**Library Transformation Protocol (perform independently for each subpool)**

1. Warm Recovery Medium in 37°C water bath for 15 minutes.
2. Warm 2 LB-ampicillin agar plate in a 37°C incubator for 30 minutes.
3. Thaw 1 vial of Endura Electrocompetent Cells and aliquot cells into 2 tubes on ice for 15 minutes
4. Place two MicroPulser Cuvettes on ice.
5. For each pool:
   1. Add 1 µL of plasmid to bacterial cells
   2. Transfer 25 µL of the bacterial cell/Gibson Assembly reaction product mixture into MicroPulser Cuvettes
   3. Place cuvette into electroporator and electroporate at 1.8 kV
   4. Quickly add 975 µL of the Recovery Medium into the cuvette and pipet up and down three times to re-suspend the cells and transfer mixture to a 1.5 mL Eppendorf tube.
   5. Place the tube in a shaking incubator for 1 hour at 37°C.
   6. Serially dilute 10 µL of the transformation mixture in Recovery Medium 4 times, using a dilution factor of 1:10 at each step.
   7. Spot 10 µL of each dilution onto an LB-ampicillin plate.
   8. Incubate plate at 30°C overnight.

Note: The number of colonies on these spots can be multiplied by 103, 104, 105, and 105, respectively, to estimate the total number of colony-forming units.

1. For the remainder of the transformation
2. Seed into a 500 mL Erlenmeyer flask containing 100 mL LB liquid media with 100 µg/mL ampicillin.
3. Incubate culture at 30°C overnight.
4. If the transformation efficiency, as assessed by the serial plating, exceeds 20-fold of the library size then prepare plasmid DNA from the bacterial culture using the Qiagen Plasmid Plus Maxi Kit according to the manufacturer’s instructions.
5. To assess recombination, run out the amplified plasmid on an ethidium bromide-stained 1% agarose gel. Visualize the plasmid DNA using a standard gel imager.