BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

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Protocol: Amplification of pDNA libraries

Watch the video protocol on Youtube at https://www.youtube.com/watch?v=piVOIxDfcS8

MATERIALS

- 100 µL electrocompetent cells (STBL4™, Thermo Fisher Scientific Cat# 11635-018)
- 400 ng library plasmid DNA per 20,000 constructs
- 4 electroporation cuvettes (0.1 cm gap, Bio-Rad Cat#165-2089)
- 10 mL SOC (1X SOC, New England BioLabs Cat# B9020S)
- Bioassay plates (500 cm^2, LB agar + antibiotic) from Broad Stock room: "LB100CARB"
    - 3 bioassays required per 20,000 constructs
- Hi-Speed Plasmid Maxi Kit (Qiagen HiSpeed Maxi Cat# 12663)
    - 2 maxi columns required per 20,000 constructs
- Biospreader (Bacti Cell Spreader, VWR International Cat# 60828-684)
- Electroporator (MicroPulser™, Bio-Rad Cat# 1652100)

PROTOCOL

Day 1 (afternoon)

1. Mix 400 ng pDNA with 100 µL Stbl4 electrocompetent cells. If your library is more than 20,000 constructs, mix another 400 ng pDNA with 100 µL cells per additional 20,000 constructs.
2. Add 35 µL of cell mixture to a cuvette, electroporate using the Ec1 setting (1.8 kV), immediately add 500 uL SOC medium and transfer to round-bottom 14 mL tube containing 2.5 mL SOC medium.
3. Repeat 2 more times for each 20,000 constructs. If library is more than 20,000 constructs, electroporate 3 more cuvettes for each additional 20,000 constructs.
4. 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 3 mL of cells on each of the 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 30 mL per plate) into 50 mL conical tube, three plates per tube. Keep tubes on ice while doing this. If the library is more than 20,000 constructs, scrape all plates together into a large flask, mix, then divide between several 50 mL tubes.
2. Spin down tubes, pour off media, and weigh the pellets. Total weight should be ~1 - 2 g. Each conical is a two maxi columns.
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.