TG1 Electrocompetent Cell Preparation For Phage Display Library Construction

Materials

- TG1 *E. coli* strain
- SOB medium (see preparation below)
- LB agar plates (see preparation below)
- Sterile 10% glycerol
- Sterile ddH_2O
- Sterile centrifuge bottles and microfuge tubes
- Electroporation cuvettes (0.1 cm gap)
- Electroporator
- Shaker incubator (37°C)
- Ice bucket

Protocol Steps

Day 1: Starter Culture

1. Streak TG1 cells on LB agar plate.
2. Incubate overnight at 37°C.
3. Inoculate 10 mL SOB medium with a single colony.
4. Grow overnight at 37°C, 200–250 rpm.

Day 2: Bulk Culture

1. Inoculate 1 L SOB with 10 mL overnight culture (1:100 dilutions).
2. Grow at 37°C, 200 rpm until OD600 = **0.4–0.5**.
3. Chill culture on ice for 15–30 minutes.

Cell Harvesting

1. Centrifuge at 4°C, 3000 × g for 10 min.
2. Discard supernatant, resuspend pellet in ice-cold 10% glycerol.
3. Repeat wash **3 times** with 10% glycerol.

4. Final resuspension: ~1 mL per 100 mL original culture.

Aliquot and Storage

1. Aliquot 50 µL into sterile microfuge tubes.
2. Snap-freeze in liquid nitrogen.
3. Store at –80°C.

Electroporation Conditions

- DNA: 10 pg control plasmid (e.g., pUC19)
- Voltage: 1.8 kV
- Capacitance: 25 µF
- Resistance: 200 Ω
- Time constant: ~5 ms
- Recovery: Add 950 µL SOC, incubate 1 hr at 37°C

Expected Efficiency

- **1-4 × 10^{10} cfu/µg DNA** with optimal conditions.

Media Preparation

LB Agar Plates

Ingredients:

- Tryptone: 10 g
- Yeast extract: 5 g
- NaCl: 10 g
- Agar: 15 g
- Distilled water: 1 L

Preparation:

1. Combine tryptone, yeast extract, NaCl, and agar in 1 L distilled water.
2. Heat with stirring until agar dissolves completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to ~50°C, pour into sterile petri dishes.
5. Allow to solidify at room temperature.
6. Store plates at 4°C, wrapped to prevent drying.

SOB Medium

Ingredients:

- Tryptone: 20 g
- Yeast extract: 5 g
- NaCl: 0.5 g
- KCl: 0.186 g
- $MgCl_2 \cdot 2H_2O$: 10 mL of 1 M stock (final 10 mM)
- $MgSO_4 \cdot 2H_2O$: 10 mL of 1 M stock (final 10 mM)
- Distilled water: 1 L

Preparation:

1. Dissolve tryptone, yeast extract, NaCl, and KCl in ~900 mL distilled water.
2. Adjust volume to 1 L with distilled water.
3. Autoclave at 121°C for 15 minutes.
4. Cool to room temperature.
5. Add sterile $MgCl_2$ and $MgSO_4$ stock solutions.
6. Mix well before use.