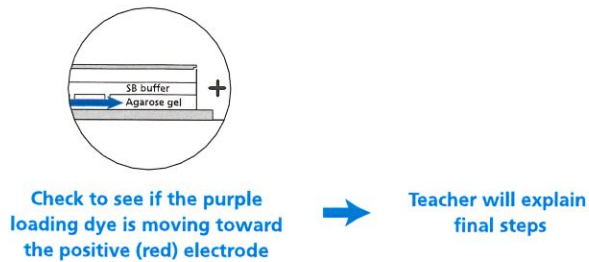
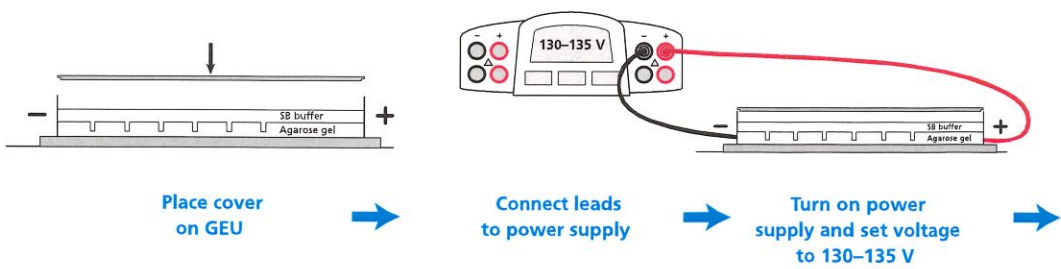
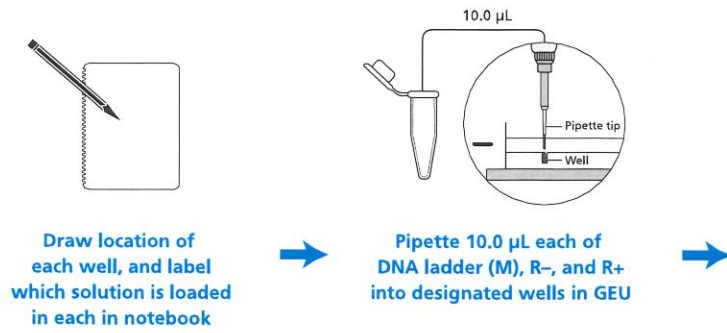
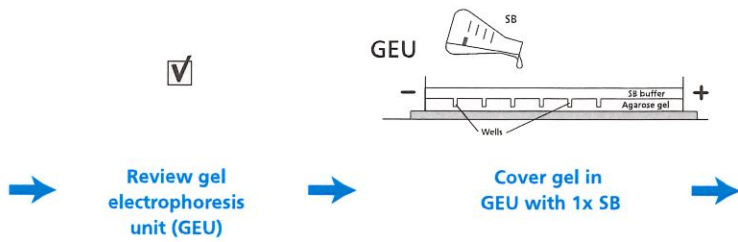
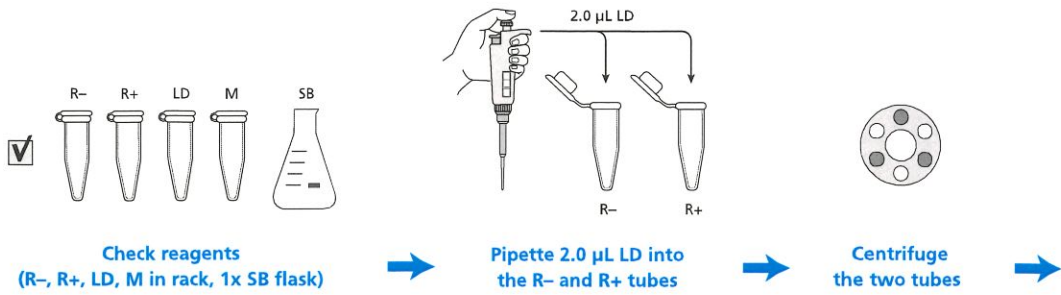
**P-20, P-200, and P-1000 pipettes may contain locks on them:** Please **UNLOCK** the pipette when

