Protocols for plasmid library amplification

*2 days before electroporation:*

Streak cloning strain onto LB plate (no antibiotic)

*1 day before electroporation:*

Pick colonies of cloning strain in 5mL LB

*Day of electroporation:*

* Inoculate 200uL o/n culture in 20mL LB without antibiotic. Grow at 37C
* When OD=~0.4, spin down cells at 8,000 rpm for >1 min. Decant supernatant.
* Wash cells with 20mL ice cold sterile diH2O
* Repeat spin and wash steps
* Resuspend cells in <1mL water and transfer to a 1.5mL tube
* Spin cells an additional 1 min at 8,000 rpm. Decant supernatant.
* Resuspend cells in 100uL sterile ice-cold diH2O. This is enough for two 50uL reactions.
* Add 1uL of 100ng/uL plasmid library
* In 1mM electrocuvette, transfer cells with DNA into gap. Pulse at 1800 V, 200 Ohm, 25 mF
* Recover in 1.2mL SOC media (LB if necessary) in 15mL pop-cap tube for 1-1.5h at 37C 230rpm
* Plate serial dilutions on LB+ampicillin (100ng/mL) plates (generally, 50uL of 1/10000 and 1/100000 dilutions are sufficient) in order to calculate transformation efficiency. Ideally, ensure that >10,000,000 cfu are obtained.
* Inoculate 1mL recovered cells in 20-50mL LB+ampicillin (100ng/mL) and grow o/n at 37C 230rpm. Mini-prep DNA libraries the next day.

*Note: this protocol can be used for transformation of DNA library into a protein expression strains as well, with the only difference being that the recovered cells grown o/n are then used a pre-culture for protein expression at the desired culture volume.*

*Also note that each re-transformation and preparation of the plasmid library may result in a loss of library diversity from the original preparation.*