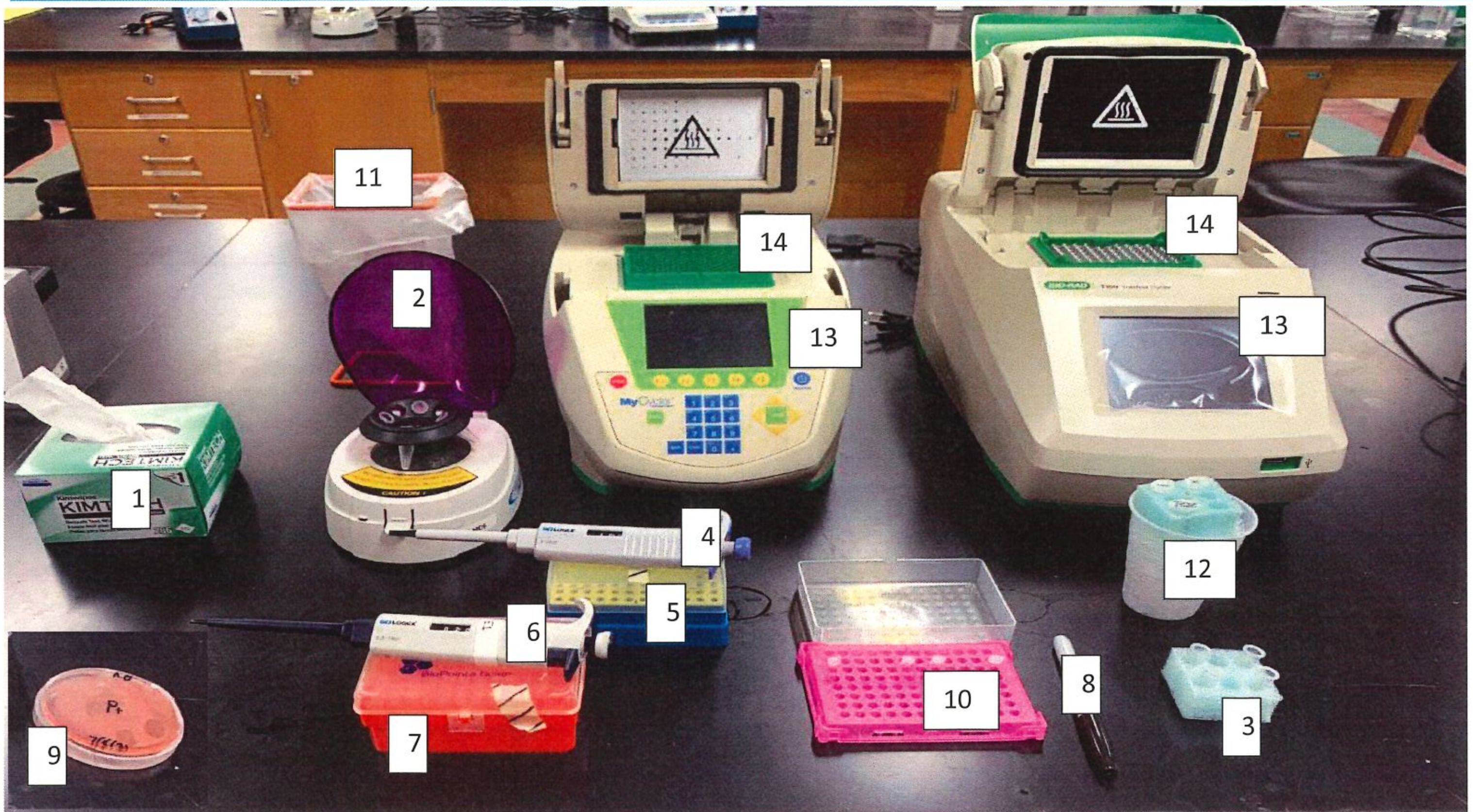
