AMGEN® Biotech Experience

Scientific Discovery for the Classroom

Rhode Island

# Resource Binder

## Contents:

1. Welcome Note
2. Reminder sheet
3. ABE Equipment Quick Guide
4. Case of the Missing Crown lab sheet
5. Resources/ Visual aids for labs 1-6

*Feel free to make copies of any paperwork you would like to keep. Please return originals with kit.

# Welcome to the 2024-2025 kit rotation!

You will find the enclosed RESOURCE BINDER containing

- ➢ ABE lab checklist for each lab listing supplies you need and aliquots you need to make with some helpful hints.
- ➢ Equipment manuals
- ➢ Travel Drive with SDS sheets for all reagents (found in resource binder)
- ➢ A pdf of the "Missing Crown"

***Feel free to make copies of anything in the resource binder. Please be sure to return originals with the kit.***
***The Contents of the Resource Binder can also be found on the Amgen Biotech Experience RI website.***

**REMINDERS:**

**-Use Distilled Water in all equipment and reagents.**
**-Store all reagents at the correct temperature.**
**-Try to check temperature of water bath and incubator the day BEFORE you need them.**
**-Please return all plastic ware for we reuse and recycle the containers.**
**-Please let us know if any equipment is not working properly or gets broken.**
**-Please return demo transformed plate and transformed broth (if you did not use**

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**-Please let us know if any equipment is not working properly or gets broken.**

**-Please return demo transformed plate and transformed broth (if you did not use)**

## TIPS:

**Lab 1.2:** Be sure to use a CLEAN flask for melting your agarose. You can make up all your gels early and store them in ziplock baggies. A gel tray uses ~30mLs of agarose.

**Lab2A and 4A:**

Pre-stain gel Method: Spin the SYBERsafe/Gel Green DNA gel stain tube and then mix with your pipettor before aliquoting it into melted agarose solution. Pipet 15µL of the SYBERSafe/Gel Green stain into 150mL of melted agarose /SB buffer solution just before poring your gel. Gently, swirl the melted agarose to mix the SYBERsafe/Gel green. Please put return all unused stock of SYBBRsafe/gelGreen (amber tube). We will need the stock tube for the next kit cycle. Keep away from light!

Post Stain Method: refer to Lab4A notes in this binder. Teachers must prepare.

**Lab 5:** The best results are obtained when the competent cells are kept as cold as possible. The adhesion zones disappear when the cells come back up to room temperature. Store the competent cells back in your freezer. Use crushed ice if possible, to place the cells in a cup submerged in the ice. If you must use cubed ice then add very cold water to it so the tubes are in contact. You can also use isopropyl alcohol that you placed in the freezer overnight in with the ice cubes - just make sure your students know which tube is which since the alcohol will erase sharpie marker. Freeze cryovial cool rack before use. When aliquoting cells for students, place aliquots of cells in the frozen cryovial cool rack and put back in the freezer.

***Some suggestions from other ABE sites***: The longer you recover the cells in the warm LB broth after the heat shock, the better your transformation efficiency. Being at 42°C is very hard on the cells, they need a "spa" day to recover. This lab can be done in two days if you have only 50-minute periods. During the recovery period, following heat shock, the students' tubes containing the cells and LB broth can be refrigerated overnight. The next class period, let plates come to room temperature before use-nobody likes the shock of being put into a refrigerator, even *E. coli.* The students can then incubate for at least 15 min at 37°C and then spread their plates.

**Lab 6:** If not using the Ready to use Transformed Broth: *Optional: To be arranged with RI program site. Transforming LB broth: Start your lab 6 culture 4-5 days BEFORE you will need it. . 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 2-3 hours) and when the broth starts to turn cloudy but not TOO cloudy), add the arabinose (1 full tube). Continue shaking overnight. We have enclosed an additional tube of Arabinose, you can add before you go home for the night. 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.* **This procedure is also found after lab 5 in the resource binder.**

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

# ABE Equipment Quick Guides

## Pipettes:

- Volume Setting: The pipet volume is shown on the handle grip window. To set volume make sure that the desired volume clicks into place, the digits are visible in the display window and that the volume is within the pipette's range.
- **WARNING: USING EXCESSIVE FORCE TO TURN THE PUSH BUTTON OUTSIDE THE PIPETS RANGE WILL JAM THE MECHANISM AND DAMAGE THE PIPET.**

## Electrophoresis Chamber:

1. Place the tray on a level surface
2. Insert the comb
3. Pour agarose gel to a 4-5mm thickness
   **Note:** When pouring agarose make sure that agarose gel has cooled before pouring into gel tray. Pouring agarose before it cools to this temperature can cause the tray to crack.
4. Add buffer to electrophoresis chamber
5. Load DNA samples into the wells.
6. Put the lid on the electrophoresis chamber
7. Plug the chamber into the power supply.
8. Turn the power supply on and set to appropriate voltage.
   **WARNING: MAKE SURE THERE IS NO LIQUID ON THE OUTSIDE SURFACE OF THE CHAMBER. DO NOT TURN ON THE POWER SUPPLY UNTIL THE COVER HAS BEEN PUT ON THE CHAMBER.**
   Make sure not to run the gel at too high of a voltage, high voltages can cause curving of the bands which could make it difficult to interpret results.

## FOTO/Phoresis UV Trans illuminator Operation:

1. Open UV blocking cover, place the mini gel on the purple filter glass and close the cover.
   - **CAUTION: DO NOT USE FOTO/PHORESIS IF COVER IS BROKEN.**
2. Turn on the power
3. View the sample
   - Only View the sample with the cover on, this protects from UV exposure.
4. Photograph gel if desired
5. Once you are done looking at the gel turn the power off, remove the gel and wipe the purple filter glass.

## Mini Centrifuge:

1. Before running the centrifuge make sure the power switch is in the "on" position.
2. To begin the run simply close the lid of the centrifuge
3. To stop rotation press down on the lid release tab located on the front of the unit.
4. After the rotor has stopped the lid can be opened by lifting the lid on the hinge.
5. Always make sure that the centrifuge is properly balanced in order to prevent rotor damage.

## Water Bath:

Status indication lights are as follows:
RUN LED: operating status
HEAT LED: operating of heating element
O/T LED: it is ON when over temperature device is active
**WARNING: DO NOT TURN ON UNIT UNTIL WATER HAS BEEN ADDED TO THE RESERVOIR**

1. Press main power switch
2. Select appropriate temperature
3. Set over temp limit to 10-15 degrees higher than the set temperature.

## Micro centrifuge Bio-Rad 16K:

1. Plug in the centrifuge, lid should click open
2. Remove rotor lid
3. Load centrifuge with appropriate sized tubes
4. MAKE SURE CENTRIFUGE IS BALANCED BEFORE RUNNING
5. Replace rotor top and close lid
6. Set desired speed and time
   **WARNING: DO NOT OPEN THE CENTRIFUGE UNTIL THE ROTOR HAS COME TO A COMPLETE STOP**

## Micro centrifuge Eppendorf:

1. Turn on centrifuge with switch located on the back by the power supply cord
2. Press open button on the front to open the lid
3. To remove rotor cover twist the handle left
4. Load centrifuge, pay careful attention that the unit is balanced
5. Once samples are loaded replace the rotor cover by twisting to the right until you hear a click
6. Close the lid
7. Set proper time and speed
8. Press the start button
9. Once cycle is complete the centrifuge lid will open on its own

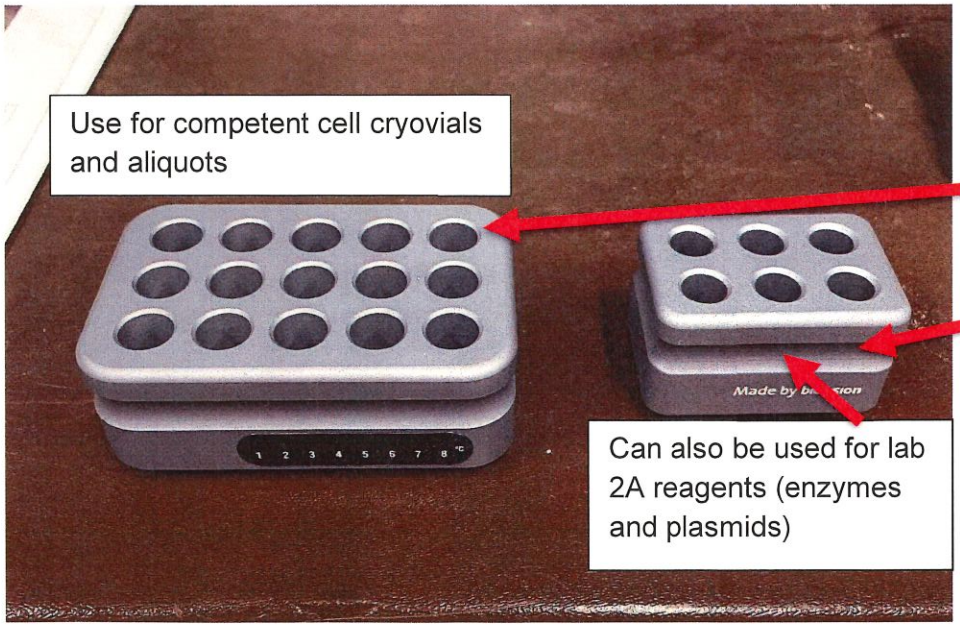
## Incubating Mini Shakers - VWR:

1. Plug in power cord once the standby light has illuminated the unit is ready for use.
2. Press the standby button to move unit from standby mode
3. Press the up/down arrows below the temperature display until you reach desired temperature.
4. Press the on/off button to start the heating function.
5. Press up/down button to adjust the speed display. The on/off button will start the shaking function.

   **CAUTION: the hot indicator light warns that the temperature of the air in the chamber is more than 40 degrees Celsius (104 F)**

# blueBox™ S Transilluminator with Imaging Hood


1. Place gel on platform.
2. Turn on unit. Caution keeps the orange lid down. Blue light is very bright.
3. Place the Black view finder on top of the box.
4. View through opening.
5. Take a picture with your phone

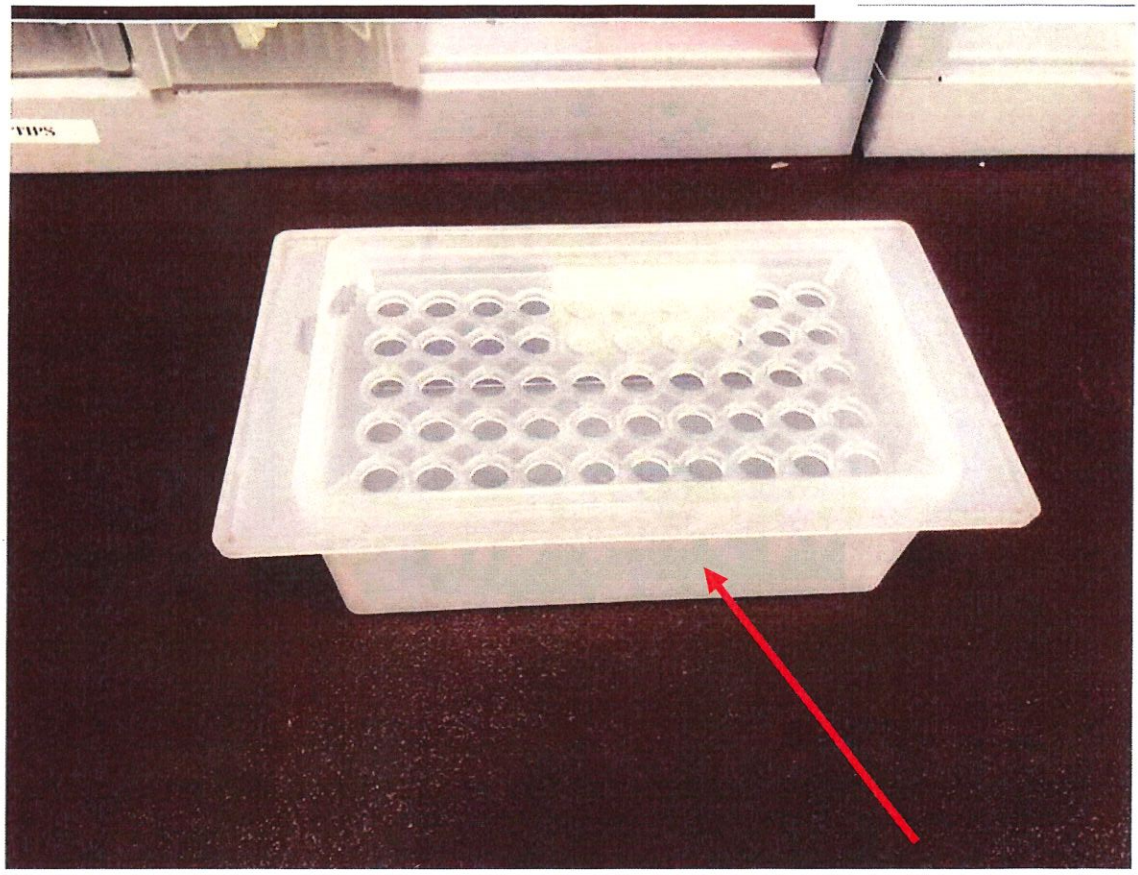

These are cold racks used to keep important reagents/aliquots cold. The **leftmost** rack is used for competent cell aliquots in **lab 5.** While the **rightmost** rack is used to hold the reagents in **colony PCR.**

The racks must be frozen prior to experiment to work properly.

**Please freeze <u>at least</u> a day before use!**

**White Ice tray for Teacher use: Fill the tray with ICE.**

**Use this tray for preparing aliquots for any lab with frozen reagents.**



Fill tray with ice before placing top/cover

# Teacher Instructions for Gel Electrophoresis Trays and Buffer

Gel electrophoresis labs require preparation of agarose, agarose gels, and 1x gel electrophoresis buffer from a stock solution of 20x sodium borate. The instructions for these additional preparation tasks follow. The instructions assume that you will provide materials for 12 groups of 2–3 students. Multiply the amounts as necessary depending on the number of students and the number of classes you are teaching. Gels are needed for Laboratory *1.2* and *Laboratory 4A and Colony PCR.*

*Make Agarose Gels*

1. Gather the following materials:
   - 6 - gel electrophoresis trays
   - 6 - 10 well combs
   - Optional: Tape
2. Set out the 6 electrophoresis gel trays and 6 combs. Prepare the trays for casting. Place a comb in each tray before adding the agarose solution.
3. Prepare the agarose solution.
   a. Gather the following materials:
      - 2 - 250mL graduated flasks, one labeled "1x SB"
      - 10 mL of 20x sodium borate buffer (20x SB)
      - 190 mL of distilled or deionized water ($dH_2O$)
      - 1.44 g of agarose for 0.8% gels (1.2, 4A, Colony PCR). Provided as 1.44g in a 50 ml conical tube.
      - Mass scale (optional)
      - 500-mL flask labeled "Gel"
      - Plastic wrap
      - Disposable pipette tip
      - Microwave
      - Heat-resistant gloves or tongs
      - 6 sandwich- or quart-sized zip-lock bags
      - Waste container for used tips and microfuge tubes

   *b.* Prepare 200 mL of 1x gel electrophoresis buffer: Add 10 mL of 20x SB to a 250 mL graduated flask, add $dH_2O$ to the 200-mL mark, and mix. (For 250ml 1X SB buffer; 12.5mL of 20X SB to 237.5 mL of water).

   c. Pour 180 mL of 1x SB into the second 250-mL graduated flask.

   d. Measure 1.44 g of agarose with the mass scale and place it in the 500-mL flask labeled "Gel." Add the 180 mL of 1x gel electrophoresis buffer from step 3c to make 0.8% agarose solution.

   e. Cover the opening of the 500-mL flask with plastic wrap. Use the pipette tip to poke a small hole in the plastic wrap.

   ***f.*** ***Cover the opening of the 500-mL flask with plastic wrap. Use the pipette tip to poke a small hole in the plastic wrap.***

   g. Place the covered flask in a microwave and heat for one minute on high. With a gloved hand, gently swirl the flask. (Alternatively, a hot plate can be used to melt the agarose, but you will need to use a double boiler.)

**Safety:** Wear heat-resistant gloves or use tongs to hold the flask.

h. Continue microwaving the flask for 5–15-second intervals until all the agarose has dissolved. To check this, hold the flask to the light and swirl the solution. Look carefully for "lenses" of agarose crystals suspended in the liquid. If no lenses are visible, the agarose is dissolved. **For lab 1.2, continue to step 4.**

i. **LAB 4A AND COLONY PCR**: Add 18 µL of Sybrsafe ™ to the 180mL melted agarose solution. Gently swirl. (1:10,000 dilution). Allow the agarose solution to cool to the point that you can safely touch the bottom of the flask (approximately 60°C; this will take around five minutes).

**Preparation tip:** Do not allow the solution to cool to the point that the agarose begins to re-solidify. If it does, simply reheat the solution as described above.

