Gibson assembly

Assembly Protocol:

1. Set up the following reaction on ice:

Recommended Amount of Fragments Used for Assembly			
	2–3 Fragment Assembly	4–6 Fragment Assembly	Positive Control**
Total Amount of Fragments	0.02–0.2 pmols*X µl	0.2–1 pmols*X µl	10 µl
Gibson Assembly Master Mix (2X)	10 µl	10 µl	10 µl
Deionized H2O	10-X µl	10-X µl	0
Total Volume	20 µl***	20 µl***	20 µl

* 50 ng of 5,000 bp dsDNA is about 0.015 pmols.

50 ng of 500 bp is about 0.15 pmols.

Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts.

Use 5 times more of inserts if size is less than 200 bps.

** Control reagents are provided for two experiments.

*** If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.

2. Incubate samples in a thermocycler at 50°C for 60 minutes. Following incubation, store samples on ice or at –20°C for subsequent transformation.

3. Remove 2 µl of the assembly product and transform into competent cells of interest.

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Transformation Protocol

1. Thaw competent cells on ice.

2. Transfer 50 µl of competent cells to a 1.5 ml microcentrifuge tube.

3. Add 2 µl of the chilled assembly product to competent cells. Mix gently by pipetting up and down or flicking the tube 4–5 times. Do not vortex.

4. Place the mixture on ice for 30 minutes. Do not mix.

5. Heat shock at 42°C for 30 seconds*. Do not mix.

6. Add 950 µl of room temperature SOC media* to the tube.

7. Place the tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.

8. Warm selection plates to 37°C.

9. Spread 100 µl of the cells onto the plates.

10. Incubate overnight at 37°C.

* Please note: Follow the manufacturer's protocols for the duration and temperature of the heat shock step, as well as the optimal medium for recovery. Transformation of our positive control assembly product will yield more than 100 colonies on an Amp plate with greater than 90% colonies containing inserts.