Library Amplification

1. Thaw one aliquot of 100 µl ElectroMax DH10B electrocompetent E. coli (ThermoFisher 18290015) on wet ice and mix by tapping the tube.
2. Chill four microfuge tubes and four 0.1 cm electroporation cuvettes on ice.
3. Place 1.0 µl containing 10 - 50 ng library DNA in chilled microfuge tubes.
4. Pipette 20. µl electrocompetent cells to each chilled tube containing DNA.
5. Electroporate each transformation:
   a. Transfer each cell/DNA mixture to an electroporation cuvette.
   b. Electroporate at 2.0 kV, 200 Ω, 25 µF.
   c. Immediately add 1.0 ml LB to each cuvette.
6. Inoculate 500 ml LB carbenicillin with transformed cells.
7. Check transformation efficiency:
   a. Withdraw 200 µl of transformation.
   b. Prepare three 10-fold serial dilutions using 20 µl plus 180 µl LB.
   c. Plate 100 µl each of the undiluted culture and serial dilutions on LB Carb plates.
   d. Incubate overnight at 37 ºC.
8. Grow cells overnight at room temperature and transfer the next day to 37 ºC, with shaking.
9. Verify coverage of 100 transformants per guide at minimum:
   For 60k guides this requires 6M transformants, i.e. 120 colonies on the 1:10 dilution plate.
10. Harvest bacteria at OD660 between 2.0 and 4.0.
11. Purify DNA from cells using a QIAGEN Plasmid Midi Kit (QIAGEN 12143).