***Electroporation transformation protocol used for ClonTracer library construction***

*(This is the protocol I used for the ClonTracer library construction. Please note that here I was using purified/concentrated ligation reactions for transformation, not a plasmid DNA pool.)*

1) 2 ul purified/concentrated ligation product was used per 22 ul MegaX DH10B™ T1R Electrocomp™ Cells (Life Technologies).  Add 2 ul ligation products\* in each microcentrifuge tube (5 tubes in total\*\*)

2) Thaw MegaX DH10B T1 electrocomp cells on ice and mix by tapping gently.

3) Add 22 ul MegaX cells into each pre-chilled tube containing ligation products.

4) Pipette the cell/DNA mixture into a chilled 0.1-cm cuvette. Use extreme caution to prevent air bubbles.

5) Electroporate at 2.0 kV, 200 Ω, 25 uF.

6) Add 1 ml Recovery Medium to each cuvette, collect and combine the mixtures to a 50 ml conical tube (~5 ml in total)

7)  Incubate transformed cells in recovery medium at 37 degrees C with shaking at 225 rpm for 1h.

8) After 1 h recovery, ~5 ml recovered culture was inoculated into 500 ml LB medium containing 100ug/ml calbenicillin. (LB agar plating step was skipped.)  Incubated at 37 degrees for 16.5 h\*\* with 175 rpm shaking.

9)  Maxiprep on the next day using four QIAGEN-tip 500 columns.

\*Since I used ligation reactions for transformation, it would be different for using plasmid DNA. Based on the manufacturer’s protocol, I think 1ul of 100 ng/ul plasmid stock per 22 ul competent cells would be reasonable. Keep in mind that 1ug ClonTracer plasmid stock (7230 bp) would contain approx. 1.3\*10^11 molecules.

\*\*If possible, try to increase the number of cuvettes/tubes to do electroporation in parallel and decrease the incubation time. It will help to maintain the barcode complexity as close as possible to the original stock.