Plasmid library amplification

1. Measure the concentration of purified DNA plasmid. Requirements: concentration ~ 80 ng/µL, 260/280: 1.8-2.0, 260/230: 1.8-2.0

2. For each sample to be electroporated: Thaw the E.coli HST08 Premium Electro-Cells (Takara) on ice, chill 0.1 cm electroporation cuvettes and FALCON 14mL Polypropylene Round-Bottom Tubes on ice, and thaw 1 ml of SOC at room temperature. 5 electroporation samples will be enough to amplify this library.

3. Add 1 µl purified products to 50 µl Electro-Cells, before transferring to pre-chill 0.1 cm cuvettes, let the mixture stand on ice for about 2 min. It is important to avoid giving birth to air bubbles before electroporation by tapping the suspension to the bottom.

4. Set up the procedure of electroporation on Gene Pulser Xcell (Bio-Rad): Voltage 1.5 kv, Resistance 200 Ω, Capacitance 25 μF, Cuvette 1 mm.

5. Place the cuvette in the ShockPod, then push the chamber lid down to close.

6. Pulse once. Check and record the pulse parameters. The time constant should be close to 5 milliseconds.

7. Remove the cuvette from the chamber and immediately add 1 ml SOC medium to the cuvette. Quickly and gently resuspend the cells with by pipetting. (The period between applying the pulse and transferring the cells to growth medium is crucial for recovering E. coli transformants (Dower et al., 1988)

8. Transfer the cell suspension to a pre-chill FALCON 14mL Polypropylene Round-Bottom Tube and incubate at 37°C for 1 hour shaking at 225 rpm.

9. Choose at least 1 tubes to measure the transformation efficiency: pick up 1 µl suspension and dilute into 200 µl liquid LB, then inoculate on LB plates with ampicillin. The number of reactions depends on the coverage compared with library size.

10. Mix all cell suspension together and incubate in 200 mL liquid LB overnight, 37°C, 220 rpm.