**Lab 5/5A/5B: Transforming Bacteria with the Ligation Products or pARA-R plasmid ed 11/22**

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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.** Hold tubes by their rims to avoid warming up the cells and return tubes to the ice quickly. |

1. Label two clean tubes “P+” and “P-” along with your group and period ID. Place them into the cup of crushed ice that contains your tube of competent cells “CC”.

2. Using a p200 micropipette set to 50µL, gently resuspend the cells in the CC tube by pumping up and down. Transfer 50µL of the cells into the P+ tube and 50µL into the P- tube. Carefully discard the tip.

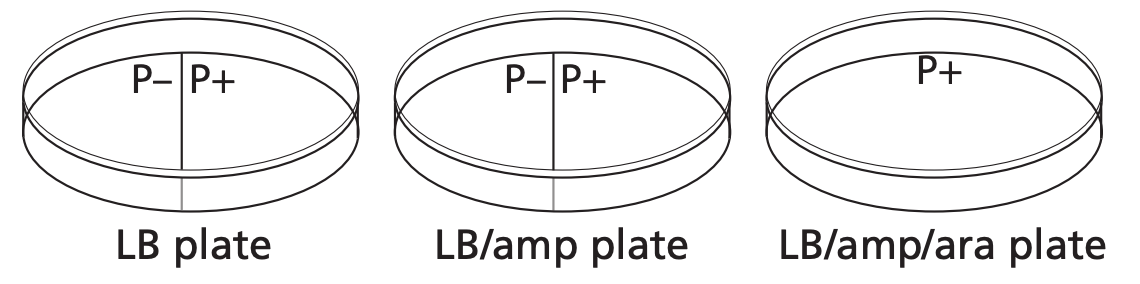
3. Using the p20 micropipette set to 10µL, add 10µL of your LIG sample to the P+ tube. Gently pump up and down to mix the solution. Discard the tip and return the tube to the ice. ***NOTE: For Lab 5A/5B add 10µL of “RP” to your P+ tube and pipet up and down to mix.***

4. Leave your tubes in the *ice* for 15 minutes. During this time label your three agar Petri plates on the bottom of each plate (the outside surface of the side containing the agar) as follows:

On the LB plate (identified by one stripe on the lid), draw a line down the center to divide the plate. Label one side “P-” and the other “P+” and label the plate “LB”. Write your group and period ID as well.

On the LBamp plate (identified by two stripes on the lid), draw a line down the center to divide the plate. Label one side “P-” and the other “P+” and label the plate “LBA”. Write your group and period ID as well.

Label the LBamp ara plate (identified by three stripes on the lid) “LBAA” and “P+”. Write your group and period ID as well.



5. After the 15 minute ice incubation, carry your ice cup containing your tubes over to the 42°C water bath (or heat block). Place the tubes at 42°C for 45 seconds. Immediately return the tubes to the *ice* cup and allow them to cool down for one minute.

6. Using the p200 micropipette, add 150µL of LB broth to the P- tube. Gently pump up and down to mix.

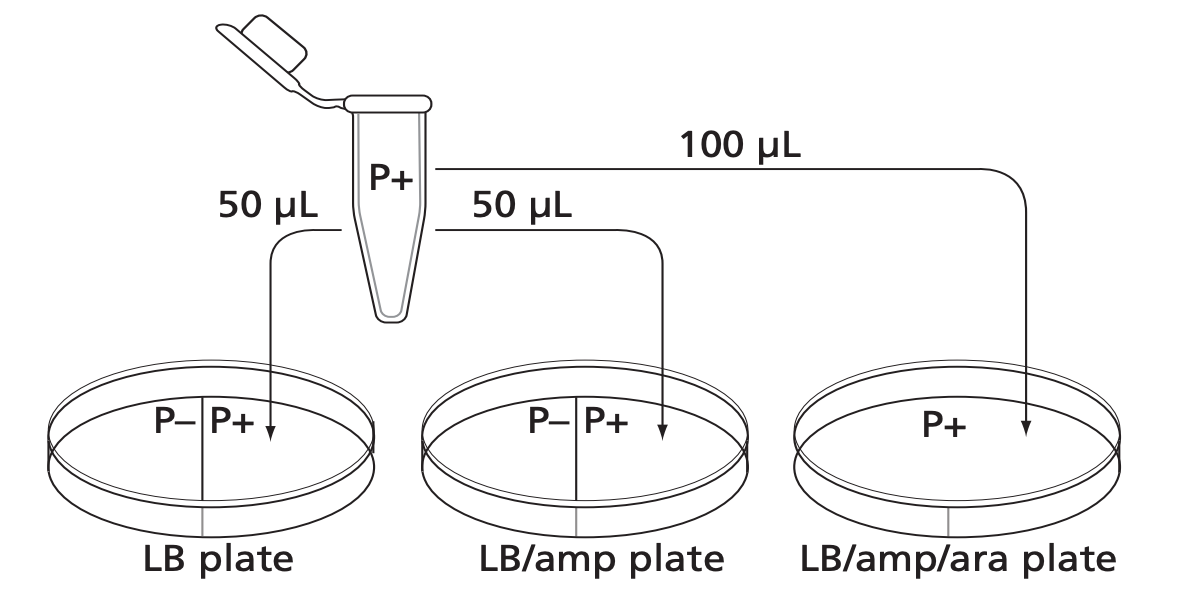
7. Using the same tip, add 150µL of LB broth to the P+ tube. Gently pump up and down to mix.

