Protocols:

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Title: Gibson Cloning (from Dr. Eric Domyan)
Rationale and background: To ligate insert and vector fragments for high efficiency plasmid transformations.
Protocol:
I: Quick Protocol
1. Thaw mix on ice.
2. Split into two tubes (7.5ul each)
3. Add 2.5ul total DNA (target 1:1:1... molar ratios but this is not critical)
4. Incubate at 50C for 1hr
5. Transform 2 ul in 50-100 ul of cells.
*If you want to chew up methylated background DNA add .5 ul DpnI and Incubate at 37 for 15 -30 min
II: Gibson reaction from set up:
1. Prepare 5X ISO buffer. Six ml of this buffer can be prepared by combining the following:
  - 3 ml of 1 M Tris-HCl pH 7.5
  - 150 µl of 2 M $MgCl_2$
  - 60 µl of 100 mM dGTP
  - 60 µl of 100 mM dATP
  - 60 µl of 100 mM dTTP
  - 60 µl of 100 mM dCTP
  - 300 µl of 1 M DTT
  - 1.5 g PEG-8000
  - 300 µl of 100 mM NAD
  - Add water to 6 ml
2. Aliquot 100 µl and store at -20 °C
3. Prepare an assembly master mixture. This can be prepared by combining the following:
  - 320 µl 5X ISO buffer
  - 0.64 µl of 10 U/ µl T5 exonuclease
  - 20 µl of 2 U/µl Phusion polymerase
  - 160 µl of 40 U/µl Taq ligase
  - Add water to 1.2 ml
4. Aliquot 15 µl and store at -20 °C. This assembly mixture can be stored at -20 °C for at least one year. The enzymes remain active following at least 10 freeze-thaw cycles.
This is ideal for the assembly of DNA molecules with 20-150 bp overlaps. For DNA molecules overlapping by larger than 150 bp, prepare the assembly mixture by using 3.2 µl of 10 U/ µl T5 exo.
5. Thaw a 15 µl assembly mixture aliquot and keep on ice until ready to be used.
6. Add 5 µl of DNA to be assembled to the master mixture. The DNA should be in equimolar amounts. Use 10-100 ng of each ~6 kb DNA fragment. For larger DNA segments, increasingly proportionate amounts of DNA should be added (e.g. 250 ng of each 150 kb DNA segment).
7. Incubate at 50 °C for 15 to 60 min (60 min is optimal).
8. If cloning is desired, transform 2 µl of the reaction mixture into 50-100 µl competent cells (DH5α) by heat shock