BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

Last reviewed: February 2016

Protocol: Amplification of pDNA libraries

MATERIALS

- 100 µL electrocompetent cells (STBL4™, Thermo Fisher Scientific Cat# 11635-018)
- 400 ng library plasmid DNA
- 4 electroporation cuvettes (0.1 cm gap, Bio-Rad Cat#165-2089)
- 10 mL SOC (1X SOC, New England BioLabs Cat# B9020S)
- 4 bioassay plates (500 cm^2, LB agar + antibiotic)
- 2 Maxi-preps (Qiagen HiSpeed Maxi Cat# 12663)
- Biospreader (Bacti Cell Spreader, VWR International Cat# 60828-684)
- Electroporator (MicroPulser™, Bio-Rad Cat# 1652100)

PROTOCOL

Day 1 (afternoon)

1. Add 400 ng pDNA to 100 µL electrocompetent cells.
2. Add 25 µL of cells to a 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 (else they will spill when shaking), shake for 1 hour at 30˚C.
5. Pre-warm bioassays and Petri dish.
6. After 1 hour, do two 1:300 dilutions of the cells (3 uL to 897 uL LB, then again). Plate 100 uL of the second dilution onto Petri dish.
7. Plate 2.5 mL of cells on each of the 4 bioassays. Distribute evenly with biospreader or glass beads.
8. 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. Count colonies on Petri Dish. Total colony yield = Count x 300 x 300 x 10 ÷ 0.1. This number should be at least 1000x greater than the number of perturbations in the library. It often takes than 16h to visualize all colonies.

5. 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.