Gibson Cloning Protocol

1. Design primers and/or gBlocks for both vector and insert. Primers and/or gBlocks should be designed such that the “vector” and “insert” pieces contain 20 bp overlap at the 3’ and 5’ ends.

2. PCR both vector and/or insert using the primers designed above
   - a. Check melting temperature of primer pair at: http://www.thermoscientificbio.com/webtools/tmc/
      - For primers >20 nt, use an annealing temp that is 3°C above the lower Tm of the primer pair.
      - For primers <20 nt, use an annealing temp equal to the lower Tm of the primer pair.
   - b. 30uL reaction
      - i. 1uL template (10 ng/uL)
      - ii. 1.5uL of each primer (10uM stock)
      - iii. 11uL water
      - iv. 15uL 2X Phusion master mix (NEB F-531)
   - c. Cycle for 30 cycles
      - i. 98ºC for 30 sec
      - ii. 98ºC for 10 sec
      - iii. 55-65ºC for 20 sec
      - iv. 72ºC for 15-30s/kb (30 sec for insert, 2-3 min for vector)
      - v. 72ºC for 10 min

3. DpnI digest PCR if it used intact plasmid as template
   - a. 1 uL DpnI
   - b. DpnI has full activity in Phusion buffer
   - c. Incubate for 15-60 minutes at 37ºC

4. PCR cleanup both vector and insert (gel purify if necessary)

5. Nanodrop vector and insert

6. If using a gBlock for your insert, dilute to 10 ng/uL with water.

7. Calculate the pmol/uL concentration for vector and insert(s)
   - a. \[
   \text{pmols/uL} = \left(\text{Concentration}_{\text{ng/ul}} \times 1000\right) \times \frac{\text{base pairs}}{650 \text{ Da}}
   \]

8. Gibson Reaction: 20uL volume
   - a. ~0.03 pmol vector (~100 ng for 5kb vector)
   - b. ~0.06 pmol insert, 2-fold molar excess (~20 ng for 500 bp insert)
   - c. Water
   - d. 10uL Gibson Master Mix (2X, NEB E5510)

9. Note: for reactions containing multiple inserts, each should be added at ~0.06 pmol.

10. Incubate at 50°C for 60 minutes

11. Transform into E. coli (XL10 Gold, DH5a, TOP10, etc.) using 2uL of assembly product