

### 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  $\text{pmol} = (\text{ng} * 1000) / (\text{bp} * 650)$ . 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.