Protocol-at-a-Glance

Stellar™ Electrocompetent Cells Protocol-at-a-Glance

(PT5057-2)

Stellar Electrocompetent Cells are an *E.coli* HST08 strain that provides high transformation efficiency paired with blue-white screening capability when used with pUC plasmid vectors. These cells are specially made for transformation using the electroporation method.

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

1. Place the vial of electrocompetent cells in a dry ice/EtOH bath immediately upon removal from –70°C freezer. Keep cells in the bath until you are ready to proceed.
2. When transforming 50 µl of electrocompetent 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.
3. When changing the scale of your experiment, optimal conditions should be considered.
4. When transferring high molecular weight DNA, transformation efficiency may decrease.
5. Use TE buffer for sample DNA preparation. High salt concentration in the sample DNA solution may decrease transformation efficiency.
6. 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.
7. Do not refreeze electrocompetent 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 50 µl of Stellar Electrocompetent Cells in an ice bath just before use.
2. Add 1–2 µl of the transforming DNA solution* directly into the thawed cell suspension. Mix gently to ensure even distribution.

   ***Note:** When the sample DNA solution contains salt, dilute with TE buffer or distilled sterilized water, or desalt by ethanol precipitation.
3. Transfer the mixture of cells and DNA to a cold 0.1-cm electroporation cuvette.
4. After applying pulse*, immediately add 1 ml of SOC medium (precooled in an ice bath), then transfer the mixture to another tube.

   ***Note:** For BIO-RAD's Gene Pulser, use the setting: 1.5 kV, 25 µF, 200 Ω. For Bio-RAD's MicroPulser, use the setting: 1.5–1.8 kV, 10 µF, 600 Ω. Users of the MicroPulser only need to set the voltage because the other values are preset.
5. Incubate by shaking (160–250 rpm) for 1 hr at 37°C.
6. 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.
7. Incubate overnight at 37°C.

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This document has been reviewed and approved by the Clontech Quality Assurance Department.

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