# Protocol: Amplification of pDNA libraries

MATERIALS

• 100 μL electrocompetent cells (STBL4TM, Thermo Fisher Scientific, 11635-018)

• 400 ng library plasmid DNA

• 4 electroporation cuvettes (0.1 cm gap, Bio-Rad, 165-2089)

• 10 mL SOC (1X SOC, New England BioLabs, B9020S)

• 4 bioassay plates (500 cm2, LB agar + antibiotic)

• 2 Maxi-preps (Qiagen HiSpeed Maxi, 12663)

• Biospreader (Bacti Cell Spreader, VWR International, 60828-684)

• Electroporator (MicroPulserTM, Bio-Rad, 1652100)

PROTOCOL

Day 1 (afternoon)

1. Add 400 ng pDNA to 100 μL electrocompetent cells.

2. Add 25 μL of cells to cuvette, electroporate using the Ec1 setting (1.8 kV), immediately add 1 mL prewarmed SOC and transfer to round-bottom 14 mL tube.

3. Repeat 3 more times, and add up to 10 mL SOC.

4. Distribute 5 mL to each of two 14 mL tubes; shake for 1 hour at 30°C.

5. Pre-warm bioassays.

6. Plate 2.5 mL of cells on each of the 4 bioassays. Distribute evenly with biospreader or glass beads.

7. Incubate at 30°C for 16 – 18h (see note).

Day 2 (morning)

1. After 16 - 18h of growth, use biospreader to scrape plates with cold LB (generally 20 mL per plate) into 50 mL conical tube, two plates per tube for a total of two tubes. Keep tubes on ice while doing this.

2. Spin down tubes, pour off media, and weigh the pellets. Total weight should be ~1 - 2 g. Each conical is a single Maxiprep.

3. Purify via Maxiprep according to manufacturer’s instructions, with two modifications: a) add P1, P2, P3 directly to the conical and centrifuge to pellet lysed debris before adding to plunger; b) warm elution buffer to 50°C before eluting.

4. Sequence library via Illumina to confirm maintenance of representation. See Illumina PCR protocol for details.

Note: For sgRNA libraries, growth at 37°C for 14 – 16h instead of 30°C is acceptable when using STBL4 cells. Many other competent cells, though, will show noticeably higher recombination rates at 37°C.