4. Cast the gels in the prepared trays by pouring 25–30 mL of the agarose solution into each electrophoresis tray. (The amount may be different depending on the trays you use.)
   a. Be sure to include the combs when casting the gels. The solution should cover about 2 mm of each comb.
   b. Once the gels solidify (which will take around 30 minutes), pull the comb out of each gel. Pull it straight out without wiggling it back and forth; this will minimize damage to the front wall of the well.
   c. Remove the gels from the gel electrophoresis trays and store them in individual zip-lock bags with a small amount of the remaining 1x gel electrophoresis buffer from step 3b. Store in the refrigerator until ready to use.
   d. **NOTE: FORE LAB 4A AND COLONY PCR GELS: BEST TO USE SYBERSAFE™ STAINED GELS WITHIN 24HRS OF PREPARING.**

*Prepare 300 mL of 1x gel electrophoresis buffer for Laboratory 1.2, 2A or Colony PCR*

1. Prepare the solution:

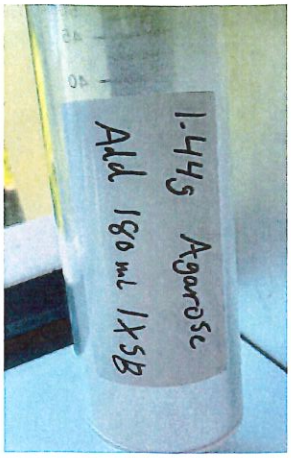
**Preparation tip:** You should prepare 1x gel electrophoresis buffer for all classes that will complete this lab—simply multiply the quantities given by the number of classes.

   a. Gather the following materials:
      - 15 mL of 20x SB
      - 500 mL graduated flask labeled "1x Buffer"
      - 285 mL (with SB) of distilled or deionized water
      - 6 - 50 mL flasks labeled "1x Buffer"
   b. Add 15 mL of 20x SB buffer to the 500 mL flask labeled "1x Buffer," add distilled or deionized water to the 300-mL mark, and mix.
   c. Pour 50 mL of solution into each of the 50 mL flasks labeled "1x Buffer."

**Note:** Once used, 1x gel electrophoresis buffer can remain in the electrophoresis box or discarded. If you have back-to-back classes, keep the buffer in the electrophoresis boxes for classes doing the same lab.

***Optional:*** *If you forget to add* Sybrsafe™ *after preparing the molten agarose and already casted the gel. You can add 10µL of* Sybrsafe™ *to1 liter of 1X SB buffer (1:10,000 dilution) and use as a post stain. After gels have finished running (electrophoresis). Place gel in a container and cover completely with 1XSB with the* Sybrsafe™. *Post stain for at least 30 minutes.*

# Lab 1.2



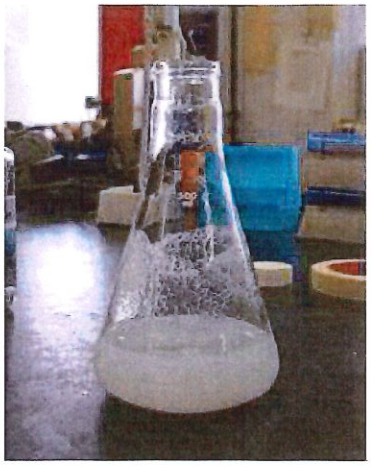

## Kit Materials:

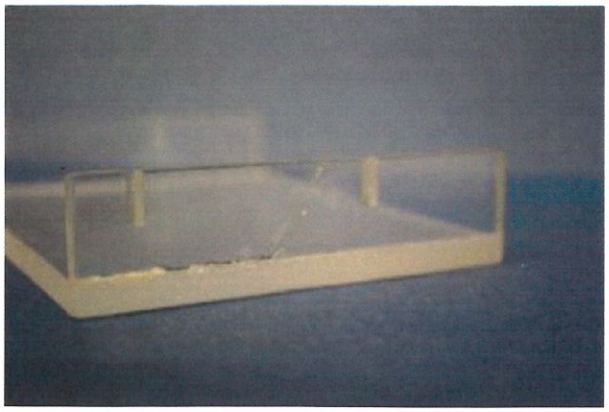
agarose, 20x Sodium Borate (SB) buffer, gel trays, combs, electrophoresis chamber, power supply, Solutions #1, 2, 3 (store at RT), red dye, P-20 micropipette and tips

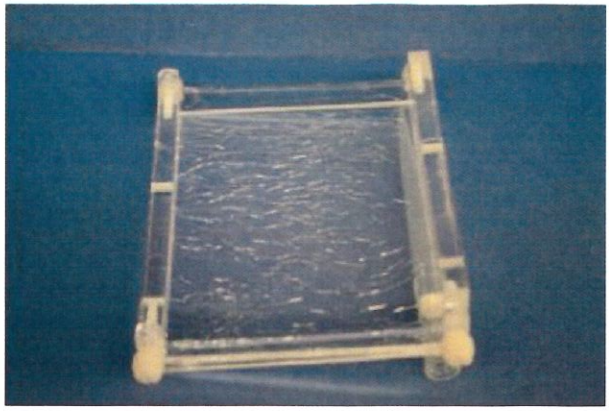
## Gel Preparation:

*Items: Agarose, 20x Sodium Borate (SB) buffer (to be diluted to 1x SB buffer), gel trays, combs*

- **Melting Agarose**
    - To prepare 0.8% agarose gel, add 180mL of 1x SB buffer to 1.44g (already measured in conical tubes unless otherwise noted) of agarose into a 500mL flask.
    - Place the covered flask in a microwave. Set the microwave for 1 minute on high. With a gloved hand, (it's hot) gently swirl the flask.
    - Place the covered flask in a microwave. Set the microwave for 1 minute on high. With a gloved hand, (it's hot) gently swirl the flask.
    - Continue this procedure, reducing the time on the microwave (5 – 15 seconds), until all of the agarose has been dissolved and the solution is clear.
    - Let the agarose cool until the flask is warm to the touch. Pouring hot liquid will warp the trays, resulting in poor electrophoresis results.







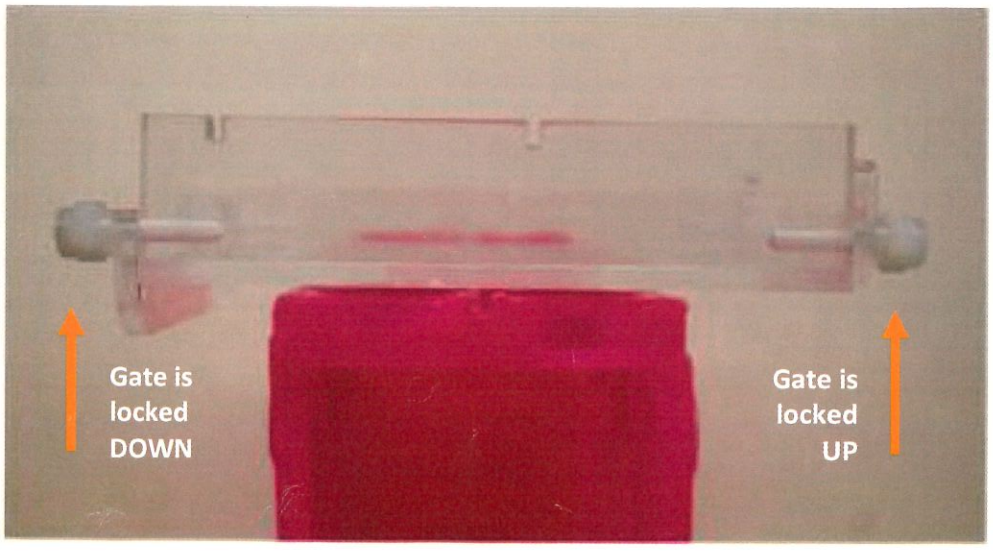
*Examples of cracked/warped trays as a result of pouring hot agar solution*

- You can keep melted agarose in a 60˚C waterbath if there is a delay before pouring the gels or if they are having students pour the gels)

- **Preparing Trays**

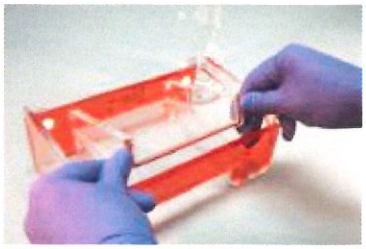
  - Prepare the trays for casting by pushing "up" the gates on the ends of each tray then tightening the screws enough so the gates seal and stay up, as well as inserting the desired number of combs.

  - Pour about 30mL of the solution into each tray, covering about 2mm of the comb.



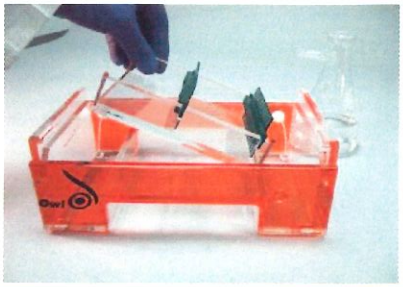
## Thermo Scientific B1A EasyCast Mini Gel System
### Casting with Owl's Gel Casting System

**1. Place UVT gel tray in buffer chamber in the casting position. Gaskets will form a seal against the walls of the chamber.**



**2. Pour warm (<60°) agarose onto tray and set combs in the desired comb slot(s).**

**3. Once solidified, turn the tray 90 degrees to the running position, remove combs, add buffer, load samples and run the gel.**





**For more information, visit: www.thermoscientifc.com/owlsci**

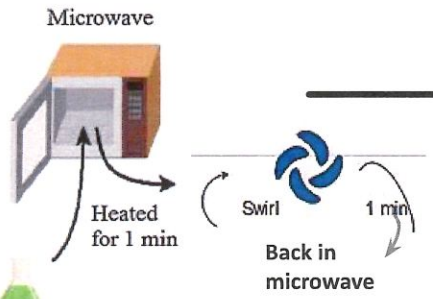
# Making Agarose Gels

**Lab 1.2: Step A 0.8% gels**
**Lab 4A and Colony: Step A 0.8 % gels**

**Lab 1.2: no SyberSafe®**
**Lab 4A and Colony PCR: add SyberSafe®**

1.44g Agarose

180mL 1X Sodium Borate buffer



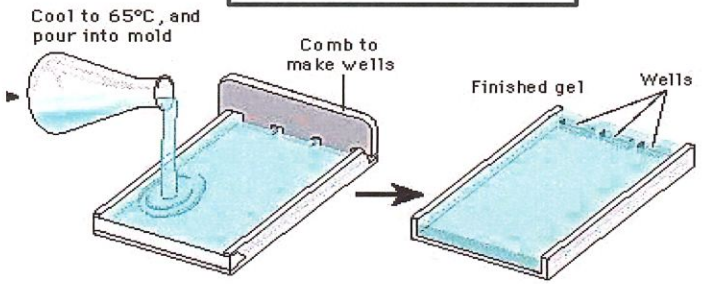

**Lab 4A, Colony PCR Step B**

Add 18µL SyberSafe® to 180mL of 1X SB or 1µL to 10mL of 1X SB Gently swirl

**Lab 1.2: Step B**

**Lab 4A/Colony PCR: Step C**

**Let the solution cool down to about 60 °C at room temperature.**




(30mLs of agarose per plate; ~ 6 gels per flask for lab 1.2 and 4A and Colony PCR.

***Running Buffer for all labs: 1X Sodium Borate; ie:10 mL 20X SB to 190 mL $dH_2O$***

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# LAB 1 RESOURCES

**ATTENTION TEACHERS:**

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

## LAB SUPPLIES/EQUIPMENT/REAGENTS CHECKLIST

| LAB 1 KIT ITEMS | LABELS | VOLUMES |
|---|---|---|
| Agarose | | Weigh out 1.44g agarose and add 180mL of 1X SB |
| Solution #1 | **S1** | |
| Solution #2 | **S2** | |
| Solution #3 | **S3** | |
| Red Practice Dye | **RD** | |
| 20x SB buffer | | |
| Extra microfuge tubes | | -- |
| P-20 micropipette | | -- |
| P-20 pipette tips | | -- |
| Electrophoresis chambers | | -- |
| Power Supply | | -- |
| Gel trays/combs | | -- |
| Spatula | | -- |
| Gloves | | -- |
| Microfuge tube racks | | -- |
| Parafilm | | |
| Sharpie markers | | -- |

**Notes:** Lab 1.2: Be sure to use a CLEAN flask before melting your agarose. You can make up all your gels early and store them in zip lock baggies. (Make sure to add SB buffer to zip lock baggies so the gels doesn't dry out) . A gel tray uses about 30mls of agarose.

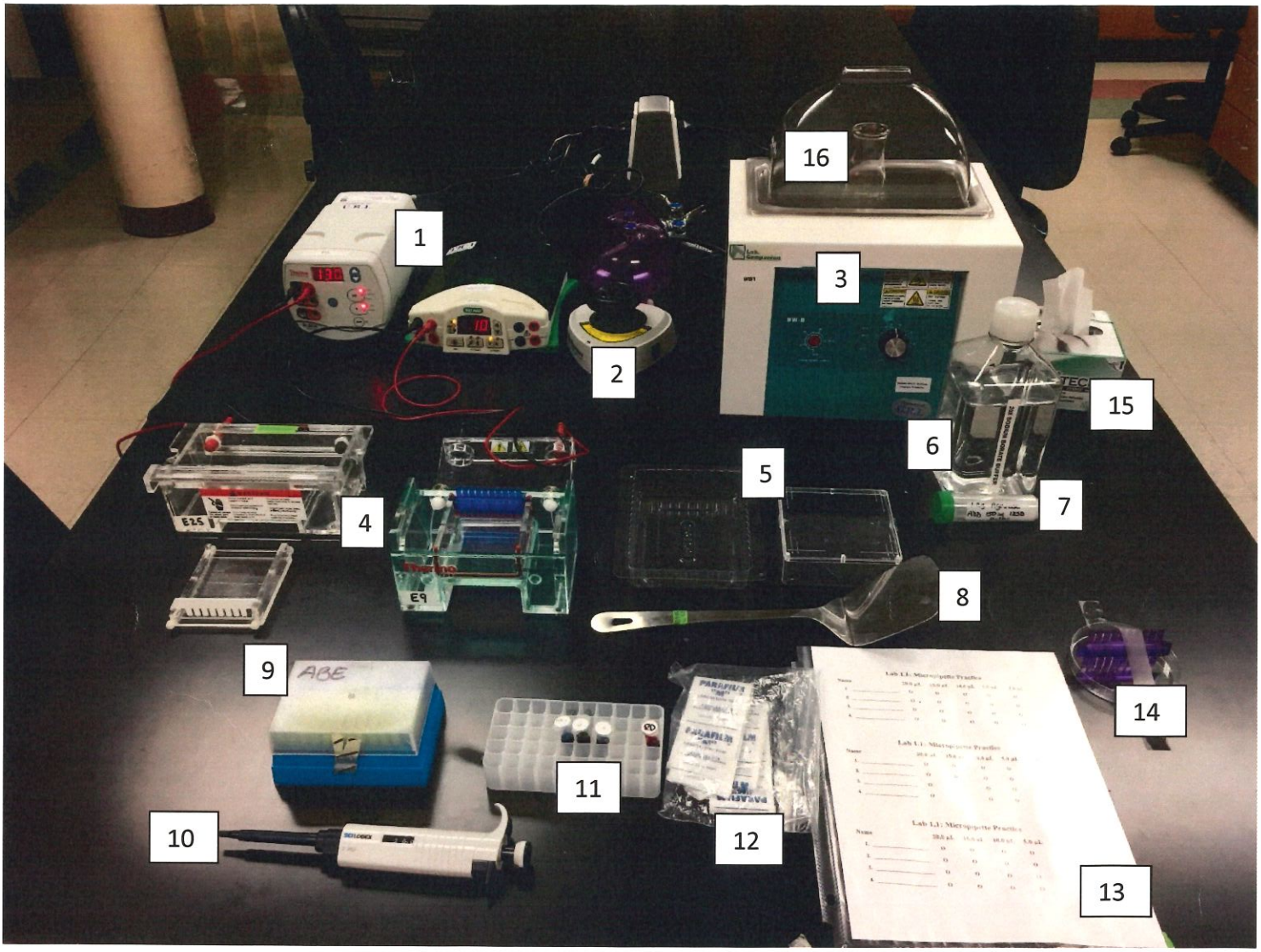
***NEW*** Take a look at the worksheet on how to read the gels, it has a picture of what the gel should look like in color.

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 33 in 2019 teacher Guide. Also refer to picture guide on Gel Making.

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

Thank you 

# Lab 1.2



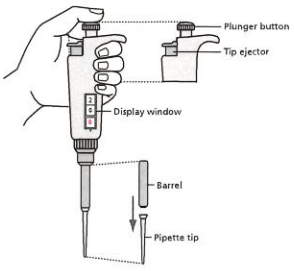

1. Electrophoretic power packs
2. Mini-micro centrifuge
3. Water bath
4. Gel electrophoretic apparatuses w/ tray and comb
5. Staining trays
6. 20x SB buffer
7. Agarose
8. Spatula
9. P20-200 pipette tips
10. P2-20 pipette
11. Solution 1,2,3 & red dye
12. Parafilm
13. Practice sheet
14. Practice petri dish
15. Kim wipes
16. Flask in water bath to cool agarose

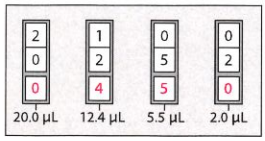
**Possible answers:**

1. Why do you think it is necessary to use very small and exact volumes of reagents in biotechnology? *In this field you would use very small amounts of the reagents and the correct measurements of reagent amounts is necessary for procedures to be successful.*

2. Read through the Methods section on pages 21 through 23 [of the Student Guide] and briefly outline the steps, using words and a flowchart.