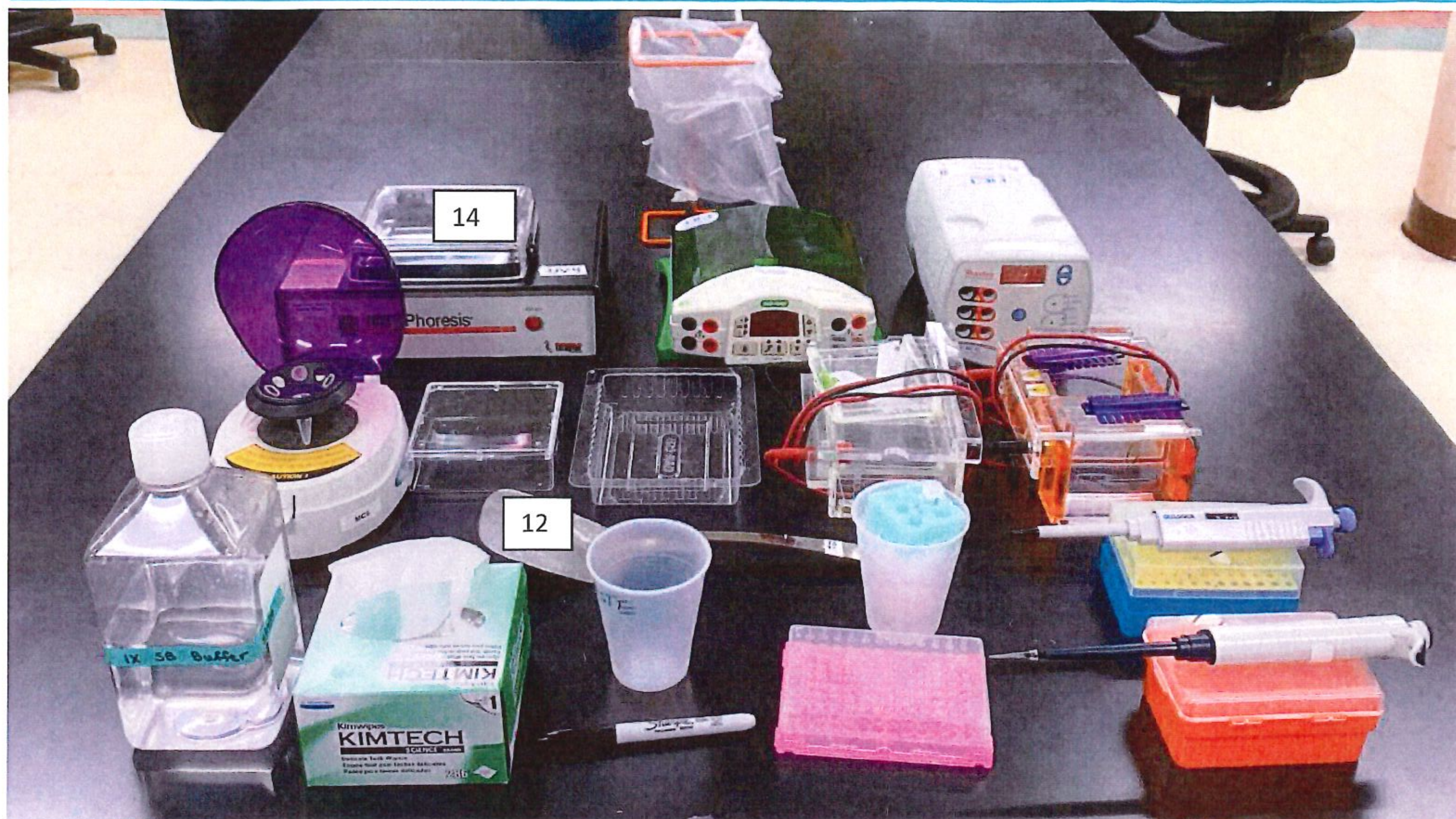
# *COLONY PCR LAB RESOURCES*

