Transformation

Aim

Transforming the plasmid of choice either with the goal to amplifying the plasmid or expressing it.

Procedure

1. Chill Eppendorf tubes on ice, heat SOC to 37 °C and let cell aliquots thaw on ice
    a. Prepare SOC by following Buffer prep protocol.

2. Gently mix cell aliquots and pipette 50μl of cells into each tube.

3. Add 10μl of 5x KCM to each tube.
    a. Prepare KCM according to Buffer prep protocol.

4. Incubate cells on ice for 10 minutes.

5. Add 5μl of plasmid and swirl tubes gently to assure good mixture.

6. Incubate on ice for 30 minutes.

7. If using Top 10 cells: heat-pulse for 60 seconds, 42 °C. If using BL21 cells: heat-pulse for 15 seconds, 42 °C.

8. Incubate cells on ice for 2 minutes.

9. Add 100μl of pre-warmed SOC to each tube.

10. Incubate cells on the shaker for 1-2 hours (37 °C, 200-250 rpm) and pre-heat plates with relevant antibiotic for selection in the incubator.

11. Place 135μl cell suspension (pipette up and down carefully to ensure good suspension) onto an agar plate with antibiotics and spread. Remaining cells

can be spread on an antibiotic-free plate to check cell viability.

12. Incubate agar plates overnight (max 18 h, ideally check for colonies after 12 h).

Note!

Competent cells are very sensitive to both mechanical disruption and temperature changes. To mix cells, do not vortex but instead pipette up and down carefully and slowly.
Always thaw cells on ice.
When performing the heat shock, the timing is very important and the tubes should be kept very still. When growing transformed cells, ideally do not leave in the incubator for >18h due to the risk of satellite colonies developing.

Sources

This protocol is a modified version of a protocol by Gunilla Karlsson Hedestam Group at Karolinska Institutet.