

Liquid Culture Re-Amplification Protocol by Chong Park

For sublibrary amplification:

1. Mix: 10-50 ng of DNA + 100 uL DH5 α high efficiency (3×10^9 cfu/ug) (1 rxn per sublibrary)
2. Transform using the manufacturer's suggested protocol, making sure to perform the full 30min ice-incubation.
3. Recover in 1 mL total with SOC for 1 hour while shaking at 37C.
4.
 - a. Take 5ul of recovery. Make serial dilutions and plate them onto LB/Amp plate to calculate transformation efficiency.
 - b. Add the rest of recovery to 500 mL LB+Carb. Grow O/N while shaking @ 37°C (16h)
5. Calculate transformation efficiency next day. If the efficiency is higher than 100~200 colonies per GBC construct in the library, harvest cells and purify the library.
6. Depending on your pellet weight use multiple Maxiprep columns (Sigma or Zymogen), a Megaprep (Qiagen or Sigma), or Gigaprep (Qiagen, Sigma, Zymogen).
7. Expect a yield ~2mg.