Gibson assembly protocol

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Materials needed
5X isothermal (ISO) buffer (1M Tris-HCl, 2M MgCl, dNTP premix or 100mM of dGTP, dATP, dTTP and dCTP, 1M DTT, PEG-8000, 100mM NAD)
T5 exonuclease (10U/μl, NEB or Epicentre)
Phusion DNA polymerase (2U/μl, NEB)
Taq DNA ligase (40U/μl, NEB or mclab)

Recipe for 5x ISO buffer

- 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
Aliquot 330 µl and store at -20 °C.

Master mixture for Gibson Assembly

	1 batch (~80 reactions)	1/2 batch (~40 reactions)
5X ISO buffer	320 μl	160 μl
T5 exonuclease (10U/μl)	0.64 μl	0.32 μl
Phusion DNA polymerase	20 μl	10 μl
Taq DNA ligase	160 μl	80 μl
H_2O	699.36 μl	349.68 μl
Total volume	1200 μl	600 μl

Aliquot 15 μl of the Master mix in individual PCR tubes and store at -20 °C.

Reaction procedure

Calculate the required amount of DNA required to have an equimolar ratio between the DNA fragments to be assembled. The total volume should not exceed 5 μl for the reactions prepared in this protocol.
The amount of total DNA added to the reaction should be around 20-200ng total.

1. Mix the DNA fragments to be assembled in a PCR tube and add PCR-grade H_2O to a final volume of 5 μl.
2. Thaw 1 vial per reaction of the Gibson assembly master mixture.
3. Immediately after thawing, transfer the 15 μl of Gibson assembly master mixture to the PCR tube containing the DNA fragments and mix the solution of a few times by pipetting up and down 2-3 times. Close the PCR cap or place lid on PCR tube.
4. Place the reaction mixture in a thermal cycler at 50°C for 30 min..
   (Note: The more fragments that needs to be joined together, the longer the reaction should run. For a standard 2 fragment assembly, I have success with 30 min.)
5. Remove sample from thermal cycler.

6. Thaw 50 μl of electrocompetent *E.coli* DH5α (or another suitable cloning strain) and add 1-2 μl of the Gibson assembly mixture.

Note: The Gibson assembly mixture is high on salts and can make the cells arch during electroporation. I routinely remove the salts and reaction enzymes using a minElute kit from Qiagen. The clean up can remove a portion of the DNA but the transformation is still efficient. I have transform with as little as 3-4ng of DNA/μl and still gotten decent transformations.

7. Following transformation, allow the cells to recover in 0.5ml LB or SOC at 37C and then plate on selective LB agar plates.