***Laboratory 1.1* Flowchart**

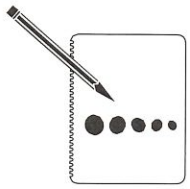




Check reagent (RD) → Review MP (micropipette) → Practice setting MP volume to 20.0 µL, 12.4 µL, 5.5 µL, 2.0 µL →

**Micropipette Practice:**

| Name | 20.0 µL | 15.0 µL | 10.0 µL | 5.0 µL | 2.0 µL |
|---|---|---|---|---|---|
| 1. _____ | ○ | ○ | ○ | ○ | ○ |
| 2. _____ | ○ | ○ | ○ | ○ | ○ |
| 3. _____ | ○ | ○ | ○ | ○ | ○ |
| 4. _____ | ○ | ○ | ○ | ○ | ○ |

Pipette 20.0 µL RD onto laminated sheet → Pipette other amounts of RD onto laminated sheet →



Draw sizes of RD amounts in notebook

# Lab 1.1: Micropipette Practice

| Name | 20.0 µL | 15.0 µL | 10.0 µL | 5.0 µL | 2.0 µL |
|---|---|---|---|---|---|
| 1. _____ | O | O | O | O | O |
| 2. _____ | O | O | O | O | O |
| 3. _____ | O | O | O | O | O |
| 4. _____ | O | O | O | O | O |


# Lab 1.1: Micropipette Practice

| Name | 20.0 µL | 15.0 µL | 10.0 µL | 5.0 µL | 2.0 µL |
|---|---|---|---|---|---|
| 1. _____ | O | O | O | O | O |
| 2. _____ | O | O | O | O | O |
| 3. _____ | O | O | O | O | O |
| 4. _____ | O | O | O | O | O |

# Lab 1.1: Micropipette Practice

| Name | 20.0 µL | 15.0 µL | 10.0 µL | 5.0 µL | 2.0 µL |
|---|---|---|---|---|---|
| 1. _____ | O | O | O | O | O |
| 2. _____ | O | O | O | O | O |
| 3. _____ | O | O | O | O | O |
| 4. _____ | O | O | O | O | O |

## SESSION 2

**Key ideas:** **Those who carry out genetic engineering use very specific tools and have well-honed laboratory skills. Gel electrophoresis allows for the visualization of minute amounts of DNA. Using this technique, scientists can separate and identify pieces of DNA they are working with.**

**Have students complete *Laboratory 1.2*. During the lab, have students share their answers to the Before the Lab and the STOP AND THINK questions and explain their thinking. (35 min.)**

Have students share their answers to the Before the Lab questions with the class.

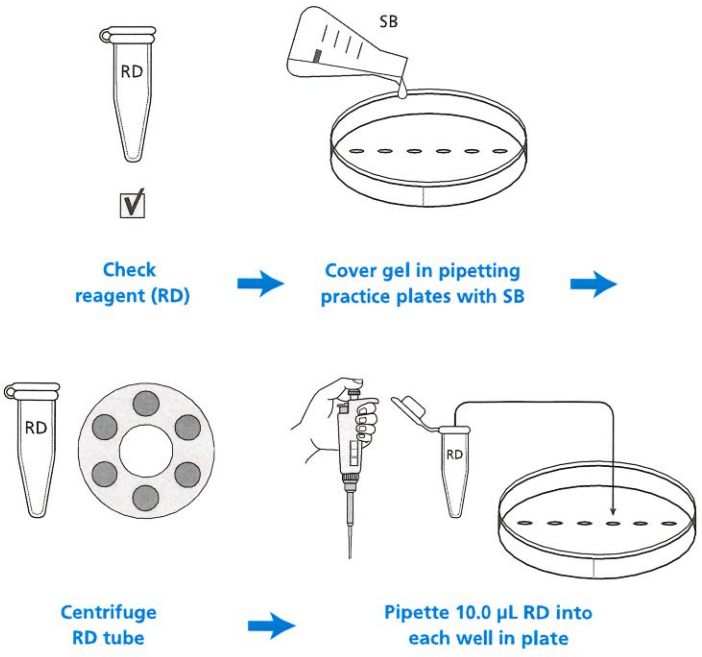
**Strategy:** **For the lab, you may want to show students the sample flowchart rather than have them create their own.**

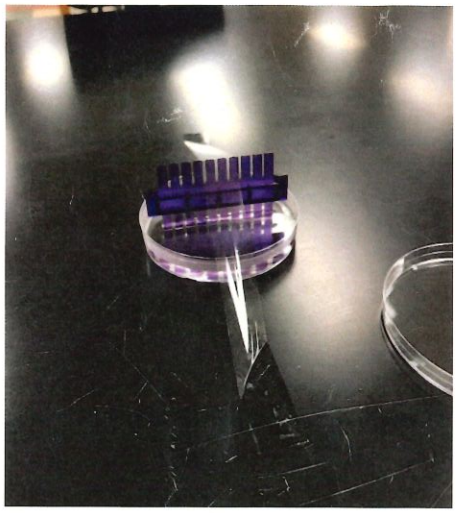
**Possible answers:**

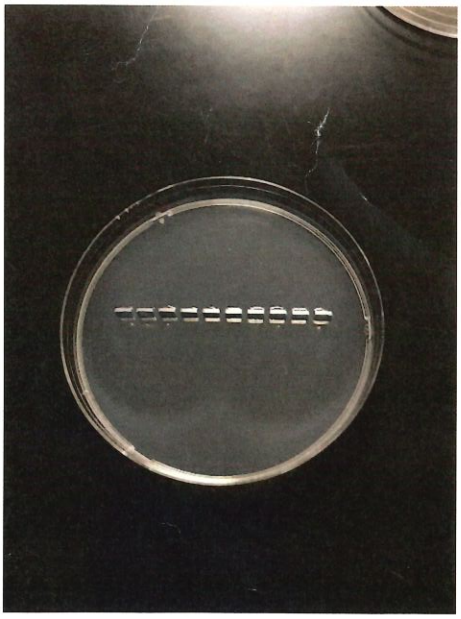
1. In what circumstances might it be important to use gel electrophoresis to separate and identify plasmids and short linear pieces of DNA? *This would be important if you are making a recombinant plasmid and have to verify that you have been successful.*

2. Read through the Methods section on pages 28 through 31 [of the Student Guide] and briefly outline the steps for *Part A* and for *Part B*, using words and a flowchart.

***Laboratory 1.2, Part A* Flowchart**







**LAB 1:**

We have provided you with some extra petri dishes if you would like to make some practice plates for the pipetting lab. You can use any leftover agarose to makes these plates.

You will need:

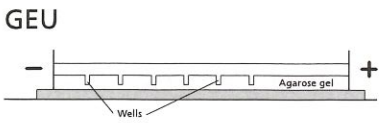
- Electrophoresis comb
- 1X SB buffer
- Tape
- Empty Petri Dish
- 15 mLs of Agarose

**Steps:**

1. Use a piece of tape to hold comb upright in petri dish. (As shown on the left)
2. Add about 15 mLs of agarose to dish.
3. Once the agarose solidifies remove the comb.
4. To store plates add 1X SB Buffer and store in the refrigerator.

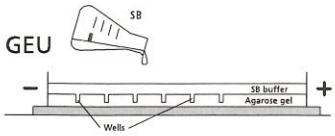
## *Laboratory 1.2, Part B* Flowchart


Check reagents (S1, S2, and S3)

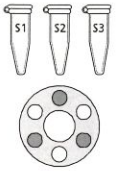



Find out which wells our group will use

Review gel electrophoresis unit (GEU)

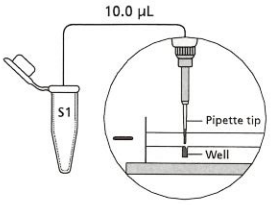



Cover gel in GEU with SB

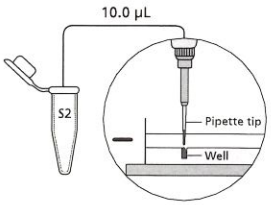



Centrifuge S1, S2, and S3

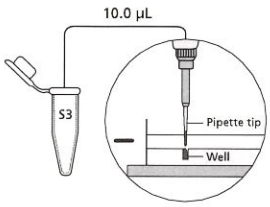
Draw location of each well, and label its solution in notebook



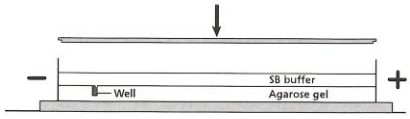

Pipette 10.0 µL S1 into GEU well



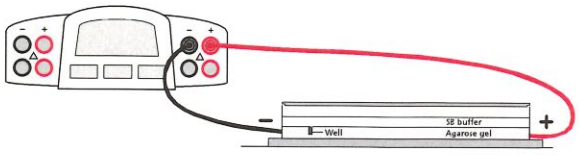

Pipette 10.0 µL S2 into GEU well




Pipette 10.0 µL S3 into GEU well



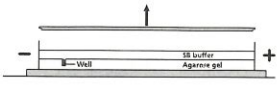

Place cover on GEU




Connect leads to power supply

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

Run gel for 10 minutes




Remove cover from GEU

Draw the relative location of the bands and their colors in notebook

# LAB 1.2 Gel Electrophoresis

## Solutions

*Dyes*

Orange G 408.40 au

Bromophenol 699.98 au

Xylene cyanole 538.62 au

*Heavier molecules move slower*

Solution 1: bromophenol blue, xylene cyanole, glycerin and water

Solution 2: bromophenol blue, xylene cyanole, orange G, glycerin and water

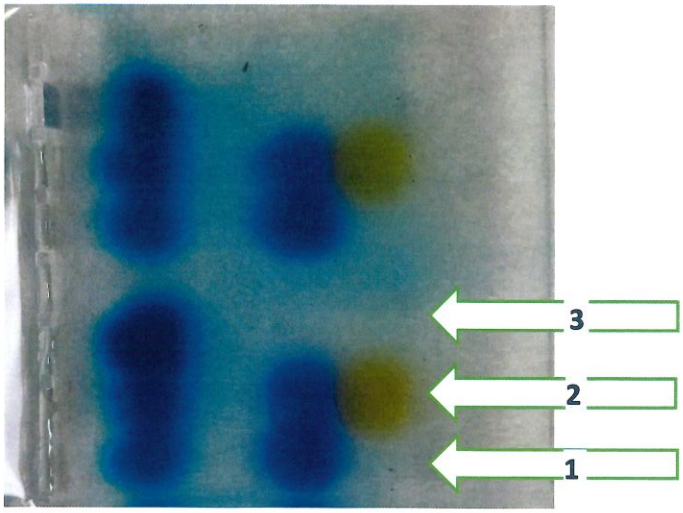
Solution 3: Xylene cyanole, glycerin and water.

## Reading the Gel:

Bromophenol blue will appear purple

Xylene cyanole will appear blue

Orange G will appear orange/yellow



# LAB 2 RESOURCES

## ATTENTION TEACHERS:

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

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

| LAB 2A KIT ITEMS | LABELS | VOLUMES to aliquot |
|---|---|---|
| P-20 micropipette | | --- |
| P-20 pipette tips | | --- |
| Minicentrifuge | | --- |
| Water bath set to 37˚C and thermometer | | --- |
| **FREEZER BOX ITEMS** | | |
| pARA-R (2a concentration) | **pARA-R 2a** | 10uL per group |
| BamH1 enzyme* | **BamHI** | 3µL-4µL per group (**note this is for RE, both BAM HI and HIND III combined)** |
| HindIII enzyme* | **HindIII** | 3µL-4µL per group (**note this is for RE, both BAM HI and HID III combined)** |
| 2.5x Restriction buffer | **2.5X Rest** | 12 µL per group |

**Notes:** *Mix BamHI and HindIII (1:1 ratio) and label as RE ie 24 µL of Bam HI and 24µLof HindIII then aliquot 4 µL into a microcentrifuge tube to each group. Each group will only use 2 µL.

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

# Lab 2A

## Kit Materials:

p-ARA plasmid (store in freezer), restriction enzymes BamHI and HindIII (store in freezer), 2.5x restriction buffer (store in freezer), dH20, water bath, thermometer, orange float, P-20 micropipette and tips

## Aliquoting:

*Items: plasmid (p-ARA), enzymes (BamHI, HindIII), 2.5x restriction buffer, water*

- Vortex and spin enzyme mix and 2.5x restriction buffer before aliquoting the tubes for student groups. If you do not have a vortex, finger flick the tube several times to mix and then spin down in the centrifuge.

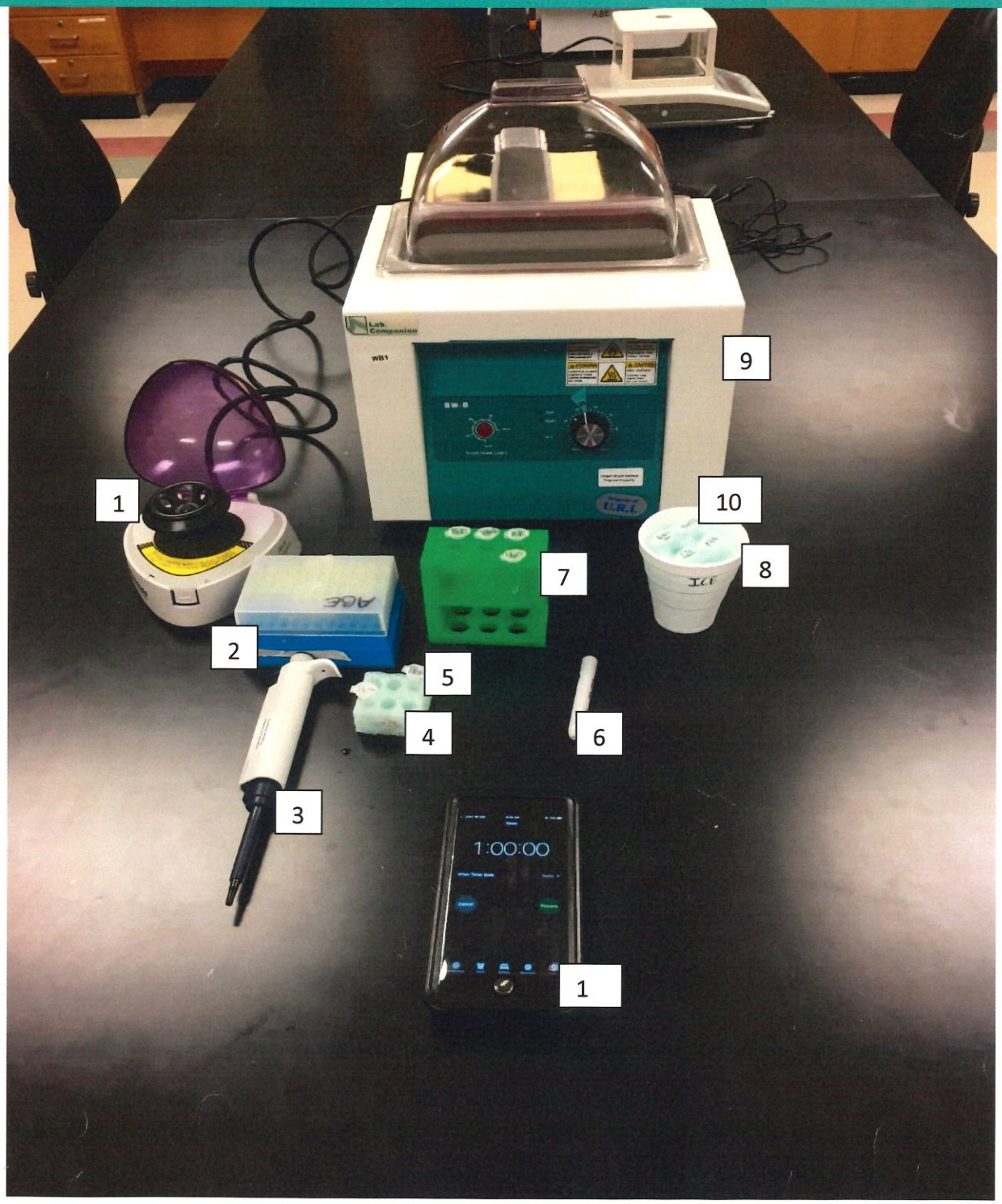
| Label Tube | Contents | Aliquot | Actually Use |
|---|---|---|---|
| 2A (RP) | pARA | 10 uL | 4 uL |
| RE | BamHI and HindIII | 3-4uL | 2 uL |
| 2.5xB | 2.5x Restriction buffer | 12 uL | 10 uL |
| dH20 | Distilled water | 1000 uL | 2uL |

## Restriction Digest

*Items: water bath, thermometer, orange float, samples to be digested*

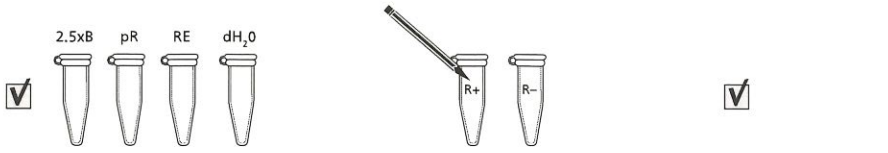
- Calibrate water bath to 37℃ the day before the lab to ensure temperature is correct for the restriction digest of student samples (60 minutes).
- Do not leave the digest in the water for over 2 hours, as BamHI will begin to cut DNA randomly.
- Store digested samples at -20℃ until you are ready to run the ligation protocol (Lab 3).
- Please empty out and wipe down the water baths before returning.

