Protocol-at-a-Glance

Stellar™ Competent Cells (dam⁻/dcm⁻) Protocol-at-a-Glance

(PT5056-2)

Stellar Competent Cells (dam⁻/dcm⁻) are an *E.coli* HST04 strain that lacks the genetic factors (dam and dcm) necessary for the methylation of DNA. Plasmids prepared using this product can be cut by restriction enzymes which are normally blocked by dam or dcm methylation. This product is not a suitable cloning host.

Genotype

F⁻, ara,Δ(lac-proAB) [Φ80d lacZΔM15], rpsL(str), thi, Δ(mrr-hsdRMS-mcrBC), ΔmcrA, dam, dcm

Please Read Before Proceeding with Transformation

1. Use no more competent cells than necessary. Transport cells on dry ice/ethanol.
2. You may use 1.5-ml microcentrifuge tubes instead of 14-ml round-bottom tubes for transformation, but this may reduce efficiency.
3. When transforming 100 µl of competent cells, do not use more than 10 ng of purified sample DNA. If you use more than 10 ng of DNA, transformation efficiency may decrease.
4. 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).
5. 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.
6. 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 (dam⁻/dcm⁻) in an ice bath just before use.
2. After thawing, mix gently to ensure even distribution, then move 100 µl of competent cells into a 14-ml round-bottom tube (falcon tube). Do not vortex.
3. Add no more than 10 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 1 ml. 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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