## Lab 4A

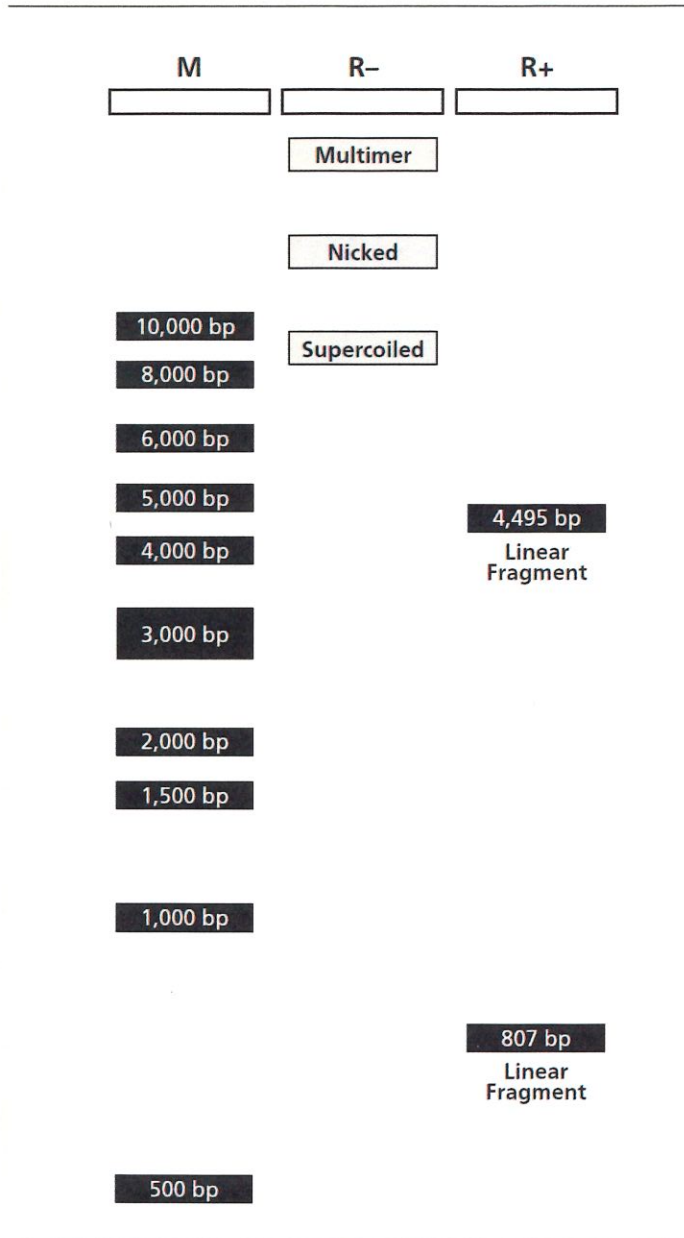


1. Electrophoresis power packs (both types that may be provided in kits are being displayed)
2. Electrophoresis chambers (both types that may be provided in kits are being displayed)
3. Trays and combs (both types that may be provided in kits are being displayed)
4. P2-20 micropipette
5. P20-200 micropipette 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°**)