# 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 4C, and use throughout the day. **Do not store overnight at 4C 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 -20C)
- 12–14 tubes with 3 µL pARA-R labeled "+" (store at 4C)
- 12–14 tubes with 3 µL pARA labeled "-" (store at 4C)
- 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 4C)
- 12– 0.025ng/µL pARA plasmid "-" control tubes (stored at 4C
- 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

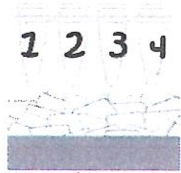
  - ➢ Some sites have had issues with satellite colonies forming on their plates during Getting a Recombinant Plasmid into Bacteria (Lab 5/5A/5B).
  - ➢ To avoid satellite colonies forming, move plates from incubator to the refrigerator right around the 24-hour mark.
  - ➢ Plates without satellite colonies 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.

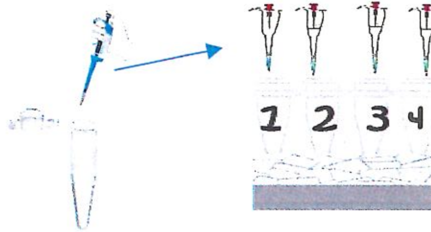
# COLONY PCR

## 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 -).*

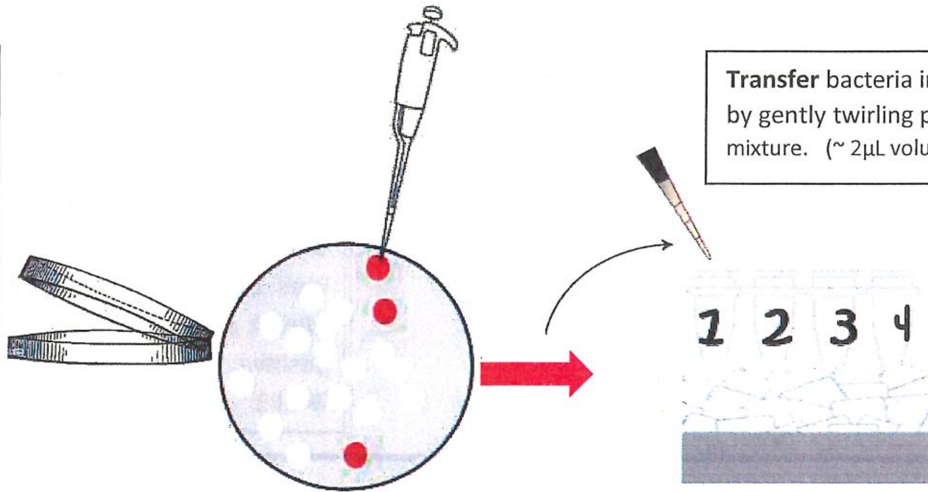



With the P-20 set to 11.5µL, **pipette** 23 µL of the master mix (2 X 11. 5µl) into to each of the tubes. Master Mix is labeled PCR.




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



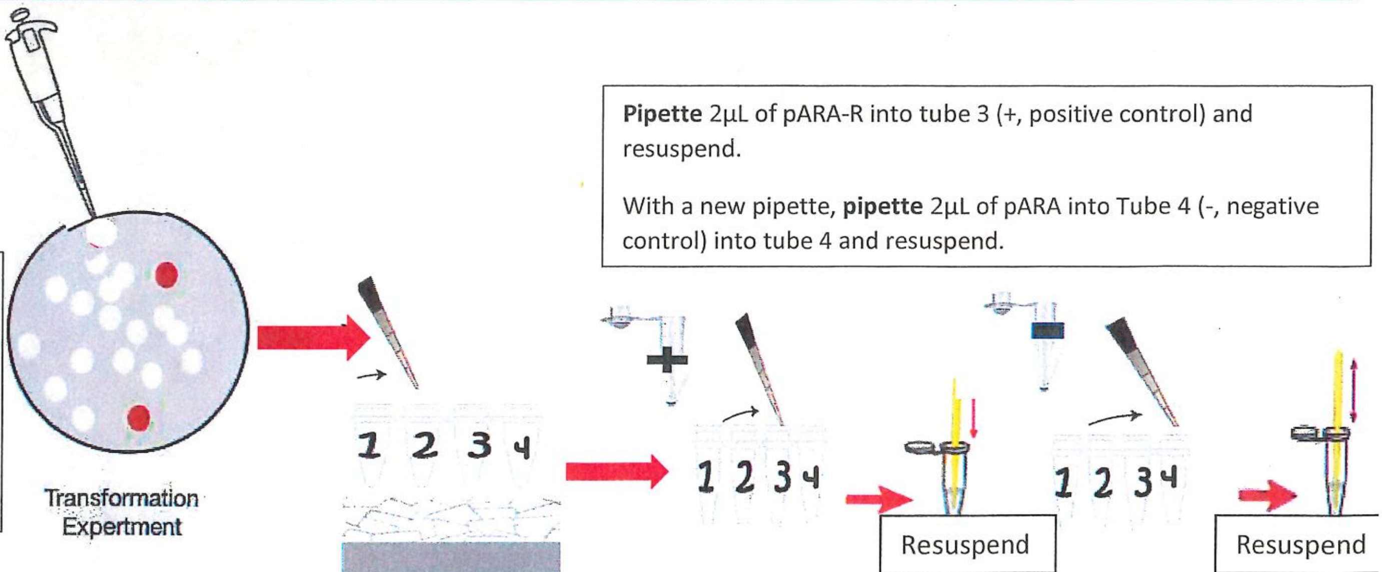

**Transfer** bacteria into PCR tube # 1 by gently twirling pipette tip in PCR mixture. (~ 2µL volume per colony).

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.

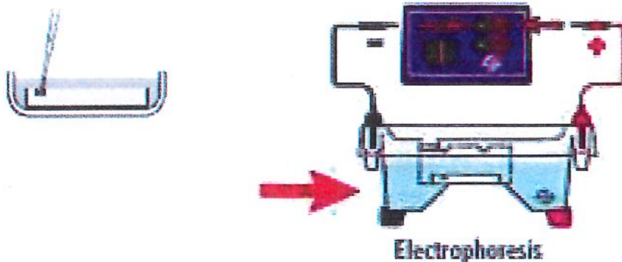
## PART B: SEPARATE PCR PRODUCTS USING GEL ELECTROPHORESIS

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

**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.




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