Gibson assembly protocol

1. Measure the DNA concentration (ng/ µl) of each assembly piece.
2. Put the 15 µl Gibson mix on ice.
3. Transfer units to pmol/µl by the following formula:

$$DNA\,Con'_{\left[\frac{Pmol}{\mu l}\right]} = \frac{DNA\,Con'_{\left[\frac{ng}{\mu l}\right]} X 1000}{Part\ size\ X\ 650}$$

4. 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)	15 ~~10~~ µl	15 ~~10~~ µl	15 ~~10~~ µl
Deionized H_2O	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.

*Use 0.05 pmol/µl of the plasmid and 0.15 pmol/µl of the insert

5. Incubate samples In a thermocycler at 50 °C for 60 minutes. Following incubation, store samples on ice or at -20 °C for subsequent transformation.
6. Remove 2-5 µl of the assembly product and transform into competent cells of interest.