Stellar™ Competent Cells (96-well Plate) Protocol-At-A-Glance

I. Genotype

F–, *endA*1, *supE*44, *thi*-1, *recA*1, *relA*1, *gyrA*96, *phoA, Φ*80d *lacZΔM15, Δ(lacZYA - argF)* U169, *Δ(mrr - hsdRMS - mcrBC), ΔmcrA,* λ–.

II. Please Read Before Proceeding with Transformation

- Transport cells on dry ice/ethanol.
- When transforming each well of competent cells, do not use more than 0.2 ng of purified sample DNA. If you use more than 0.2 ng of DNA, transformation efficiency may decrease.
- When adding X-Gal to medium, do so as follows:
  Add 20 mg/ml X-Gal (dissolved in dimethylformamide) into 200 µl/100 ml agar medium.
- Do not refreeze competent cells once thawed. If necessary, freeze the cells in dry ice and stock at –70°C. However, the transformation efficiency may decrease more than one order of magnitude.

III. Transformation Protocol

The plate can be subdivided into 24-well (3 x 8) sections immediately after removal from the –70°C freezer (do not allow the cells to thaw). Bend the plate at the scored junctions (visible from underside of plate) to break the plastic plate. Cut the foil seal between the sections. Promptly store the unused sections at –70°C.

1. Thaw the plate (or plate section) on ice. Mix gently by tapping to ensure that the cells are suspended. Return the cells to ice. Thaw SOC Medium and equilibrate to room temperature.
2. Remove foil seal entirely and add 1 µl DNA solution (no more than 0.2 ng of DNA) to each well. Be sure to pipette the DNA directly into the cells, not on the wall of well. After adding the DNA solution, stir gently with pipette tip. Cover wells with the 8-Cap Strips or Plate Lid.

NOTE: Rough treatment can decrease transformation efficiency. Do not vortex or pipette up and down to mix.

3. Incubate the transformation mixtures (DNA + cells) on ice for 5 min.
4. Heat shock the cells for 10–30 sec at 42°C.

NOTE: A heat block is recommended.

5. Place the cells directly on ice for 1–2 min.
6. Remove the 8-Cap Strips or Plate Lid. Add 80 µl of room-temperature SOC Medium to each well. Cover wells with the 8-Cap Strips or Plate Lid.
7. Incubate for 1 hr at 37°C (shaking is not necessary).
8. Plate an appropriate amount of culture on selective medium.

NOTE: For a plate with a diameter of 9 cm, plate more than 50 µl of culture. Plating is accomplished by spreading cells on selective medium [e.g., LB agar + Ampicillin (50–100 µg/ml)]. The medium should also contain X-Gal (40 µg/ml) for plasmids that permit blue/white screening of transformants.

9. Incubate overnight at 37°C.

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