

Electroporation with NEB 10-betas:

- place two 0.1cm gap cuvettes, two eppie tubes on ice
- prewarm six LBamp plates 37C. (Note, we use "low salt" LB plates, 5g NaCl/L.)
- prewarm the NEB10beta "Stable Outgrowth Medium" to 37C. Need ~500uL per reaction.
- Dilute lib1 maxi prep stock to 5 ng/uL in molecular grade water, as well as a ctrl plasmid (wildtype SARS-CoV-2 RBD yeast display plasmid)
- Thaw one aliquot NEB10-beta electrocomps (need 20uL per electroporation, each tube is 100uL). Keep the cells as chilled as possible throughout the protocol, and don't do anything too vigorous with pipetting etc.
- add 1uL of diluted DNA to each tube
- add 20uL of electrocompetent cells (NEB10beta)
- transfer to cuvette between electroporation plates, avoiding bubbles
- shock cells 1.8kV (time constant should be >5ms)
- *immediately* add 480uL pre-warmed SOM, use the volume to gently wash the cells out from between plates and transfer to tube.
- incubate 37°C 1hr recovery in shaker incubator
- Make 10^{-2} - 10^{-4} dilutions in LB, plate 100uL of the 10^{-2} , 10^{-3} , 10^{-4} dilutions onto LB+amp, incubate O/N

Yields from 5ng plasmid transformations:

Electroporation yields:

- library: 140 cfu on 10^{-4} plate, estimated $7e6$ total transformants
- ctrl: 204 cfu on 10^{-4} plate, estimated $1e7$ total transformants