Stellar™ Competent Cells
Protocol PT5055-2

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Genotype

F–, endA1, supE44, thi-1, recA1, relA1, gyrA96, phoA, Φ80d lacZΔ M15, Δ (lacZYA - argF) U169, Δ (mrr - hsdRMS - mcrBC), ΔmcrA, λ–

Please Read Before Proceeding with Transformation

- Use no more competent cells than necessary. Transport cells on dry ice/ethanol.
- You may use 1.5-ml microcentrifuge tubes instead of 14-ml round-bottom tubes for transformation (see Transformation Protocol Step 2, below), but this may reduce efficiency.
- When transforming 50 µl of competent cells, do not use more than 5 ng of purified sample DNA. If you use more than 5 ng of DNA, transformation efficiency may decrease.
- If you change the amount of competent cells, or types of tubes used, it might be necessary to reevaluate the optimal conditions. For example, when using 1.5-ml microcentrifuge tubes, heat shock for 60 seconds at 42°C (see Transformation Protocol Step 5, below).
- 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.

Transformation Protocol

1. Thaw Stellar Competent Cells in an ice bath just before use.
2. After thawing, mix gently to ensure even distribution, and then move 50 µl of competent cells into a 14-ml round-bottom tube (falcon tube). Do not vortex.
3. Add no more than 5 ng of DNA for transformation.
4. Place tubes on ice for 30 min.
5. Heat shock the cells for exactly 45 sec at 42°C.
6. Place tubes on ice for 1–2 min.
7. Add SOC medium to bring the final volume to 500 µl. SOC medium should be warmed to 37°C before using.
8. Incubate by shaking (160–225 rpm) for 1 hr at 37°C.
9. Plate an appropriate amount of culture on selective medium.

NOTE: For a plate with a diameter of 9 cm, plate 100 µl. 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.

10. Incubate overnight at 37°C.

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