Gibson Assembly® Protocol (E5510)

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	0.02–0.5 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

* 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. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.
** Control reagents are provided for 5 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 15 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled. Following incubation, store samples on ice or at –20°C for subsequent transformation.

Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases (for further details see FAQ section).

3. Transform NEB 5-alpha Competent E. coli cells (provided with the kit) with 2 µl of the assembly reaction, following the transformation protocol.

Gibson Assembly® Chemical Transformation Protocol (E5510)

1. Thaw competent cells on ice.

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

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

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

5. Transfer tubes to ice for 2 minutes.

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

7. Incubate 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 selection plates. Use Amp plates for positive control sample.

10. Incubate overnight at 37°C.