Molecular cloning and genetic engineering – Transformation

Aim

Transformations are essential to amplify the plasmid or express it.

Materials

Plasmids to be transformed

Competent cells DH5α(for cloning) or BL21(for expressing)

LB medium

Procedure

1. Label 1.5ml tubes with part name or well location. Fill lab ice bucket with ice, and pre-chill 1.5ml tubes in a floating foam tube rack.

2. Thaw competent cells on ice. Dispose of unused competent cells. Do not refreeze unused thawed cells, as it will drastically reduce transformation efficiency.

3. Pipette 50µl of competent cells into 1.5ml tube: 50µl in a 1.5ml tube per transformation. Keep all tubes on ice.

4. Pipette 1µl of resuspended DNA or your plasmids of choice into 1.5ml tube. Gently pipette up and down a few times. Keep all tubes on ice.

5. Close 1.5ml tubes, incubate on ice for 30min: Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.

6. Heat shock tubes at 42°C for 45 sec.

7. Incubate on ice for 5min: Return transformation tubes to ice bucket.

8. Pipette 950µl LB media to each transformation.