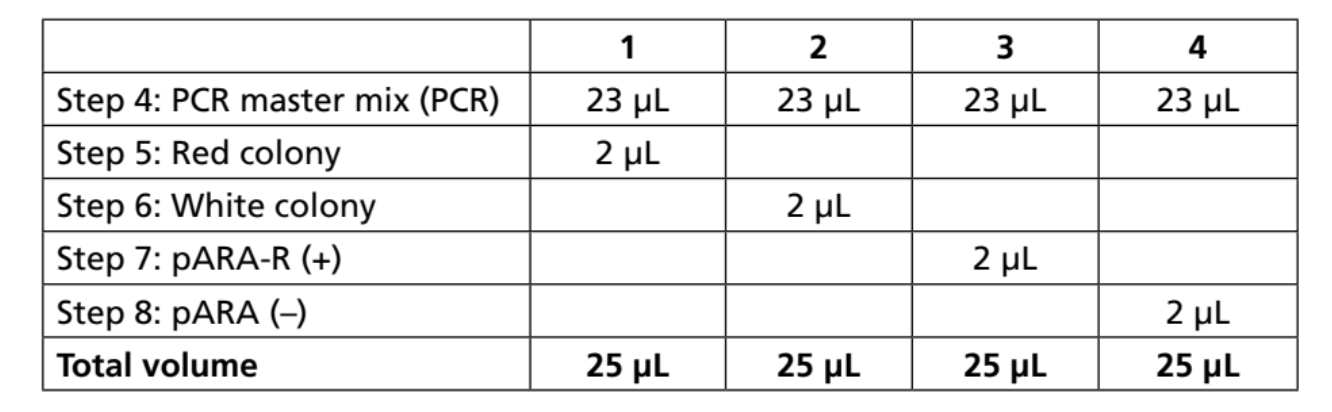
**Colony PCR Part A**

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| **LAB TECHNIQUE NOTES: All appropriate safety precautions and attire required for a science laboratory should be used. Please refer to your teacher’s instructions. Use *aseptic technique* when working with E. coli bacteria. Discard all tubes and tips from this lab into the WASTE containers indicated by your teacher.** |

1. Label the four PCR tubes 1-4 on the sides. Place them in the ice cup or empty tip rack.



2. Using the p20 micropipette set at 11.5µL, add 23µL (2 x 11.5µL) of [PCR] solution to each of the tubes. Pipet down into the bottom of the tube and try not to introduce air bubbles.

3. Locate a well isolated Red colony on the LBAA plate. While wearing gloves, use a pipette tip to gently touch the colony to pick up a tiny amount of cells. Place the end of the tip containing the cells into tube #1 and twirl it in the PCR solution several times. Discard the tip in the WASTE container. Do not gouge the agar and try not to create bubbles in the PCR solution.

4. Do the same for a White colony and tube #2.

5. Use a new tip and transfer 2µL of “+” (the pARA-R plasmid) to tube #3. Gently pump up and down to mix the solution.

6. Use a new tip and transfer 2µL of “-” (the pARA plasmid) to tube #4. Gently pump up and down to mix the solution.

7. Quickly spin the tubes in a microcentrifuge with a PCR tube rotor to pool the reagents or remove any bubbles in the solutions. Be sure to balance tubes.

8. If the class is sharing a thermal cycler, take the ice cup containing your tubes up to your teacher to be placed in the thermal cycler. If using smaller thermal cyclers, place your tubes into the unit and follow your teacher’s instructions on setting the program.

**Colony PCR Part B**

1. Using a new tip each time, add 2µL of LD to each of your four PCR tubes and the DNA marker [M].

2. Correctly place a gel in the buffer tank (wells up and near the “-” electrode). Cover the gel with 1X buffer.

3. Using a new tip for each sample, load 10µL of each sample in the following order:

M R W + -



4. Be sure to draw a gel map of your samples.

5. Cover the gel tank and run the gel.

If using a mini one or blue gel unit, turn the unit on but leave the light off and run the gel for 20-30 minutes. Be sure to check the gel every 5-10 minutes.

If using a separate gel box and power supply, plug the electrodes into their corresponding ports on the power supply. Turn the power supply on and set the voltage to 130V and press Run. Allow the gel to run for 15-30 minutes.

7. Examine your gel using the transilluminator, take a photo and record your observations.