Liquid Culture Library Plasmid Re-amp Protocol

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April 3 2017

1) Thaw Lucigen Endura ElectroCompetent cells (Lucigen cat # 60242-2) on ice for 15 minutes and pre-chill 0.1 cm electroporation cuvettes (USA Scientific cat # 9104-1050) on ice.

2) Combine 1 µL of 25 ng/µL library plasmid DNA with ~50 µL Lucigen cells (1 vial) and flick to mix. Incubate on ice for 30 mins.

3) Transfer to pre chilled cuvette (without creating bubbles!)

4) Electroporation: Using Bio-Rad Gene Pulser Xcell (room L310) electroporate at 1.8 kV, 600 Ω , 10 uF in 0.1 cm cuvette

5) Immediately add 2 mL (1 mL at a time) Lucigen recovery media to electroplated cells and transfer into a 14 mL round bottom culture tube

6) Shake at 37° C for 2 hours at 225 rpm

7) Prepare 2-3 dilution LB-carb plates to check transformation efficiency (Usually 5,000-100,000x dilution of recovery volume) and use the rest of recovery to inoculate 0.5 L LB-carb media in a 2L flask

8) Shake at 37° C for 16 hours at 225 rpm

9) Check transformation efficiency from dilution plates, if coverage is at least 30x can proceed to Maxi Prep. (For example, a library of ~15,000 elements should have at least ~10 colonies in a 50,000x dilution plate [coverage = # colonies * dilution factor / # elements] , however it is not unusual to get much higher colony counts (50-100+) in a 50,000x dilution plate)

Spin down culture at 6084 x g for 20 min at 4° C and maxi prep pellet using Qiagen HiSpeed Plasmid Maxi Kit

Note: liquid culture re-amplification of libraries has been verified to have no diversity loss compared to LB agar plates for libraries up to ~20,000 elements.