# Lab 2A

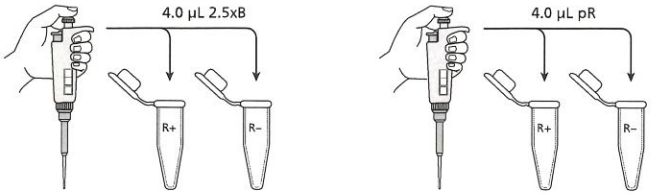



1. Mini-centrifuge
2. P20-200 pipette tips
3. P-20 pipette
4. Green microfuge tube float holding tubes labeled **R+** and **R-**
5. Microfuge tubes
6. Sharpie marker
7. Microfuge tube rack holder holding tubes labeled **2.5xB**, **pR**, **RE**, **$dH_2O$ (** should be aliquoted by **teacher**)
8. Cup to hold ice
9. Water bath (set to 37°C for this specific experiment)
10. Microfuge tubes that **must** be on ice containing either of the following: **BamH1, HindIII, pARA 2A & 2.5x restriction buff**
11. Timer **(WE DO NOT PROVIDE!)**

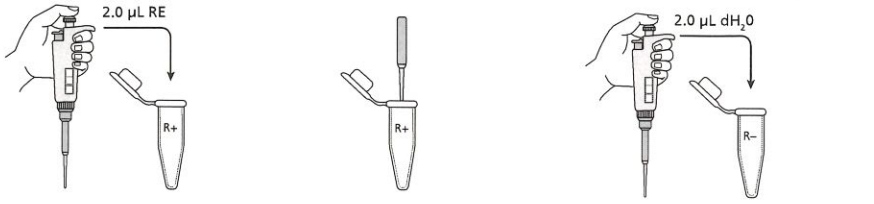
## *Laboratory 2A* Flowchart



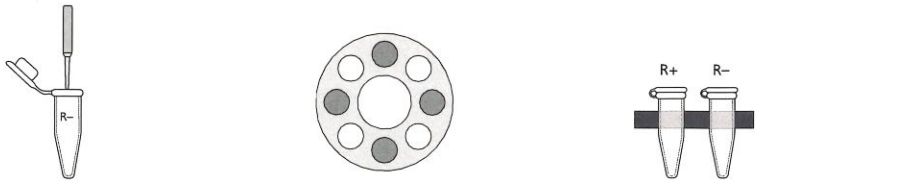

Check reagents (2.5xB, pR, RE, $dH_2O$) → Label 2 tubes R+ and R– → Review Table 2A.2 →



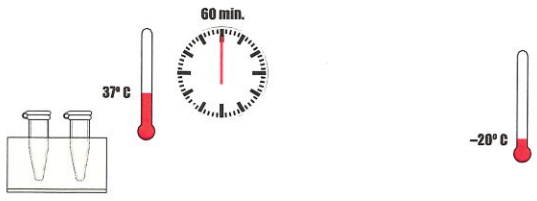

Pipette 4.0 µL 2.5xB into R+ and R– tubes → Pipette 4.0 µL pR into R+ and R– tubes →




Pipette 2.0 µL RE into R+ tube → Use pipette to mix reagents in the R+ tube → Pipette 2.0 µL $dH_2O$ into R– tube →




Use pipette to mix reagents in the R– tube → Centrifuge the R+ and R– tubes → Place tubes in floating microfuge tube rack →




Teacher will: Place R+ and R– tubes into 37°C water bath and incubate for 60 minutes → Teacher will: Place the R+ and R– tubes in the freezer at –20°C

# LAB 4 RESOURCES

## ATTENTION TEACHERS:

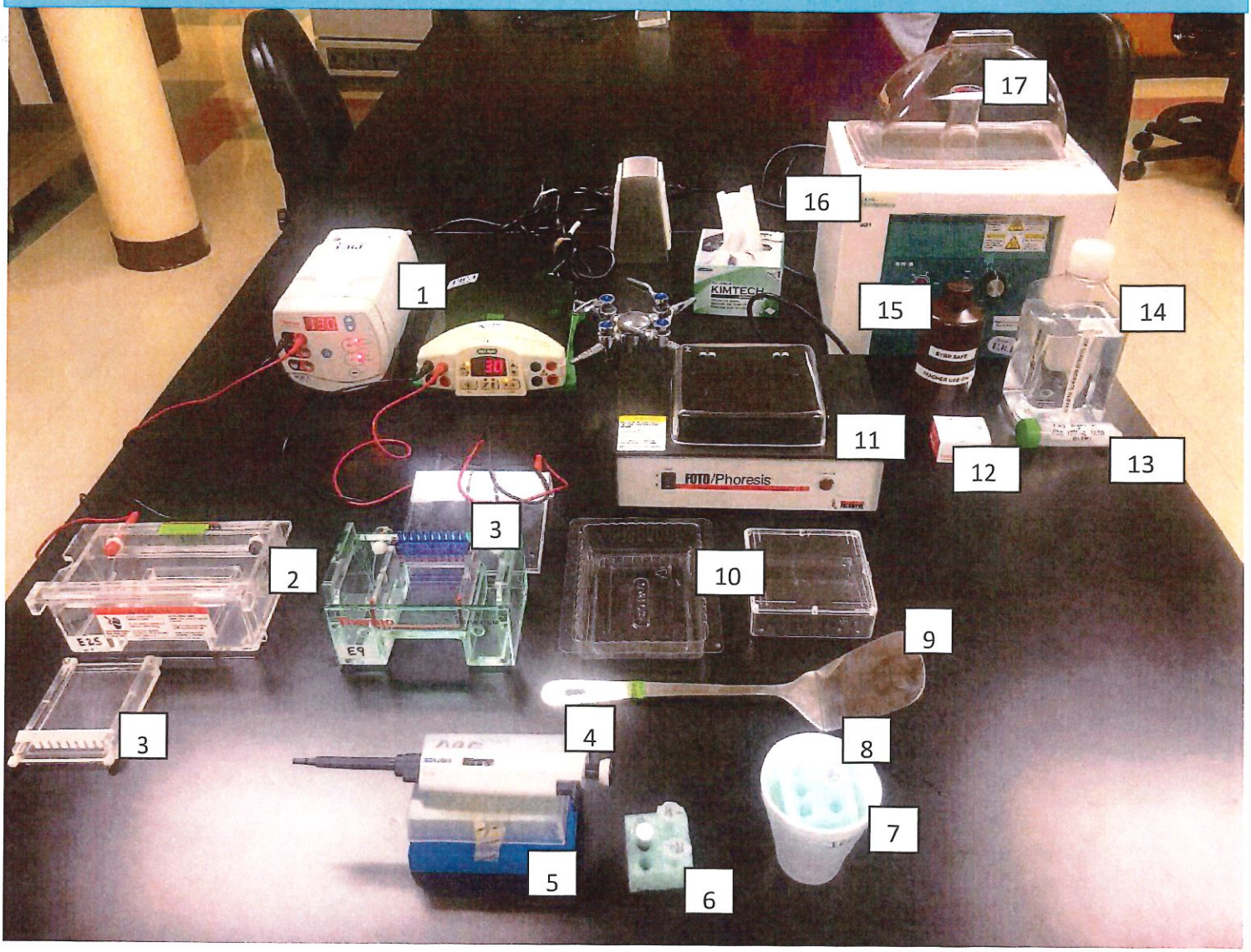
**Please have your students know how to use a pipette before proceeding to do this lab!**

| 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.

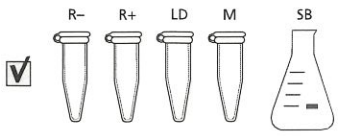
Post Stain Method. Teacher must prepare if needed. **NOT PROVIDED**
Diluting 20x SB Buffer to 1x SB buffer---- Mix 25mLs of 20x SB Buffer with 475 mLs of deionized water and then add 50µL of SyberSafe. Keep away from light. Solution 2 is the same reagent as LD.
**P-20, P-200, and P-1000 pipettes may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

# Lab 4A

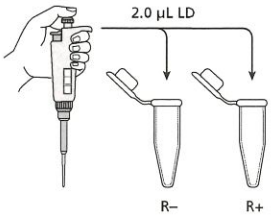



1. Electrophoresis power packs (both types that may be provided in kits are being displayed)
2. Electrophoresis chambers (both types that may be provided in kits are being displayed)
3. Trays and combs (both types that may be provided in kits are being displayed)
4. P2-20 micropipette
5. P20-200 micropipette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

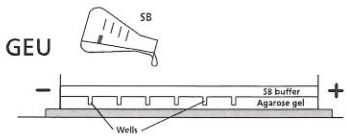



Check reagents
(R–, R+, LD, M in rack, 1x SB flask)




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

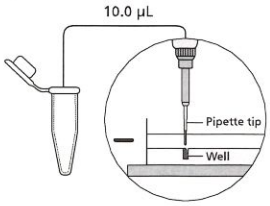
Centrifuge the two tubes



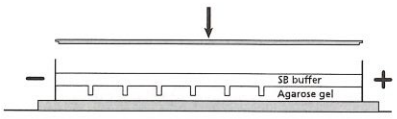

Review gel electrophoresis unit (GEU)

Cover gel in GEU with 1x SB

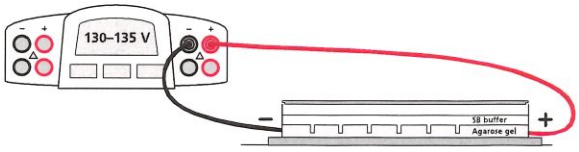
Draw location of each well, and label which solution is loaded in each in notebook




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

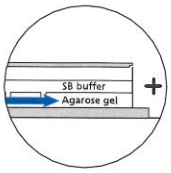



Place cover on GEU




Connect leads to power supply

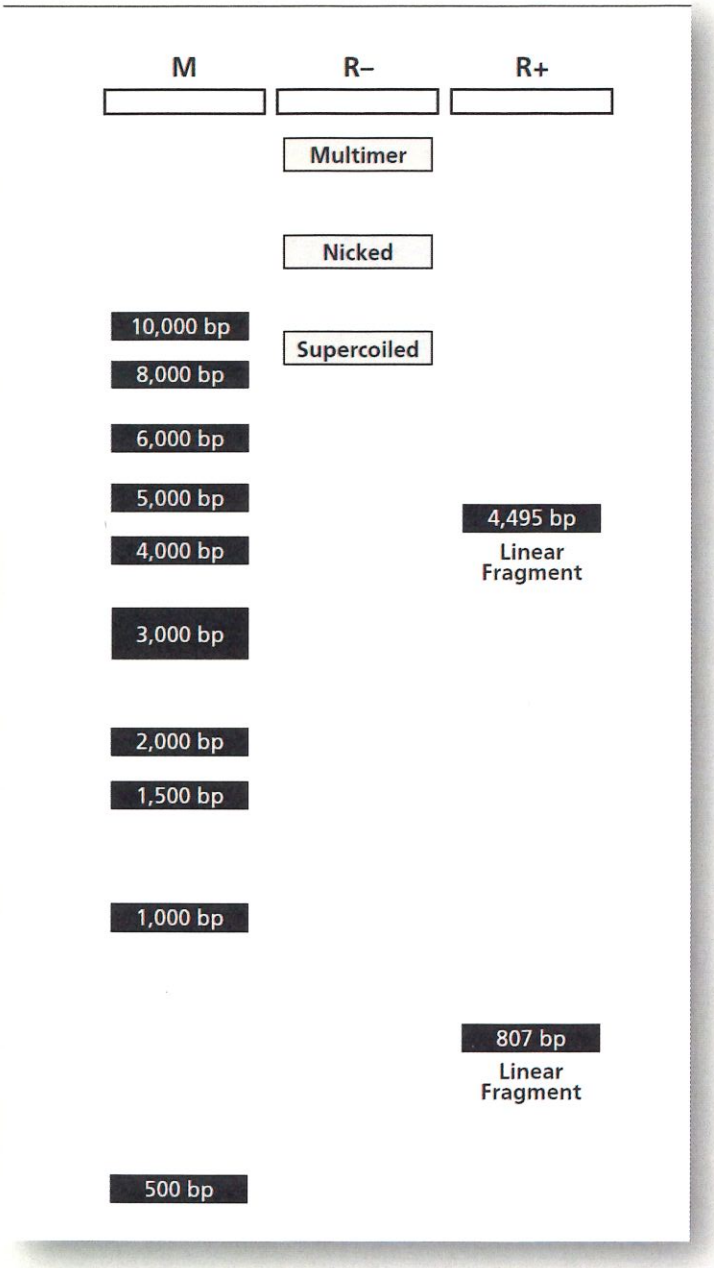
Turn on power supply and set voltage to 130–135 V




