CITIZEN SCIENCE
Bard College

Transformation of Bacteria

Bacteria reproduce asexually, which means they divide in half and make two identical bacterial cells. But they are still able to share genetic information through the use of small circular pieces of DNA called plasmids. Today you will be able to put a plasmid into bacteria that will give them new abilities.

Materials

2 LB ampicillin plates
1.5 ml of LB broth
Z competent cells (Will be handed out when needed)
2 μl pGreen Tir plasmid
Sterile glass beads
pipettors with sterile tips

Protocol

1. Label microcentrifuge tube containing 2 μl plasmid DNA with your initials.
2. Pipette the 25 μl aliquot of Z competent cells from the microcentrifuge tube and add it to the microcentrifuge tube containing 2 μl plasmid DNA.
3. Mix by **GENTLY** flicking bottom of tube.
4. Incubate on ice for 5 minutes.
5. Set up 2 LB agar plates supplemented with 100 μg/ml ampicillin.
   Label BOTTOM of plate with:
   - Initials
   - Date
   - 1 plate 10 μl and the other plate 50 μl
6. Add 1 mL LB broth to the plasmid/cell mixture that has been incubated 5 minutes on ice.
7. Pipette 10 μl of this cell suspension on the appropriately labeled LB agar plate.
8. Pipette 50 μl of this cell suspension on the appropriately labeled LB agar plate.
9. Sprinkle 5-10 sterile glass beads onto each plate, and gently agitate beads so that the entire surface of the plate is covered with cell suspension. Avoid swirling the beads in a circular motion or only the outside of the plate will be covered. Do not resuse beads.
10. Pour glass beads into container on the bench containing Wescodine disinfectant to be washed and re-sterilized.
11. Incubate plates inverted overnight at 37° C.

What results do you expect?

What do you think is in the plasmid?