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 3ºC 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. $\frac{pmols}{uL} = \frac{\left(Concentration\frac{ng}{uL}\right)*1000}{base\ pairs * 650\ Da}$
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 50ºC for 60 minutes
11. Transform into E. coli (XL10 Gold, DH5a, TOP10, etc.) using 2uL of assembly product