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

Teacher will explain final steps

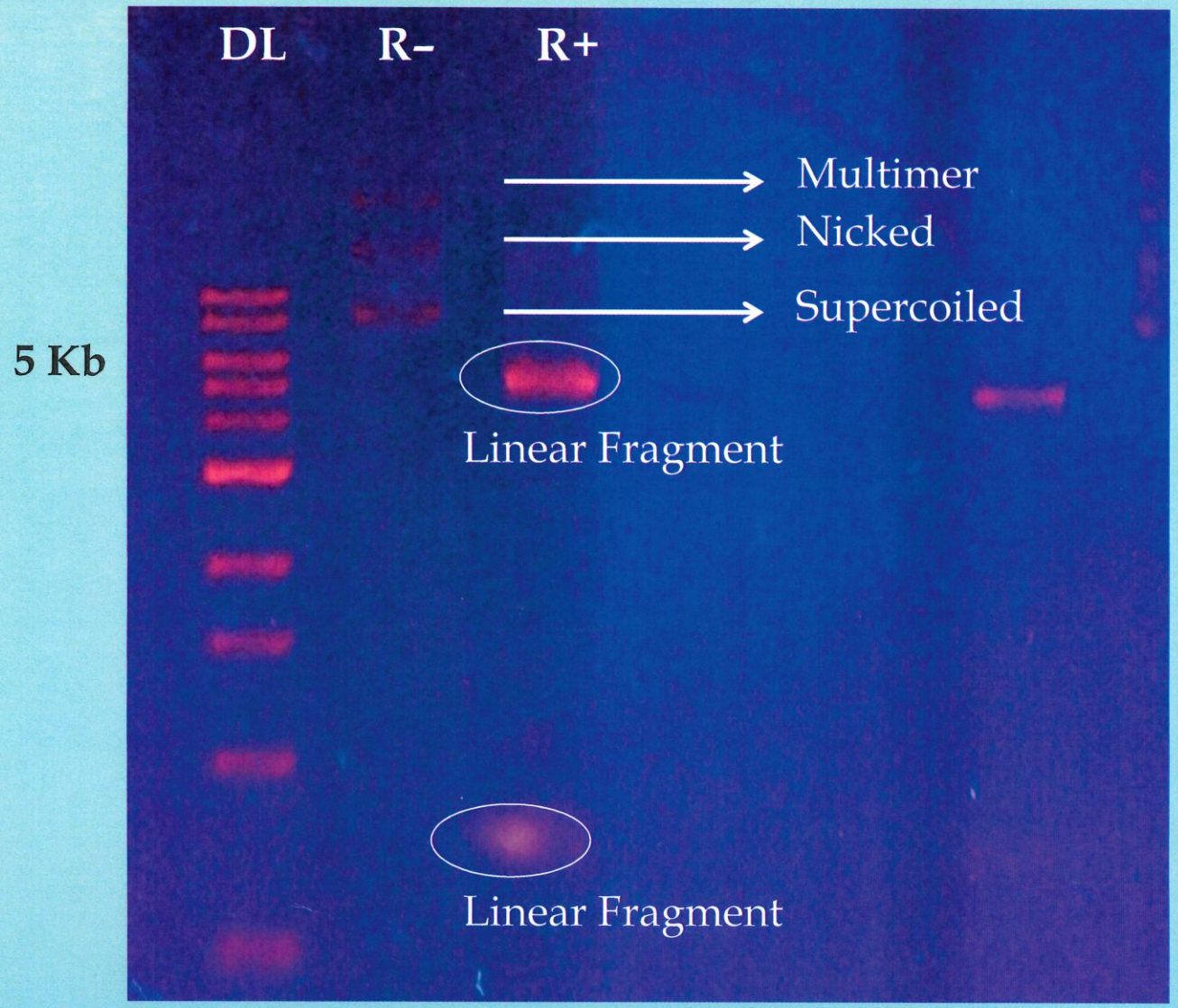
# LAB 4A DNA LADDER DIAGRAM

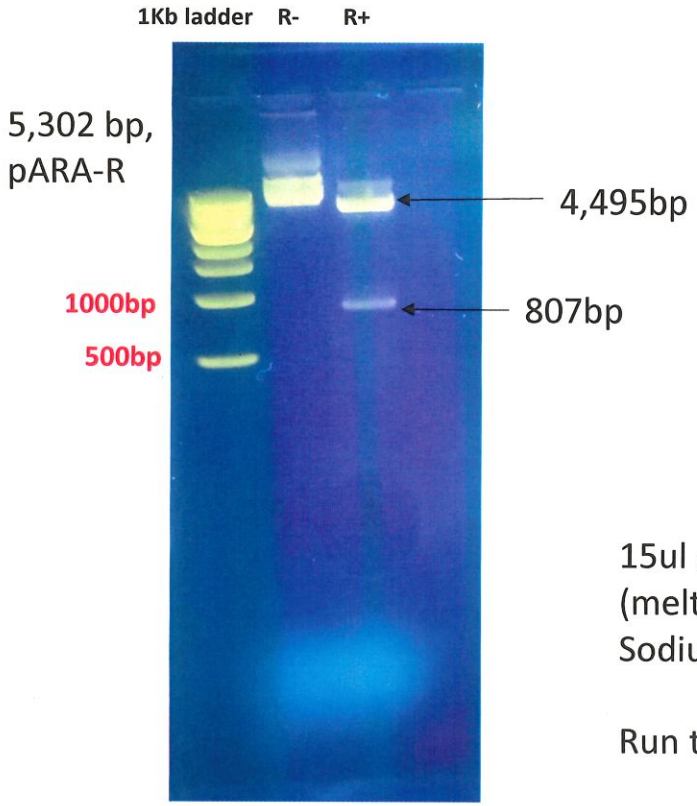


Amgen Biotech Experience

Scientific Discovery for the Classroom

# 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!

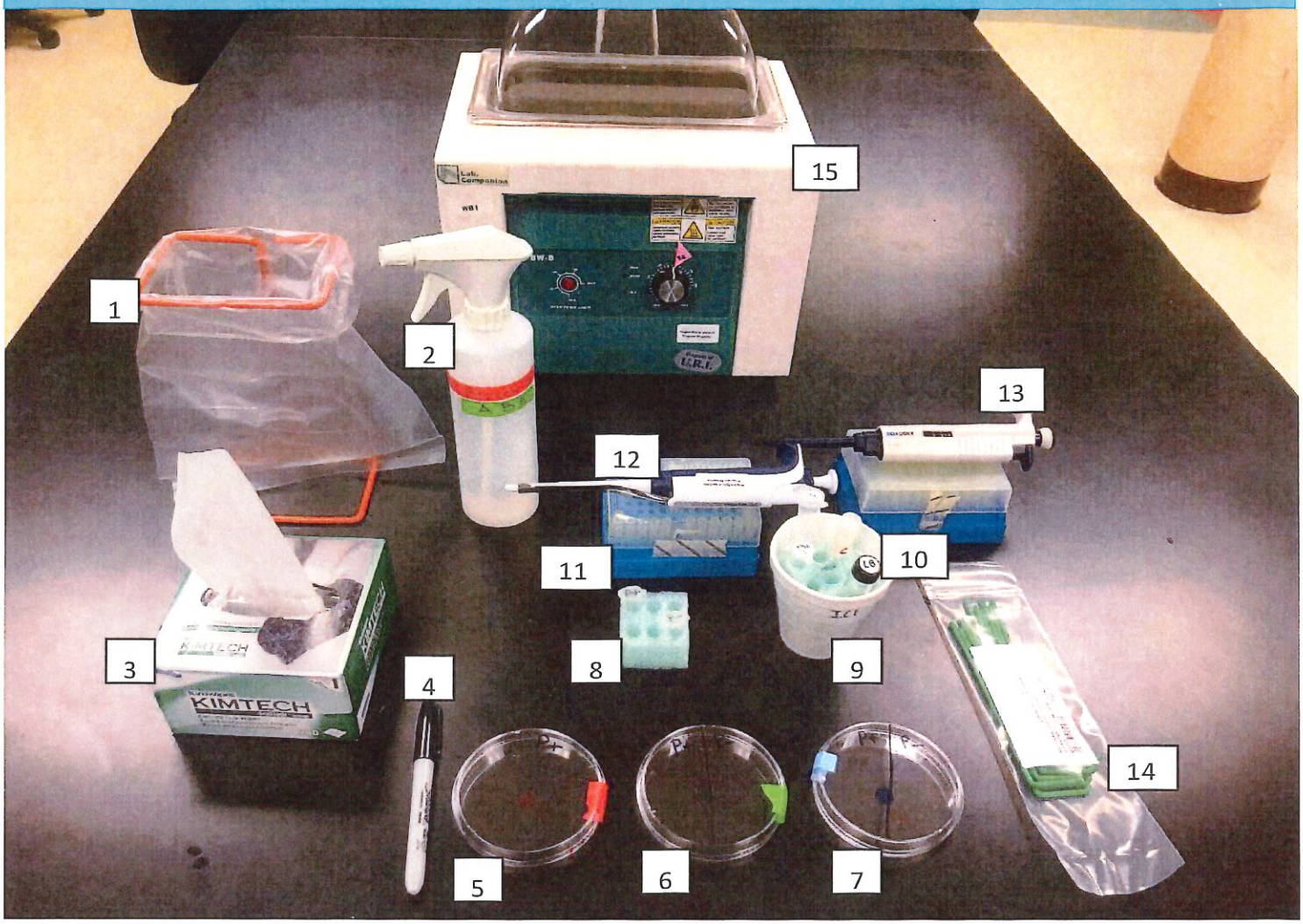
| LAB 5A KIT ITEMS | LABELS | VOLUMES to aliquot |
|---|---|---|
| LB plates | **1 blue line** | --- |
| LB/amp plates | **2green lines** | --- |
| LB/amp/ara plates | **3 red lines** | --- |
| P-20 micropipette | | --- |
| P-20 pipette tips | | --- |
| P-200 micropipette | | --- |
| P-200 pipette tips | | --- |
| 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.

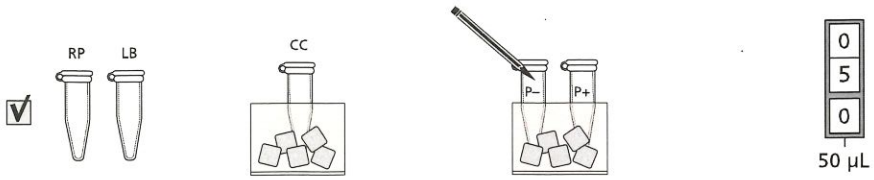
**P-20, P-200, and P-1000 pipettes may contain locks on them:** Please **UNLOCK** the pipette when adjusting the measurement

# Lab 5

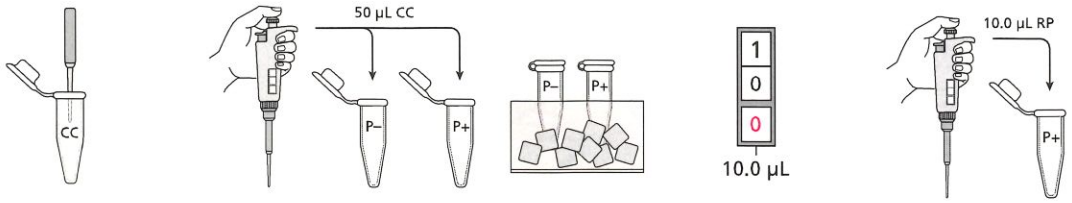



1. Biohazard bag and waste stand
2. 10% bleach bottle
3. Kimwipes
4. Sharpie marker
5. LB/AMP/ARA plate (marked by **3 red** stripes)
6. LB/AMP plate (marked by **2 green** stripes)
7. LB plate (marked by **1 blue** stripe)
8. Green float: used to hold tubes marked **P+ & P-**
9. Ice cup
10. **LB glass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

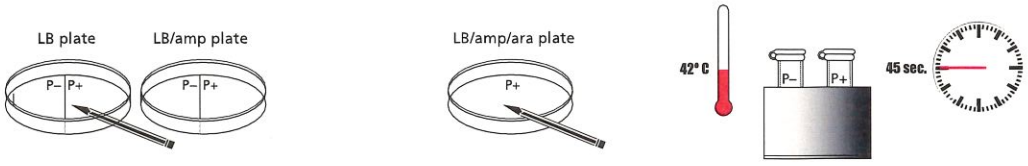



Check reagents (RP, LB) → Get CC (on ice) → Label two tubes P– and P+ and put tubes on ice → Set P-200 micropipette to 50 µL →

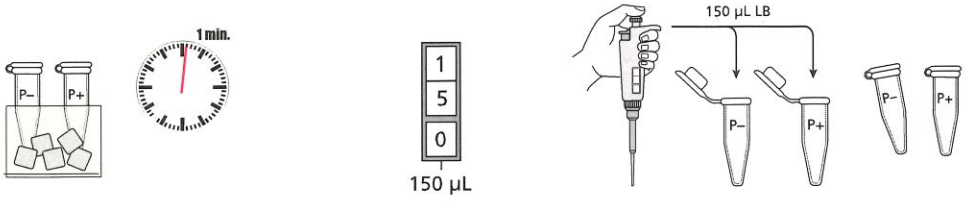



Suspend cells in CC tube by gently pumping cells in and out → Pipette 50 µL CC into P– and P+ tubes; hold tubes at the rim to keep cold and return quickly to ice → Set P-20 micropipette to 10.0 µL → Pipette 10.0 µL RP into P+ tube, holding P+ tube at the rim to keep cold →


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 →

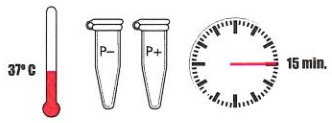



Draw a line on LB plate and LB/amp plates dividing them in middle; label one half "P–" and the other "P+" → Label LB/amp/ara plate "P+" → Carry cup with P– and P+ tubes on ice to the 42°C water bath, and place tubes in bath for exactly 45 sec. →

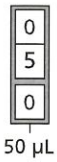



Immediately place tubes back on ice, and keep there for a minute → Set P-200 micropipette to 150 µL → Pipette 150 µL of LB into P– and P+ tubes, cap tubes, and gently flick to mix →

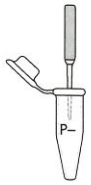
## *Laboratory 5A* Flowchart (Continued)



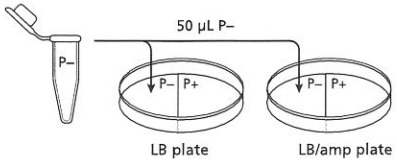

Incubate P– and P+ tubes at room temperature or at 37°C for 15 min.



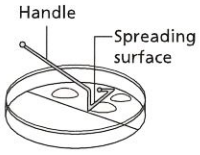

Set P-200 micropipette to 50 µL



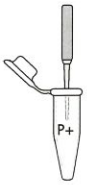

Gently pump pipette a couple of times in P– tube



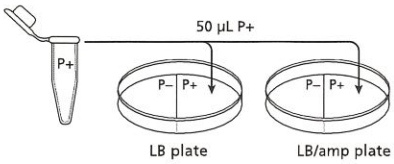

Pipette 50 µL of P– onto P– halves of LB and LB/amp plates, and close lids immediately after dispensing P–




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

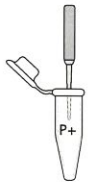



With new pipette tip, gently pump pipette a couple of times in P+ tube

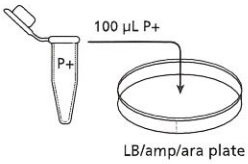



Pipette 50 µL of P+ onto P+ halves of LB and LB/amp plates, and close lids after dispensing P+

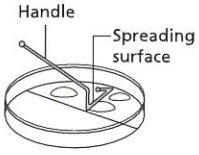

Set P-200 micropipette to 100 µL




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

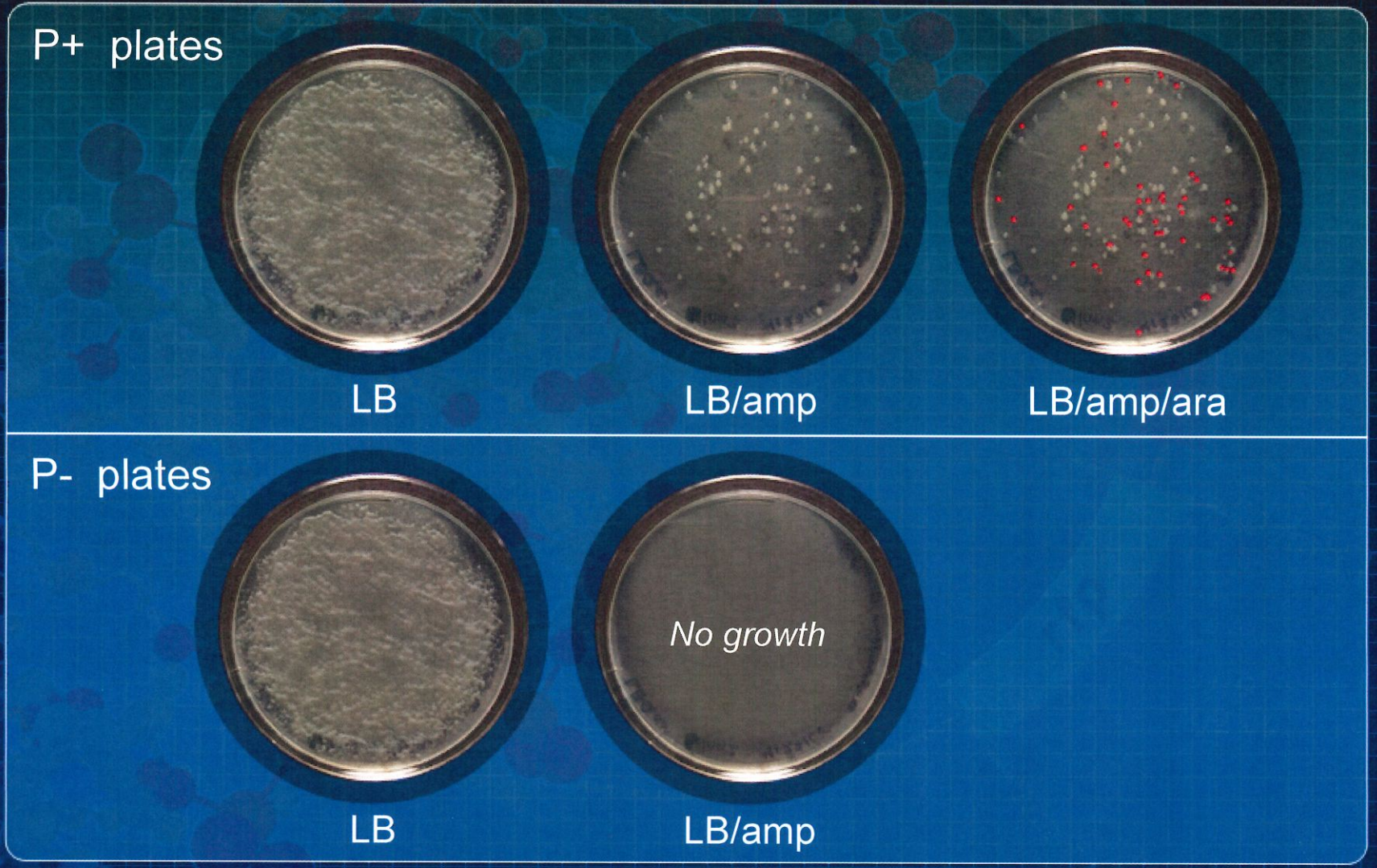

Place plates in the 37°C incubator upside down


Incubate plates for 24–36 hours at 37°C

Examine plates and record the amount of growth on each

AMGEN

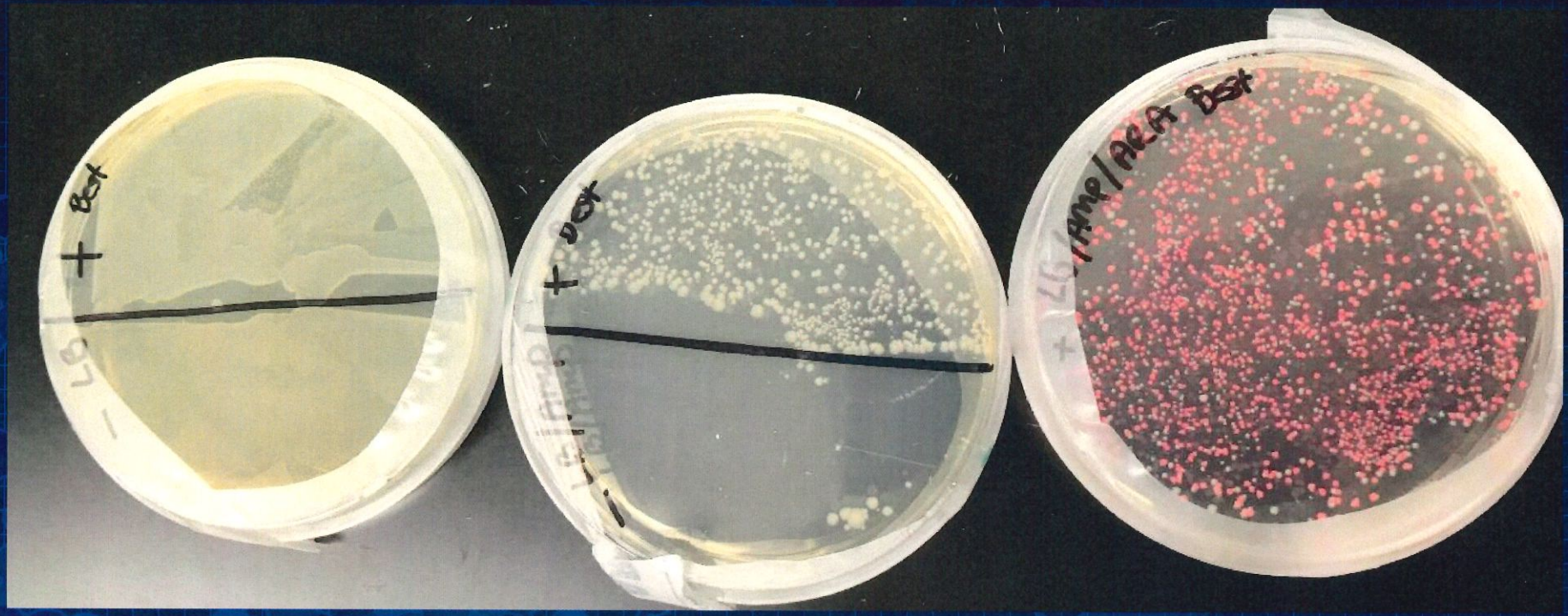
# growth of transformed bacteria on various plates



