Gibson Assembly Method

Gibson Assembly Protocol

1. Set up the following reaction on ice:

	2-3 Fragment assembly	**4-6 Fragment assembly**	**Positive control**
Total amount of fragments	0,02-0,5pmols X µl	0,2-1pmols X µl	10 µl
Gibson assembly min (2X)	10 µl	10 µl	10 µl
Deionized H_2O	10 - X µl	10 - X µl	0 µl
Total volume	**20 µl**	**20 µl**	**20 µl**

2. Incubate samples in a thermocycler at 50°C for 15 minutes when 2-3 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.
3. Transform DH 5-alpha competent E. coli cells with 2 µl of the assembly reaction, following the transformation protocol.

Gibson Assembly Transformation Protocol

1. Thaw chemically 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 vortex.
4. Heat shock at 42°C for 30 seconds. Do not vortex.
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.