Protocol for Bacteria Transformation

Aim

To transform competent bacteria with plasmids.

Material

- 500 µl tube of competent bacteria (competent cells stored at -80°C)
- Plasmid to transform
- 1.5 ml Eppendorf tubes
- P1000, P200, P10 pipettes + paired cones
- Petri dishes with LB agar/CARB for carbenicillin (an equivalent of ampicillin, resistant to temperature)
- Water-bath at 42°C
- Incubator at 37°C

Protocol

Plasmid transformed
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Competent cells are extremely sensitive; always handle the tubes on ice.

You must operate in the vicinity of the Bunsen burner when manipulating bacterial cultures.
Name the tubes with the transformed cells: Cell type/name, plasmid (vector and composition), initials of the operator: First Name/Last Name, date.

Protocol for Bacteria Transformation

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1. Split the tube of competent cells in aliquots of 50 µl, in 1.5 Eppendorf tubes placed on ice.

For maximum competency:

2. Add 1µl of β-mercapto-ethanol only to subcloning grade competent cells DH5alpha.
3. Mix by gently tapping the bottom of the tubes.
4. Add …… µl = …… pg of plasmid to transform.
5. Mix by gently tapping the bottom of the tubes.
6. Let it rest for 30 minutes on ice.

In the meantime, check that the water-bath is at 42°C, and place the SOC media at 37°C for warming.

7. Put the tubes in the floats.
8. Place the floats in the 42°C water-bath for 40 s, then remove the floats quickly.
9. Place the tubes on ice for 3 min.
10. Add 650 µl of SOC media per tube.
11. Incubate and mix the tubes at 150 rpm at 37°C for 40 min.

In the meantime, place the labelled LB/AMP petri dishes at 37°C.

12. Generate 2 petri dishes with each tube: one dish containing 200 µl and the other 500 µl.
13. Spread the bacteria using an inoculator.
14. Wait for the dishes to dry.
15. Store the dishes with agar side up in the incubator at 37°C for approximately 16 h (overnight).