Transformation_DH5a

Introduction

This is used to transform cloned plasmids into cells.

Materials

- LB Agar Plates containing (1:1000) concentration of antibiotics
- SOC Medium
- Competent Cells (e.g. DH5-alpha)

Procedure

Before Starting

Equilibrate a water bath to 42°C
Warm the vial of SOC medium to room temperature
Place the plates in a 37°C incubator to remove excess moisture (use two plates for each transformation)
Defrost chemically competent cells on ice. One vial of cells per transformation

Cell Transformation

1. Add 5 µL of plasmid DNA to 50 µL of competent cells (1:10 Dilution). Mix by tapping not pipetting.
2. Incubate the vial(s) on ice for 30 minutes.
3. Heat shock: Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
4. Remove vial(s) from the 42°C bath and quickly place on ice for 30s.
5. Add 250 µl of pre-warmed SOC medium to the vial(s).

   Note: SOC is a rich medium; practice good sterile technique to avoid contamination. (LB broth can also be used).
6. Incubate the vials for 1hr at 37C, 250rpm.

Plate pouring and cell seeding (for two plates, conducted in the hood)

7. Add 50mL of LB agar into a 50mL falcon tube; add 500uL of Xgal (20 mg/mL); add 50uL IPTG (1 M); add 50uL of chloramphenicol (1:1000 dilution) (34 mg/mL).
8. Pour 25 mL of solution per plate and wait 1hr for it to cool down: leave the lid half open to avoid condensation.

9. pipette 100uL of transformed cell onto each plate; spead the cell evenly with the spreader.

10. Place the LB agar plate overnight at 37˚C. Make sure the plates are placed upside down in the incubator to prevent water disturbing the colonies, store in fridge if not using so colonies won't grow (4C), not freezer.