BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

Protocol: Amplification of pDNA libraries

MATERIALS

- 100 µL electrocompetent cells (STBL4™, 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 cm^2, LB agar + antibiotic)
- 2 Maxi-preps (Qiagen HiSpeed Maxi, 12663)
- Biospreader (Bacti Cell Spreader, VWR International, 60828-684)
- Electroporator (MicroPulser™, 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 pre-warmed 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.