

Gibson Assembly® Protocol (E5510)

Protocols.io also provides an [interactive version of this protocol](#) where you can discover and share optimizations with the research community.

Optimal Quantities

NEB recommends a total of 0.02–0.5 pmols of DNA fragments when 1 or 2 fragments are being assembled into a vector and 0.2–1.0 pmols of DNA fragments when 4–6 fragments are being assembled. Efficiency of assembly decreases as the number or length of fragments increases. To calculate the number of pmols of each fragment for optimal assembly, based on fragment length and weight, we recommend using NEB's online tool, [NEBioCalculator](#), or using the following formula:

$$\text{pmols} = (\text{weight in ng}) \times 1,000 / (\text{base pairs} \times 650 \text{ daltons})$$

50 ng of 5000 bp dsDNA is about 0.015 pmols.

50 ng of 500 bp dsDNA is about 0.15 pmols.

The mass of each fragment can be measured using the NanoDrop instrument, absorbance at 260 nm or estimated from agarose gel electrophoresis followed by ethidium bromide staining.

Recommended overlap of fragments: 15-40 (NEB)

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	0.02–0.5 pmols* X µl	0.2–1 pmols* X µl	10 µl
Gibson Assembly Master Mix (2X)	10 µl	10 µl	10 µl

Deionized H ₂ O	10-X µl	10-X µl	0
Total Volume	20 µl***	20 µl***	20 µl

2. * 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.

3. 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).

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