AMGEN

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

Colony isolation and culture



LB LB/amp LB/amp/ara

# 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 to aliquot |
|---|---|---|
| Shaker | | --- |
| Inoculating loops | | --- |
| Arabinose (for flask) | **ARA** | -- |
| Sterile LB/amp flask | | --- |
| Columns | | --- |
| Lysis buffer | **LYS** | 160µL 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) Pink broth in 0range cap bottle.** | 2 (2@ 1mL aliquots) mL per column |

**Notes:**

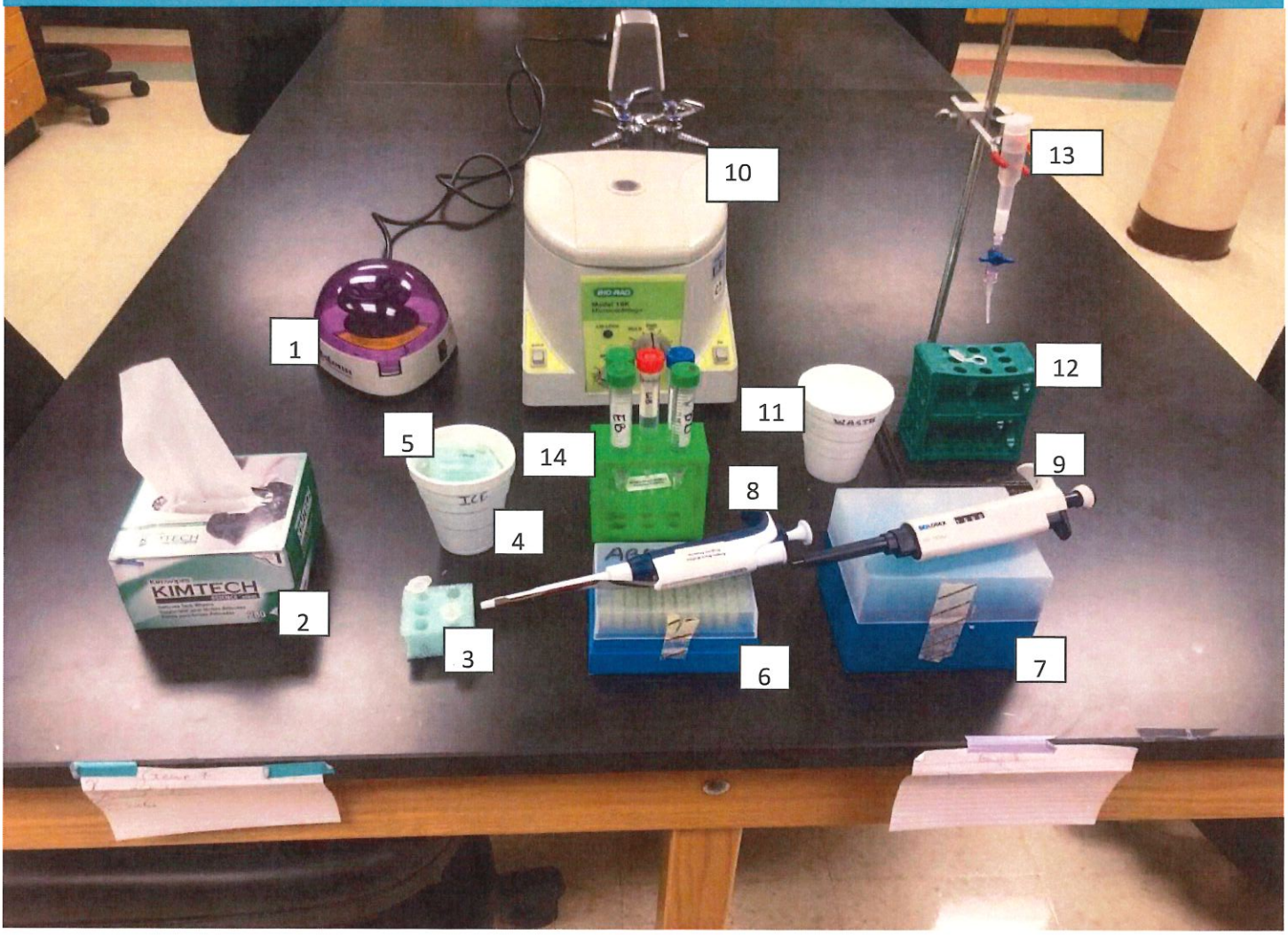
Only if you request this step other wise use the Pink "Transformed " Broth provided to perform 6A!

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. Add another vial of arabinose before you leave. 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.

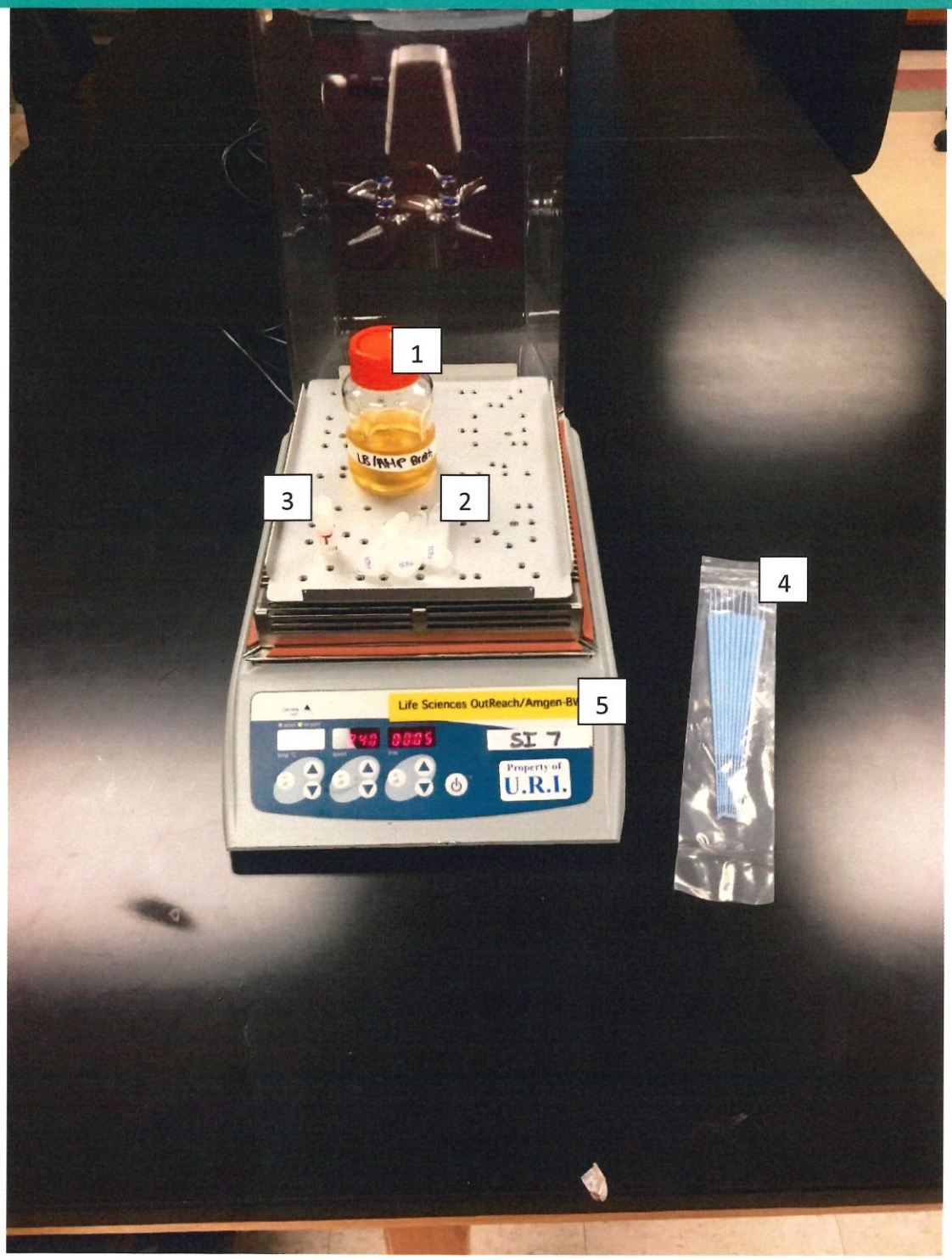
6A: Lysing cells: Optimal lysing can be achieved if you are able to do multiple free/thaw/steps. After freezing, place cell in $37^0$C (you can use the water bath) or room temperature if you do not have access to $37^0$C. If you have access to a vortex or use the plastic micro centrifuge tube rack provided, 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

# Lab 6




1. Mini centrifuge
2. Kimwipes
3. Greet float tube holder: holds tubes throughout the experiment
4. Ice cup
5. Tube labeled **LYB**; 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**




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

# Grow Bacteria for Protein Purification

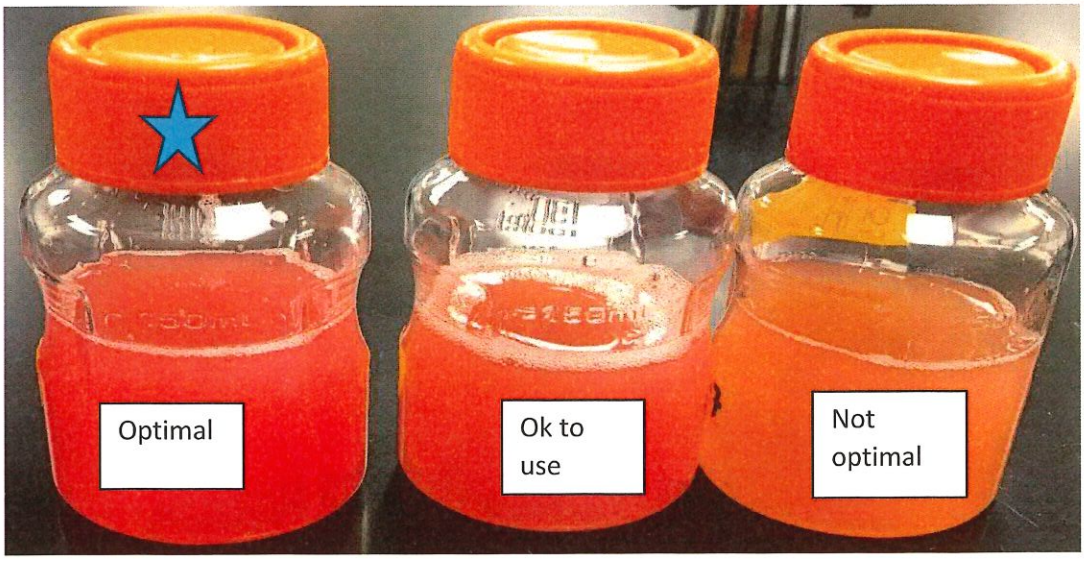
**To be arranged with the RI program site.**

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

**Materials:**

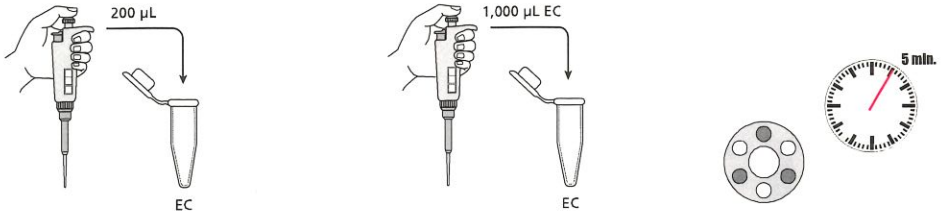
- 1000ul Pipette
- Transformed cells (provided in kit)
- Sterile flask containing LB/amp broth
- Shaker
- 3 tubes of sterile arabinose

1. **Start your lab 6 culture 4-5 days BEFORE you will need it. It can be stored in the refrigerator until the lab.**
2. **Inoculate the LB amp broth with a vial of transformed cells when you get to school in the morning.**
3. **After several hours of shaking (This can be anywhere from 2-3 hours) and when the broth starts to turn cloudy but not TOO cloudy), add the arabinose (1 full tube).**
4. **Continue shaking overnight. We have enclosed an additional tube of Arabinose; you can add before you go home for the night.**
5. **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. Compare the suspension culture:**
6. Optimal

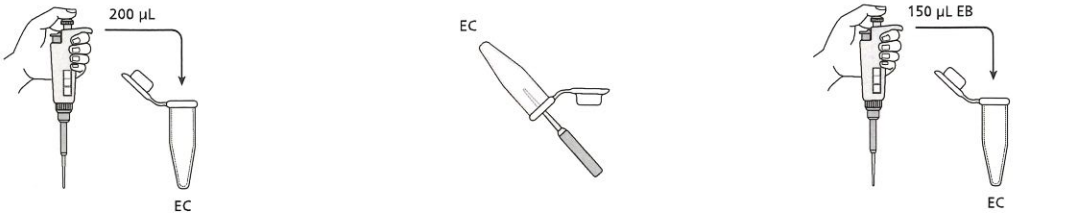


## *Laboratory 6, Part A* Flowchart

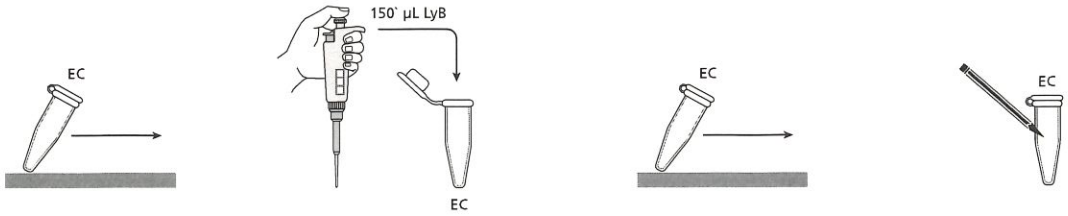

Assign tasks → Check reagents (EC, EB, LyB) → Record color of EC tube → Centrifuge EC tube for 5 minutes →




Use P-200 micropipette set to 200 µL to remove supernatant from EC tube → Get 1,000 µL (1 mL) of additional EC solution from teacher; add to EC tube → Centrifuge EC tube for 5 minutes →



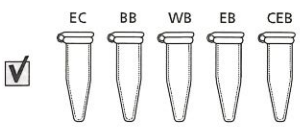

Use P-200 micropipette set to 200 µL to remove supernatant from EC tube → Invert EC tube and use P-200 micropipette to remove as much liquid as possible without disturbing cell pellet → Pipette 150 µL EB into EC tube →




Cap EC tube and drag vigorously across microfuge tube rack until cells are resuspended and not clumped → Pipette 150 µL LyB into EC tube → Cap EC tube and drag vigorously across microfuge tube rack to mix → Label EC tube with group number and class period, and give to teacher

## *Laboratory 6, Part B* Flowchart

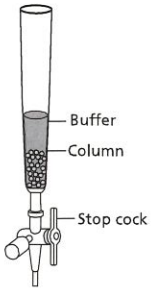
**One group member:**



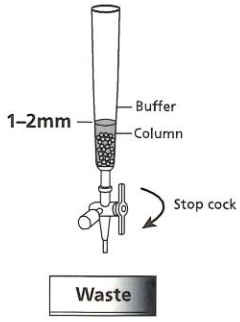

Check reagents (EC, BB, WB, EB, CEB)


Label microfuge tubes (SUPER, RFP)

**One group member:**

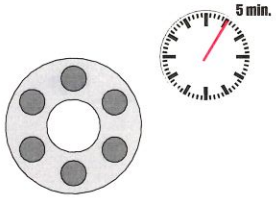



Set up chromatography column



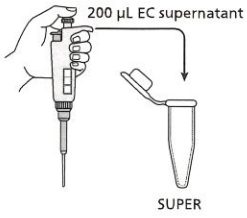

Set waste collection container under column stopcock, open column by turning stopcock valve, allow buffer to drain from column, and close valve when 1–2 mm of liquid is left above resin

**One group member:**

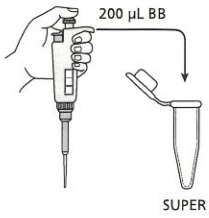



Centrifuge EC tube for 5 minutes


Examine tube



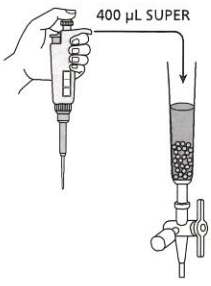

Pipette 200 µL EC supernatant into SUPER tube



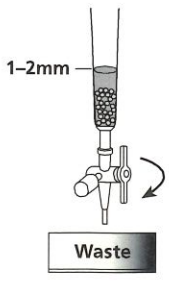

Pipette 200 µL BB into SUPER tube

**Whole group:**


Mix reagents in SUPER tube by gently pumping solution in and out

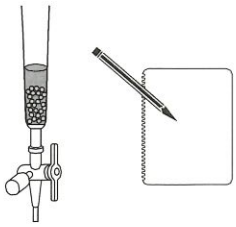



Pipette 400 µL SUPER down side of chromatography column

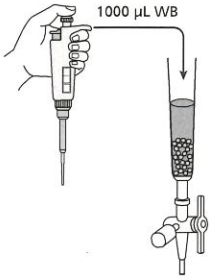



Set waste collection container under stopcock, open valve, allow buffer to drain, and close valve when 1–2 mm of liquid is left above resin

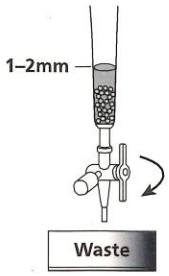
## *Laboratory 6, Part B* Flowchart (Continued)



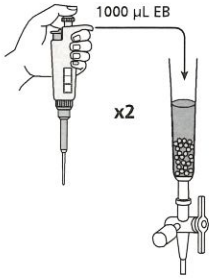
Locate RFP and record observations in notebook