8. Incubate both tubes in your tube rack at *room temperature* for 15 minutes.

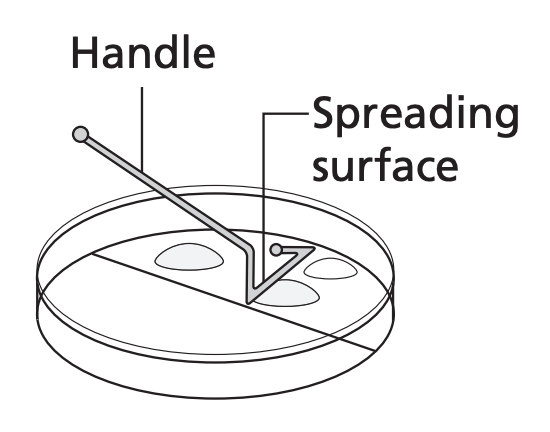
NOTE: Your teacher may collect your tubes at this point and place them in the refrigerator overnight

so that you can continue the lab tomorrow. If not, proceed to step 9.

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| **LAB TECHNIQUE NOTES: Place your three agar plates with their lids up in this order: LB, LBA, LBAA. Use the “clamshell” technique to open the lids only when necessary. Keep the plates covered as much as possible.** |



9. Using the p200 micropipette, gently resuspend the cells in the P- tube and transfer 50µL to the P- side of the LB plate. Using the same tip, transfer 50µL to the P- side of the LBA plate. Discard the tip.



10. Using a sterile cell spreader, first spread the cells across the entire P- half of the LB plate. Using the same spreader, spread the P- cells on the LBA plate. Discard the spreader.

11. Using the p200 micropipette, gently resuspend the cells in the P+ tube and first transfer 50µL to the P+ side of the LB plate. Then transfer 50µL to the P+ side of the LBA plate. Finally transfer 50µL *twice* (for a total of 100µL) to the LBAA plate. Discard the tip.

12. Using a sterile cell spreader, first spread the cells across the entire P+ half of the LB plate. Then spread the P+ cells on the LBA plate and finally on the LBAA plate. Discard the spreader.

13. Allow the plates to sit for several minutes. Then stack the plates with the LBAA plate on the bottom and tape them together. The lids should all be facing the same direction.

14. Place the plates in the 37°C incubator upside down (bottoms up). Incubate for 24-36 hours at 37°C.

15. Disinfect your lab station following your teacher’s instructions. Tubes, tips and spreaders should all be placed in the WASTE cup and emptied into the AUTOCLAVE BAG.

16. Predict how much bacterial growth you will see on each plate. Mark the plate/plate section with +++ (for high growth), ++ (for medium growth), + (for low growth) or - (for no growth).

17. Examine your plates the next day and make detailed observations in your lab notebook. Take photos of your plates and send them to your teacher. When finished, place your plates in the AUTOCLAVE BAG as instructed by your teacher. *If doing the Colony PCR lab, SAVE YOUR LBAA PLATE, your teacher will refrigerate it.*