Competent Cells

Overview

This protocol covers making chemically competent cells for the transformation of plasmids. Keep cells on ice at all points after centrifugation. Protocol can be scaled up or down as needed[i]

Materials

- LB Agar plate
    - Should be antibiotic free unless strain is resistant to a particular antibiotic
- Inoculation Loop
- LB
- Glycerol stock or Existing Competent Cells
- Sorvall centrifuge tubes
- TSS Buffer
    - Composed of: 10% w/v PEG-8000, 20mM $MgCl_2$, 5% v/v DMSO, with the remainder as LB
- KCM Buffer
    - Composed of: 0.5M KCl, 0.15M $CaCl_2$, 0.25M $MgCl_2$
- 1.7mL Microcentrifuge tubes or Screw cap tubes[ii]
    - Recommended to label these on the sides with something short (e.g. JS or 5a), this keeps the top free for transformation keys.

Procedure

1. Streak out cells on agar plate and incubate overnight or until colonies form
2. Pick a single colony into 5mL of LB and grow overnight to saturation
3. Dilute cells 1:100 into 50mL fresh LB.
4. Grow cells to OD 0.5-0.7 (~2-3 hours) [can take less time for JS006 or other wild type strains].[iii]
    - This step is critical, too high or low OD will lead to poor quality competent cells.
    - Don't forget to blank with LB
5. When cells are close to fully grown, pre-chill the following:
    - Centrifuge rotor (4C)

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[i] This protocol comes from Murray Lab. The original publication from which it's derived is (Chung et al. PNAS, 1989).

[ii] Screwcaps are less liable to have the top burst off at -80.

[iii] In Chung et al. they use a lower OD600 (0.3-0.4) for optimal efficiency, but note that transformation efficiency is good at higher OD600. However, their method does not use KCM.

- Sorvall Tube (on ice)
- TSS (on ice)
- KCM (on ice)
- 1.7mL Tubes (on ice)

6. Move 42.5mL of cells to the pre-chilled Sorvall tube and centrifuge at 3500 RPM, 4C for 5 minutes.
    - Make sure to balance centrifuge.
7. Resuspend cells in 4.25mL of ice cold TSS.
    - Do not vortex
8. Add 0.85mL ice cold KCM to tube.
9. Aliquot 60µL of cells on ice into each final tube. Store at -80C.
10. Bleach Sorvall tubes then rinse well with Millipore. Dry and store.
11. It is recommended to test competency of cells before use.