

Protocol 3: Antiobiotic Resistance

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PROTOCOL CITATION

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Forked from Protocol 3: Antiobiotic Resistance, Alyssa Ayala

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MATERIALS TEXT

Perishables

1 - Sterile Water Tube

Non-perishables

- 1 10-100 µL Variable Volume Adjustable Pipette
- 1 Box 1-200 µL Pipette Tips
- 14 Petri Plates
- 1 Microcentrifuge Tube Rack
- 5 Inoculation Loops
- 1 Bag of Microcentrifuge Tubes

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BEFORE STARTING

The final step is testing the success of the bacterial transformation. There are multiple ways bacterial cells can become antibiotic reiststant. As you may recognize in the video, the E Coli in this experiment is made resistant to streptomycin through modifying the antibiotic binding target (rpsL gene) through transformation.

- 1 Plate the CRISPR solution. Take LB Strep/Kan/Arabinose agar plate out of the fridge and let sit to adjust to room temperature. Shake the microcentrifuge tube containing the CRISPR experiment and then pour or pipette contents onto the agar plate. Use an inoculation loop to spread contents onto the plate. Let dry for a few minutes. Only let the plate dry in a sanitary space or other contaminents may join the plate.
- 2 **Incubate contents on LB plate.** Put the lid back on, flip the plate over, make sure to label it, and leave the bacteria to culture in room temperature. It will take 1 to 2 days to grow.
- 3 Congratulations! You have now completed your first CRISPR experiment! Go you.
- 4 Take a picture of your end culture and input it on your Lab Notebook. If you did not see any growth, identify factors that may have contributed to this result.