Materials Provided
HB2151 Electroporation-Competent Cells, 50 tubes

Storage Conditions
Place cells at the bottom of a -80°C freezer directly from the shipping container. Do not store in liquid nitrogen. Competent cells are sensitive to small temperature changes. Transferring tubes between freezers may result in a loss of efficiency.

Guaranteed Efficiency
$1.0 \times 10^9$ cfu/µg control plasmid DNA

Test Conditions
Transformations are performed both with and without control plasmid DNA using the protocol outlined below. Following transformation, samples of the culture are plated on LB agar plates with 15 µg/ml tetracycline. The plates are incubated at 37°C overnight and the efficiency is calculated based on the average number of colonies per plate.

Electroporation Protocol
1. Pre-chill sterile electroporation cuvettes (0.2-cm gap) and sterile 1.5 ml microcentrifuge tubes thoroughly on ice.
2. Set the Bio-Rad electroporator to 200 ohms resistance, 25 µF capacitance, and field strength of 2.5 kV/cm. For best results, chill the electroporator or perform the electroporation in a cold room.
3. Thaw the cells on ice for approximately 10 minutes.
4. Add the DNA to be transformed to the pre-chilled microcentrifuge tubes. For optimal efficiency, add 2 µl of plasmid DNA (100 pg/µl in a low ionic strength buffer or water).
5. When cells are thawed, gently mix the cells and transfer 50 µl of cells to the DNA-containing microcentrifuge tubes and mix gently.
6. Transfer the cell-DNA mixture to a chilled electroporation cuvette, tapping the cuvette until the mixture settles evenly to the bottom.
7. Wipe excess moisture from the cuvette, then slide the cuvette into the electroporation chamber until the cuvette sits flush against the electrical contacts.
8. Pulse the sample once, then quickly remove the cuvette. Immediately add 850 µl of SOC medium (held at 37°C), and pipette up-and-down twice to resuspend the cells.
9. Transfer the cells to a sterile 14 ml BD Falcon polypropylene round-bottom tube (BD Biosciences Catalog #352059). Incubate the tube at 37°C for 1 hour with shaking at 225-250 rpm.
10. Plate the transformation mixture on LB agar plates containing the appropriate antibiotic. For the control transformation, plate the transformation mixture on LB-tetracycline agar plates.
**Preparation of Media and Reagents**

**SOB Medium (per Liter)**
- 20.0 g of tryptone
- 5.0 g of yeast extract
- 0.5 g of NaCl

Add dH₂O to a final volume of 1 liter and then autoclave.

Add 10 ml of filter-sterilized 1 M MgCl₂ and 10 ml of filter-sterilized 1 M MgSO₄ prior to use.

**SOC Medium (per 100 ml)**
- Prepare immediately before use
- 2 ml of filter-sterilized 20% (w/v) glucose or 1 ml of filter-sterilized 2 M glucose

SOB medium (autoclaved) to a final volume of 100 ml.

**LB Agar (per Liter)**
- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract
- 20 g of agar

Add deionized H₂O to a final volume of 1 liter.

Adjust pH to 7.0 with 5 N NaOH and then autoclave.

Pour into petri dishes (~25 ml/100-mm plate).

**LB-Tetracycline Agar (per Liter)**
- 1 liter of LB agar, autoclaved and cooled to 55°C
- Add 1.2 ml of 12.5 mg/ml filter-sterilized tetracycline

Pour into petri dishes (~25 ml/100-mm plate).