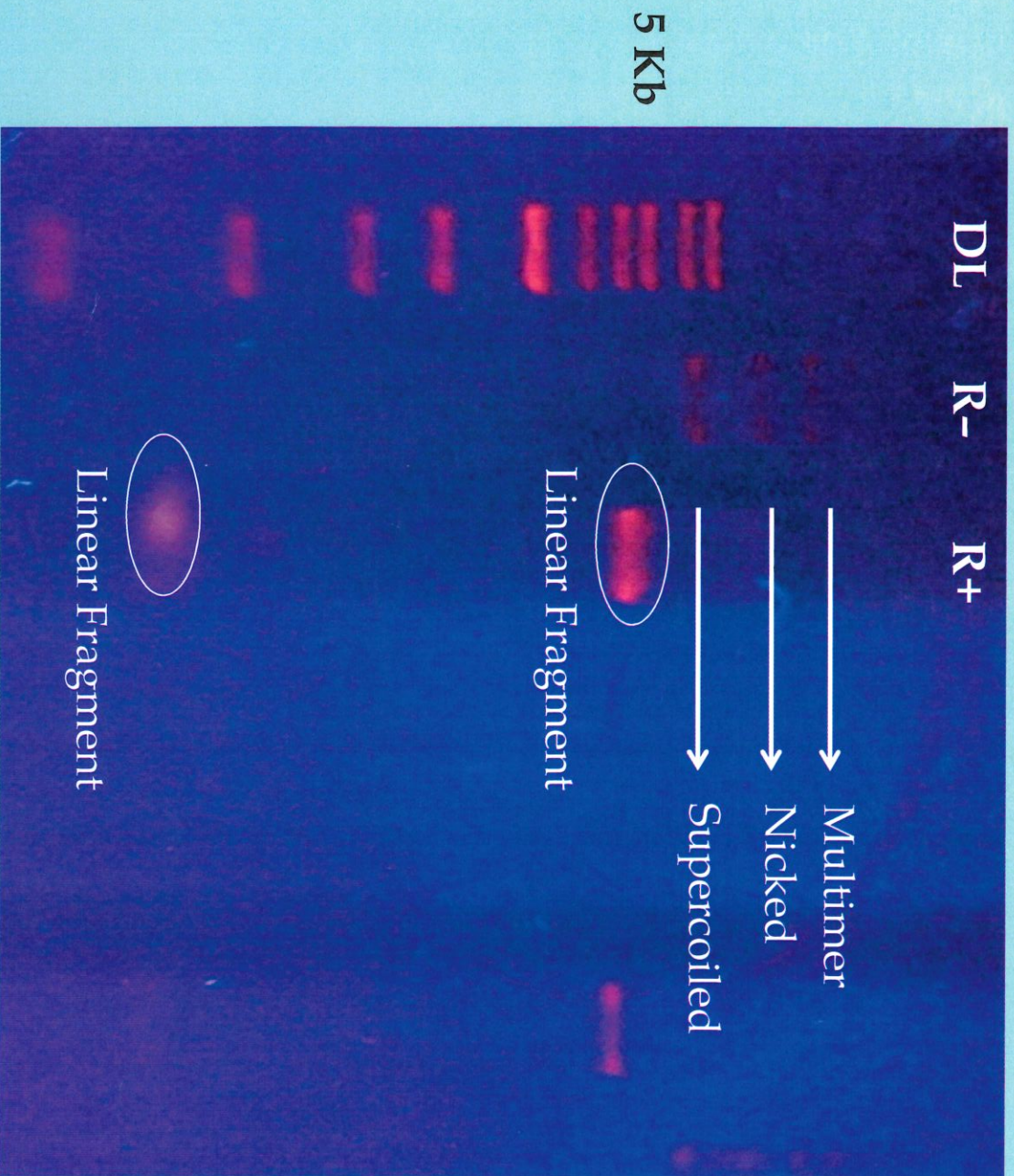
### Laboratory 4A Flowchart

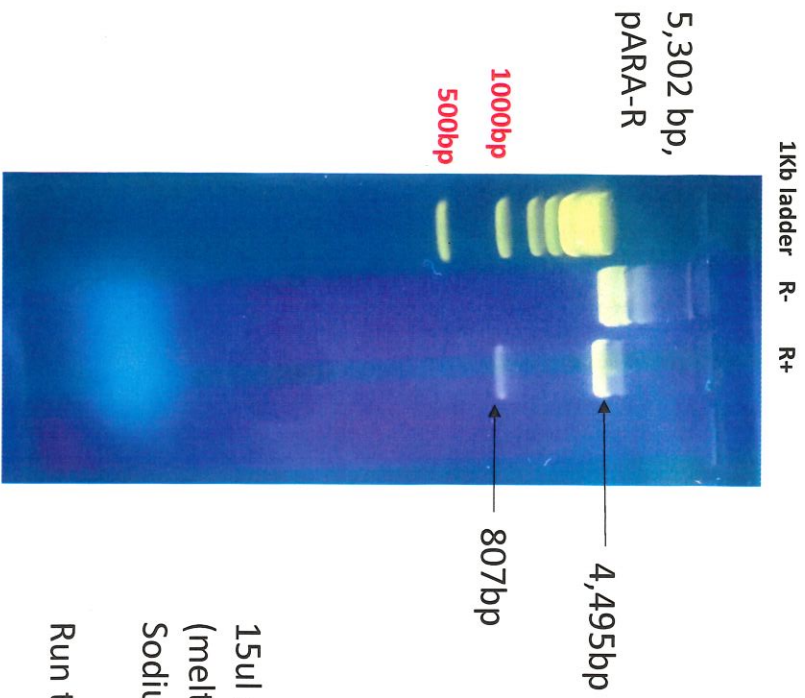


# LAB 4A DNA LADDER DIAGRAM



# Lab 4A Gel Results





15ul of sybrsafe to 150ml of  
(melted) 8% agarose gel in 1X  
Sodium Borate Buffer  
Run time: 40 minutes