Gibson Assembly protocol & transformation

1. PCR products with overlaps. Gel check and extract. Confirm 260/280 > 1.8, 260/230 > 2.
2. Choose a mole ratio so the selectable marker (typically antibiotic resistance or amino acid) is in the lowest proportion. Calculate pmoles by pmol=(ng*1000)/(bp*650). Typically use ~10-15 ng of selectable marker, 2:1 mole ratio insert : selectable marker or 2:2:1 if doing 3 piece Gibson.
    a. Approx example: insert=3461 bp, half backbone=2093 bp, half backbone with selectable marker=2217 bp
        i. Insert=40 ng (.0178 pmol), half backbone=30 ng (.022 pmol), selectable=15 ng (.0104 pmol).
3. Dilute products in PCR tube and add so that products + additional water =2.5 uL. Add 7.5 uL Gibson Master mix.
4. Run in Thermocycler 50˚C for 60 minutes.
5. Add to TOP10 (I use 5 uL).
6. Continue with transformation protocol.
7. Colony PCR transformed cells with primers targeting both in and outside of the insert(s) to confirm correct assembly.
    a. Colony PCR—add ~35 uL sterile water to PCR tube. Pick colony, swirl cells/pipet up and down. Add 15 uL to LB for culturing, remainder can be PCR'ed with 2 uL water/cell mix as template.