Gibson Assembly® Protocol (E5510)

Materials Required but not Supplied

Gibson Assembly® Cloning Kit

- Recommended DNA polymerase options for PCR
  - Q5® High-Fidelity DNA Polymerase (NEB #M0491)
  - Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493)
  - Q5 Hot Start High-Fidelity 2X Master Mix (NEB #M0494)
- LB (Luria-Bertani) plates with appropriate antibiotic.

Overview

Gibson Assembly efficiently joins multiple overlapping DNA fragments in a single-tube isothermal reaction. The Gibson Assembly Master Mix includes three different enzymatic activities that perform in a single buffer:

- The exonuclease creates single-stranded 3´ overhangs that facilitate the annealing of fragments that share complementarity at one end (overlap region).
- The proprietary DNA polymerase fills in gaps within each annealed fragment.
- The DNA ligase seals nicks in the assembled DNA.

The end result is a double-stranded fully sealed DNA molecule that can serve as template for PCR, RCA or a variety of other molecular biology applications, including direct transformation.

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 pmoles 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:

pmols = (weight in ng) x 1,000 / (base pairs x 650 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.

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_2O	10-X µl	10-X µl	0
Total Volume	20 µl***	20 µl***	20 µl

* Optimized cloning efficiency is 50–100 ng of vector with 2-3 fold molar excess of each insert. Use 5-fold molar excess of any insert(s) less than 200 bp.To achieve optimal assembly efficiency using in 4-6 fragment assemblies, use a 1:1 molar ratio of each insert:vector.Total volume of unpurified PCR fragments in the 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.