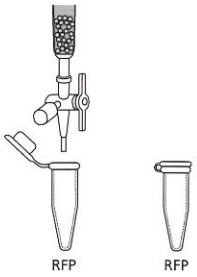

Pipette 1,000 µL WB down side of chromatography column



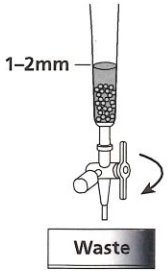

Set waste collection container under stopcock, open valve, allow buffer to drain, and close valve when 1–2 mm of liquid is left above resin



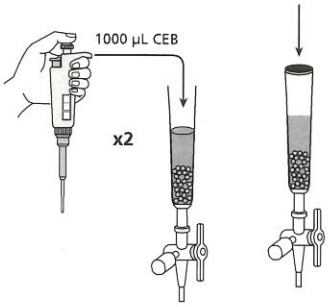

Twice more, pipette 1,000 µL EB down side of chromatography column



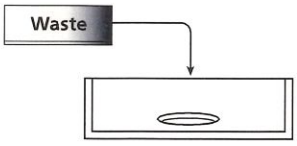

Set RFP tube under stopcock, open valve, allow only red part of eluate to drain, close valve, and cap tube tightly



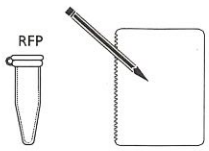

Set waste collection container under stopcock, open valve, allow buffer to drain, and close valve when 1–2 mm of liquid is left above resin




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. 2 tubes of sterile arabinose

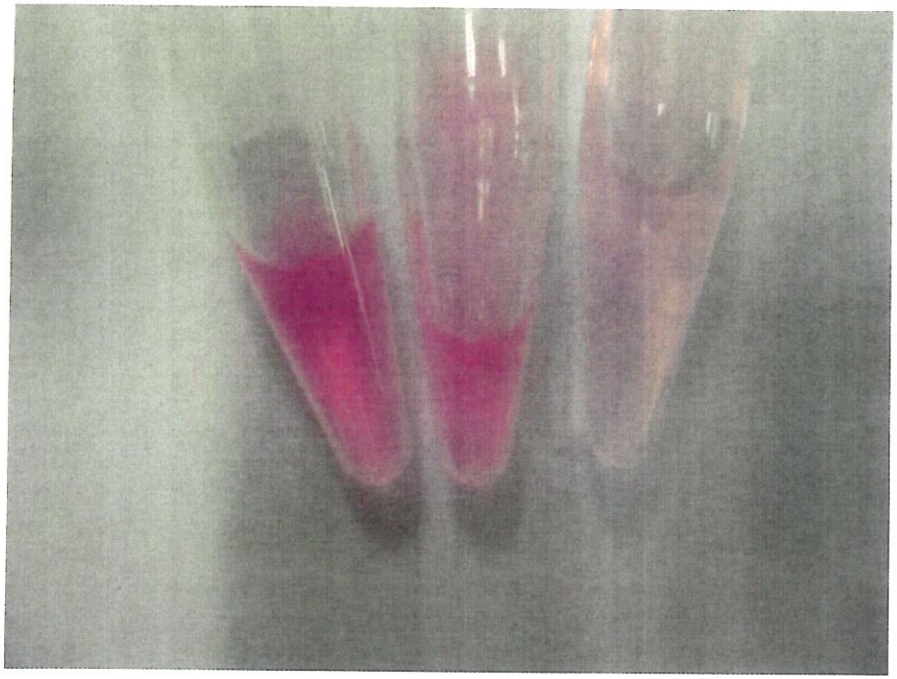
**Prepare the suspension culture:**

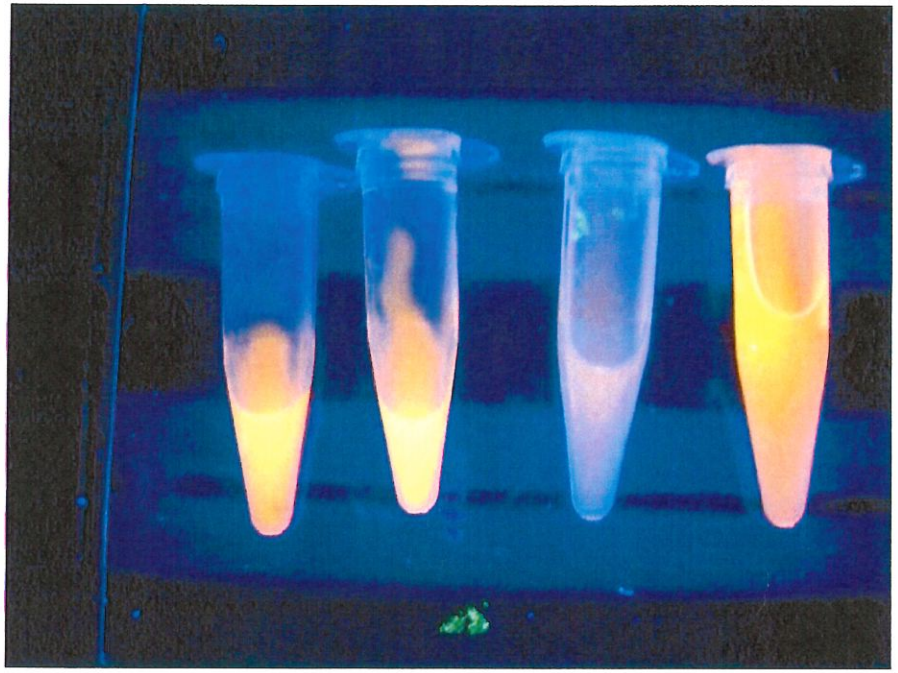
1. Using the pipette, aseptically transfer transformed cells into the sterile flask containing LB/amp broth.
2. Replace the cap, make sure to loosen the cap ¼ of a turn.
3. Shake and incubate the flask (at 37°C) for four to five hours. The LB/amp broth should become cloudy, indicating the cells are growing.
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.

Amgen Biotech Experience

Scientific Discovery for the Classroom

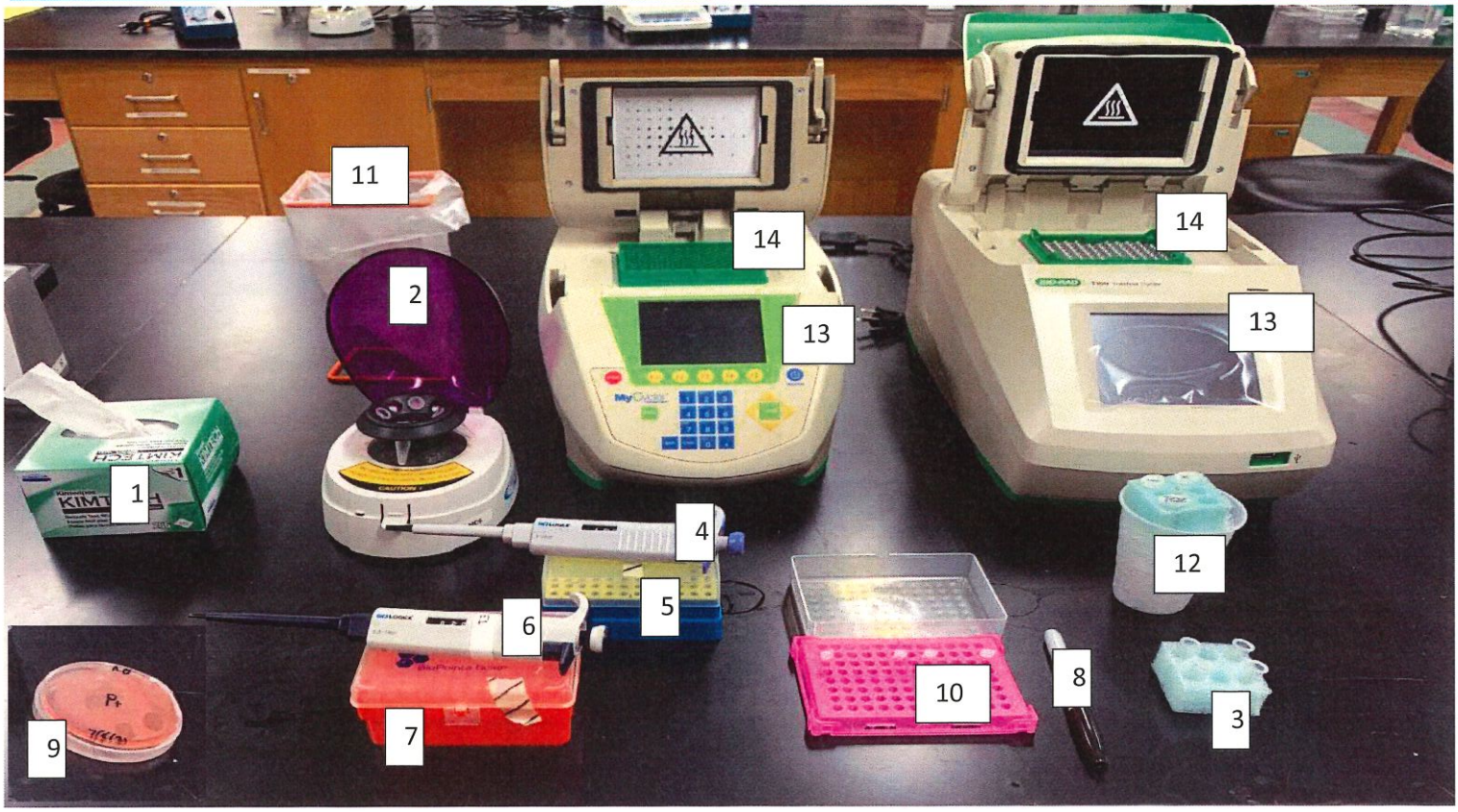
# Lab 6 Results





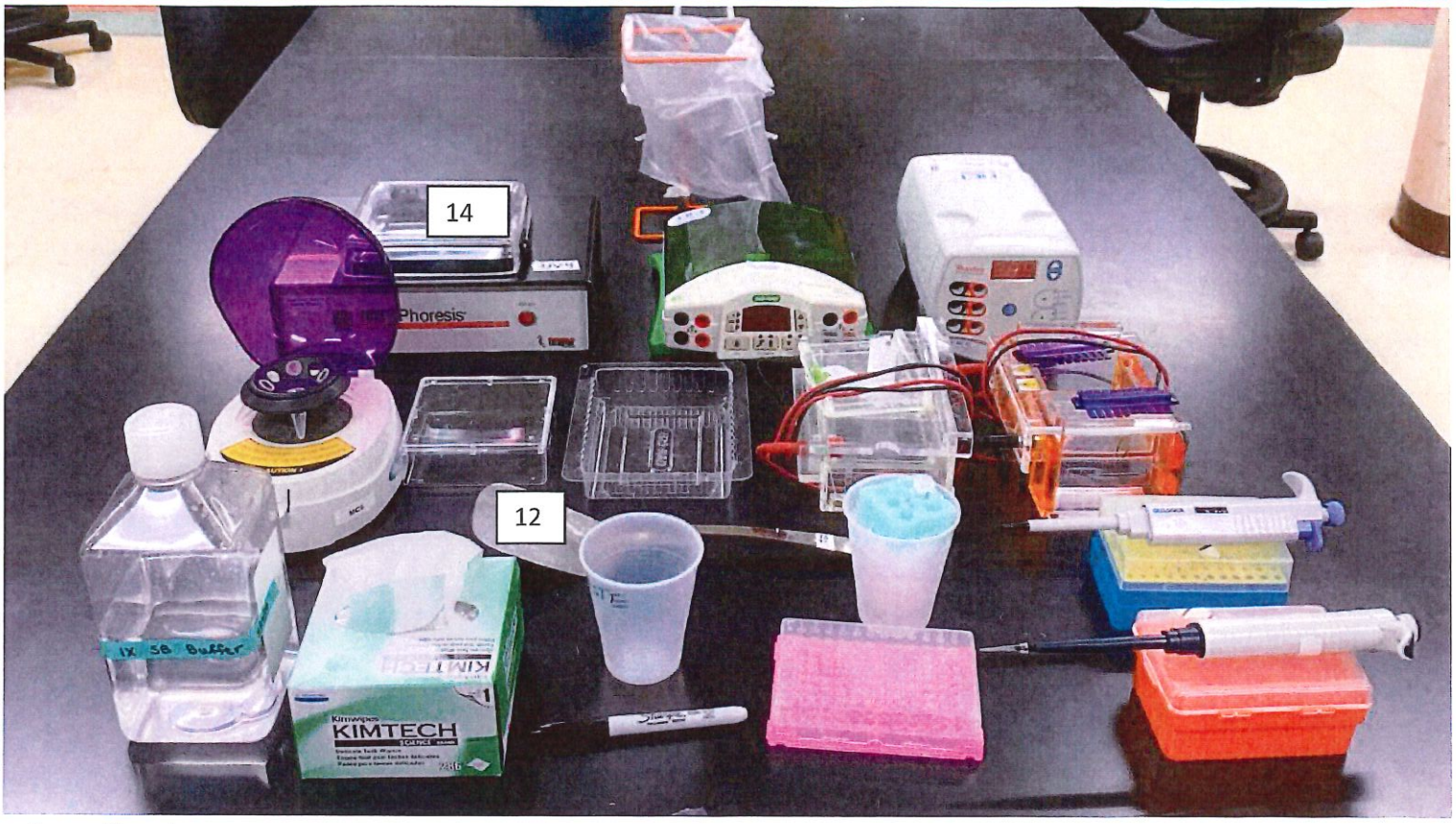
# *COLONY PCR LAB RESOURCES*

# Lab Colony PCR Part I (A)




1. Kimwipes
2. Mini centrifuge
3. Cap-less tubes & forceps & green foam float rack: **cap-less tubes** should be used to keep small PCR tubes safe when centrifuging, **forceps** should be used to help retrieve PCR tubes and cap-less tubes from either the mini centrifuge or the large centrifuge.
4. P0.5-10 micropipette
5. P10 pipette tips
6. P20 micropette
7. P20-200 pipette tips
8. Sharpie marker
9. Lab5 Transformed P+ colonies the arabinose/ampicillin plate: "Pink colonies"
10. PCR tube holder with 4 sample tubes from each group
11. Waste Bag and holder
12. Ice cup: should hold tubes labeled: Positive (+), Negative (-), and PCR (contains TAQ and Primers)
13. Thermocycler (PCR)- both types that may be provided in kits are being displayed
14. PCR green adapter- both types that may be provided in kits are being displayed

# Lab Colony PCR Part II (B) Gel Electrophoresis




1. Kim wipes
2. Mini centrifuge with capless tubes
3. 1X Sodium Borate Buffer (Made up earlier from 20X Sodium Borate stock).
4. Electrophoresis power packs (both types that may be provided in kits are being displayed)
5. Electrophoresis chambers (both types that may be provided in kits are being displayed)
6. Trays and combs (both types that may be provided in kits are being displayed)
7. P0.5-10 pipette
8. P-10 pipette tips
9. P20 micropette
10. P20-200 pipette tips
11. Ice cup: holding the 100bp ladder: (M-100) and green float
12. Spatula
13. Gel trays (both types that may be provided in kits are being displayed)
14. UV trans illuminator
15. Waste receptacle
16. Waste cup
17. PCR tube holder (with PCR samples 4 per group)
18. Sharpie marker

***Not Shown but needed for Gel preparation***

***1.44g of agarose tubes***

***20x SB buffer***

***Sybrsafe in tube ( keep away from light)***

***Water bath (used to cool down the agarose- set temp at 55-60°C)***

***Flask containing the hot agarose should be placed in the water bath to cool down before being poured.***

## ATTENTION TEACHERS:

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

| Colony PCR LAB KIT ITEMS | LABELS | VOLUMES: Amounts per 1 Group (2-4 students) |
|---|---|---|
| Thermocycler | | 1 for class |
| Large Micro-centrifuge | | 1 for class |
| Mini centrifuge | | 1 for class |
| P--20 micropipette | | 12 (1 per group) |
| P--20 pipette tips | | 1 box per group |
| P--10 micropipette | | 12(1 per group) |
| P--10 pipette tips | | 1 box per group |
| P--200 micropipette | | 12 |
| P--200 pipette tips | | 1 box per group (same as P20) |
| Light box/ amber filter | | 1 for class |
| 0.2mL **Clear** Microfuge tubes (PCR Tubes) used for **PCR reaction** | | 4 tubes per group |
| Fine-tip Permanent Marker | | 12 (1 per group) |
| Waste cups | | 6 (1 per 2 groups) |
| Gel Electrophoresis system; **(includes trays, combs, and boxes)** | | 6-boxes and trays<br>12-combs (share 1 unit with two groups) |
| Power supply | | 3 for class |
| Biohazard Bags and stands | | 2 for class |
| 1.5mL **Clear** Microfuge tubes for aliquots | | Bag (4 per group) |
| Capless microfuge tubes used as adapters for centrifuge and to hold tubes | | bag |
| Staining trays | | 6 (share) |
| Spatula | | 1 (share) |
| Microfuge floats/microfuge rack | | 6 (1 per 2 groups) |
| **FREEZER BOX ITEMS:** | | |
| One Taq Quick Load<br>**TEACHER USE** | **TAQ** | See above for Master Mix preparation. |
| pARA-R -0.025ng/µL | + | Aliquot 12 tubes with 3 µL. Actual use 2µL. |
| pARA -0.025ng/µL | – | Aliquot 12 tubes with 3µL per group. Actual use 2µL.<br>Keep on Ice or frozen until ready to use. |
| PCR Master Mix: **(TEACHER MUST ALIQUOT: 100µL of Master Mix per group of 2-4 students; actual use 92µL per 4 samples.** | **PCR** | For 48 samples; 12 student groups (i.e., 24 students running 4 samples: 54 samples x10µL of Primer =540µL and 54 samples x 13 µL of NEB Master Mix =702 µL. |

## ATTENTION TEACHERS:

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

| Prepare morning of the lab. | | TEACHER MUST PREPARE DAY OF LAB |
|---|---|---|
| DNA (100bp) ladder- **TEACHER MUST ALIQUOT** | **M or M100** | Aliquot 12 tubes with 9µL. Actual use 8µL. |
| **ROOM TEMPERATURE** | | |
| 1X Sodium Borate-**TEACHER MUST PREPARE** | | 20mL of 20X SB to 380mL of DI water |
| Distilled or deionized water-(used to dilute the 20X SB buffer provided into 1X SB.) **NOT SUPPLIED!** | | 380mL of DI water to 20 mL of 20x SB to make 1x Sb |
| Agarose- (1.20g agarose to 150mL 1X SB) | | ~30mL per casting tray |
| **KEEP AWAY FROM LIGHT** | | |
| Sybr Safe or gel green (gel stain) **TEACHER MUST PREPARE** | | 15 µL in Agarose solution (180mLSB Buffer + 1.44g of agarose). |

**Notes:**

- Label three 1.5mL microfuge tubes as follows: **PCR, +, and – for each group.**

- Pipet the PCR master mix + Taq up and down several times to mix it thoroughly, then aliquot 100 µL into each microfuge tube marked "PCR." Store at 4°C. You can prepare this mixture the morning of the lab, store it at 4℃, and use throughout the day. **Do not store overnight at 4℃ or you risk the quality of the PCR reaction.**

- **If you have 12 student groups running four samples each for the ABE Colony PCR lab, you will need to run a total of 54 samples. We suggest that when you make the primer + master mix, you increase the sample number for extra volume.**

**When centrifuging samples:** Please do not spin cap-less microfuge tubes at the highest speed, press the **QUICK** button only when spinning they will crack if spun at the highest speed.

