CRISPR library re-amplification

1) Mix: 100 ng library + 75 uL DH5α (3x10^8 cfu/ug)

2) Heatshock 30s, 42°C

3) Recover for 30 min in 5 mL, LB+Carb

4) Take 5ul of recovery. Make serial dilutions and plate with beads to calculate transformation efficiency. Calculate transformation efficiency next day. If the efficiency is higher than 1000 colonies per sgRNA construct in the library, harvest cells and purify the library.

5) Add recovery to 500 mL LB+Carb. Grow O/N while shaking @ 37°C (16h)

6) Pellet Cells

7) Depending on your pellet weight use multiple Maxiprep columns (Sigma or Zymogen), a Megaprep (Qiagen or Sigma), or Gigaprep (Sigma, Zymogen).

8) Expect a yield ~2-3 mg

* We strongly recommend deep sequencing the amplified libraries before use.