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

# Colony PCR Laboratory

Colony PCR uses what is referred to as a "Master Mix," and New England Biolabs provides ABE programs with OneTaq® 2X Master Mix with Standard Buffer. This includes the dNTPs, magnesium, and Taq polymerase all in one tube. All you have to do is add the appropriate volume of forward and reverse primers, as well as your DNA (in this case, your bacterial colony).

## How to Use New England Biolabs OneTaq® 2X Master Mix with Standard Buffer

Now, you will have to combine this working concentration primer with the New England Biolabs OneTaq® 2X Master Mix with Standard Buffer using the following recipe:

- For every PCR reaction that will be run, add together:
  - 10 µL of the primer mix: [5µL F primer (1 nm/µL) and 5 µL of R primer (1 nm/µL)]
  - 13 µL of NEB OneTaq master mix

### Tips:

- Teachers will add primer to the Master Mix
- Add the primers to a PCR master mix immediately before use so that students only need to add their DNA to assemble their reactions.
- If you have 12 student groups running four samples each for the ABE Colony PCR lab, you will need to run a total of 48 samples. We suggest that when you make the primer + TAq solution, you increase the sample number for extra volume.
  - For example: If you have 48 samples, add at least four samples to account for errors, so use 54 to calculate volumes.
    - 54 samples x 10 µL/sample for primer = 540 µL of ABE Colony Primer
    - 54 samples x 13 µL/sample = 702 µL of NEB OneTaq quick load (Labeled TAQ). Note this Taq solution contains the buffers and nucleotides as well as the Taq enzyme.
  - Each student group will need at least 92 µL of this primer + TAQ mix ( labeled PCR)to run four PCR. Students will aliquot 23 µL to each tube (4 tubes @ 23 µL=92 µL)

- Aliquot 100µL of the Master Mix per group to allow for some wiggle room.
- Be certain to thaw and re-suspend reagents completely before aliquoting. Mix well and keep on (wet) ice. Students will add ~2 µL of their DNA (colony from plate) or 2 µL of control plasmid to the tube with the master mix. and set it up in the PCR machine.

## One or Two Days before Colony PCR Lab

### Programming the Thermocycler

The chart below explains how to program the thermocycler.

| | | Temperature (°C) | Time (sec) |
|---|---|---|---|
| Initial hold | | 4 | Indefinite |
| Initial denaturation | | 95 | 270 |
| 30 cycles | Denaturation | 95 | 30 |
| | Annealing | 53 | 30 |
| | Extension | 68 | 60 |
| Final extension | | 68 | 300 |
| Hold | | 4 | Indefinite |

NOTE: Not all Thermocycler models will allow for a 4°C hold.

### Day of Colony PCR (Prior to Part I PCR class)

### Preparing the Master Mix

Once the master mix is thawed, it's very important to keep the mixture in wet ice. If allowed to sit at room temperature, it's possible to produce not only primer dimers but additional unintended amplification products.

Pipet the PCR master mix + Taq up and down several times to mix it thoroughly, then aliquot 100 µL into each microfuge tube marked "PCR." **Store at 4°C. You can prepare this mixture the morning of the lab, store it at 4°C, and use throughout the day. Do not store overnight at 4°C or you risk the quality of the PCR reaction.**

### Day of Gel Electrophoresis (Prior to Part II Gel Electrophoresis class)

**Prepare 6 gels.** (The video *Making an Agarose Gel* on the ABE program website walks you through the process of making an agarose gel and casting it as described below.) **Refer to Instruction sheet in Resource Binder. We also have a gel preparation video on the RI Amgen**

Prepare the following materials:

- 6 gels with SyberSafe® or GelGreen® using 10-well comb
- PCR master mix with primers and Taq polymerase (store at -20℃)
- 12–14 tubes with 3 µL pARA-R labeled "+" (store at 4℃)
- 12–14 tubes with 3 µL pARA labeled "-" (store at 4℃)
- Plastic container full of water and crushed ice
- Fine-tip marking pen
- 12- P-20 pipettes or P10
- P-20 pipette tips or P10 tips
- 6 Cups with disinfectant for tip disposal
- Storage container for prepared gels

## Laboratory Setup

Supplies needed for a class of 24 students (12 groups of 2):

PART A – PCR REACTION

- 12- P-20 micropipettes with tips
- 12P-10 micropipettes with tips
- 12–cups (or ice buckets)
- 12- fine-tip permanent markers
- Ice (crushed preferable)
- Deionized or distilled Water
- 3 or more LB/amp/ARA plates with transformed colonies
- 12–tubes of PCR Master Mix + Taq (labeled "PCR")
- 12- 0.025 ng/µL pARA-R plasmid "+" control tubes (stored at 4℃)
- 12– 0.025ng/µL pARA plasmid "-" control tubes (stored at 4℃
- PCR tube strips and 48 caps (or small PCR tubes) – 4 tubes/student or group
- Waste containers (for used tips and microfuge tubes)
- Thermocycler
- Microcentrifuge with PCR tubes adapters

PART B – GEL ELECTROPHORESIS

- 12- P-20 micropipettes
- 6 prepared gels (using 10-well comb )
- 6 electrophoresis chambers and power supplies
- 1X sodium borate buffer
- 12 microfuge tubes (1.5-mL) with DNA Ladder (labeled "M")
- 24 copies of DNA Ladder Diagram (RM E)
- Transilluminator

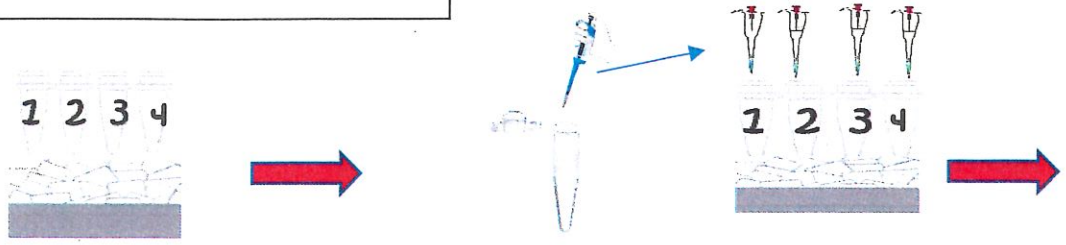
## Tips

- Colony PCR Lab Prep
  - If necessary, the master mix and primers can be mixed and frozen up to 3 weeks prior to the lab with good amplification results. However, this should only be done for reliable teachers as the mixed reagents will be wasted if not used.
- Picking a colony
  - Students often want to pick up a large amount of cells when picking up a colony off the plate; only a tiny bit is necessary (turbidity/too many cells will negatively affect the PCR amplification).
  - The student picking the colony should be the same student holding the plate.
  - Remind students to collect cells only, and NOT to pick up the agar!
  - Model for students how to use a precise colony-picking motion rather than a "digging" motion.
  - Try using just the pipette tip to pick up the cells; it's much easier than holding an entire pipette.
- Satellite Colonies
  - Some sites have had issues with satellite colonies forming on their plates during Getting a Recombinant Plasmid into Bacteria (Lab 5/5A/5B).
  - To avoid satellite colonies forming, move plates from incubator to the refrigerator right around the 24-hour mark.
  - Plates without satellite colonies allow students to isolate a colony much easier.
- Trouble Shooting
  - Always check that the thermocycler is set to the correct program before running students' samples. If you use miniPCR machines, you should check student setups for the correct protocol as well.

# COLONY PCR
## PART A: PERFORMING PCR

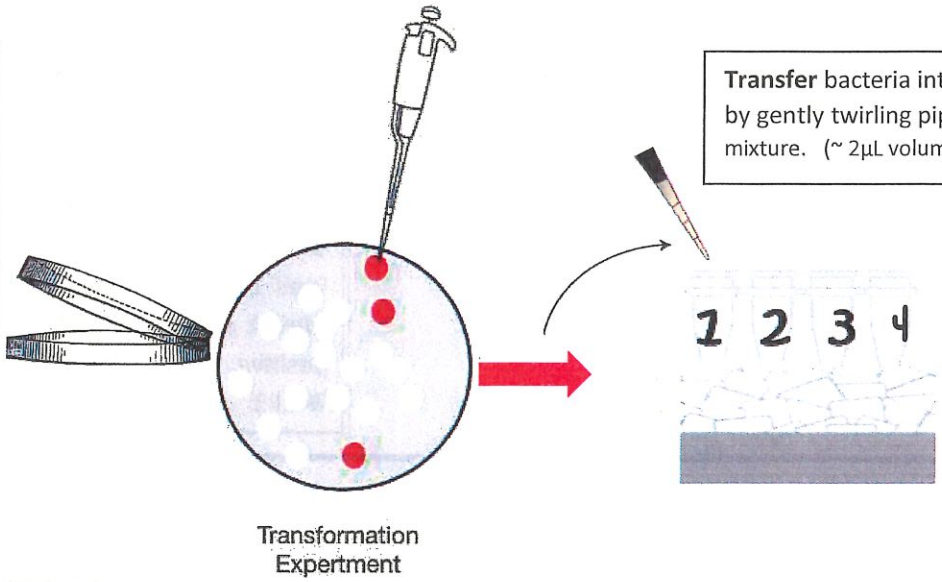
**Label** 4 empty tubes 1-4 and initial them. Label both sides and top. Keep on ice. *Tube 1 will have a pink colony; tube 2 white or another pink colony, tube 3 for pARA-R (labeled +) and Tube 4 for pARA (labeled -).*

With the P-20 set to 11.5µL, **pipette** 23 µL of the master mix (2 X 11. 5µl) into to each of the tubes. Master Mix is labeled PCR.



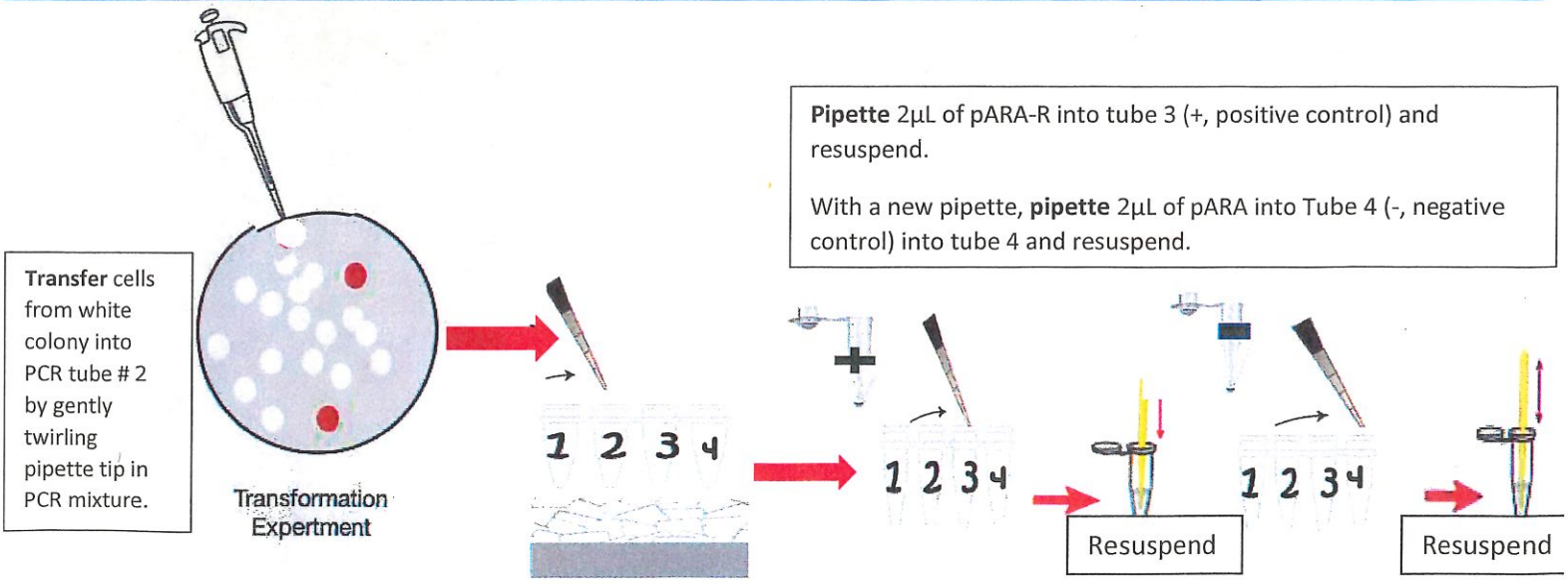

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Step 4: PCR master mix (PCR) | 23 µL | 23 µL | 23 µL | 23 µL |
| Step 5: Red colony | 2µL | | | |
| Step 6: White colony | 2µL | | | |
| Step 7: pARA-R (+) | 2µL | | | |
| Step 8: pARA (-) | 2µL | | | |
| Total volume | 25 µL | 25 µL | 25 µL | 25 µL |

**Locate** a red colony that is isolated from the other colonies. Open petri dish like clam shell and use the pipette tip to **lightly touch** the colony.




Transformation Expertment

**Transfer** bacteria into PCR tube # 1 by gently twirling pipette tip in PCR mixture. (~ 2µL volume per colony).



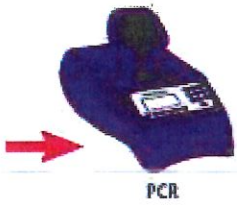

Transformation Expertment

**Transfer** cells from white colony into PCR tube # 2 by gently twirling pipette tip in PCR mixture.

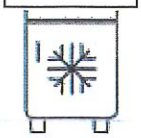
**Pipette** 2µL of pARA-R into tube 3 (+, positive control) and resuspend.

With a new pipette, **pipette** 2µL of pARA into Tube 4 (-, negative control) into tube 4 and resuspend.

**Cap samples** and take ice tray/cup with PCR tubes to your teacher to **place** in the thermal cycler. The PREPROGRAMMED thermocycler will run for ~ 70 minutes. Thermocycler will **hold** samples at **4$^0$C** until the samples are transferred to the **freeze**, where they are stored until agarose gel electrophoresis is performed.

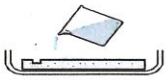

PCR

-20$^0$C



## PART B: SEPARATE PCR PRODUCTS USING GEL ELECTROPHORESIS

**POUR** gel (8% agarose with 1:10,000 dilution of dilution of Sybersafe/gel green dye in 1X Sodium Borate buffer)

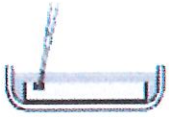


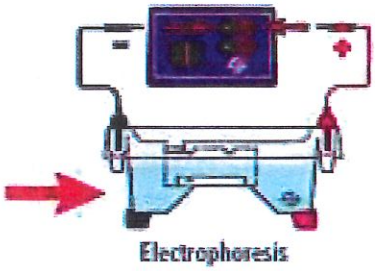
Let gel set

**Make a drawing** in your notebook or paper that shows the location of the wells in the electrophoresis box. Order of samples in each well should be as follows:

Well 1: DNA ladder (M)
Well 2: Red colony (Tube 1)
Well 3: White colony (Tube 2)
Well 4: pARA-R (or pBAD-R)(Tube 3), 1092 bp, positive control ( Tube 3)
Well 5: pARA (Tube 4), 662 bp, negative control

Using a fresh pipette tip for each sample, **dispense** 10µL of each prepared sample and the DNA ladder (M) into their designated wells.




Electrophoresis

**Run** 120-130 V for 30 min