Gibson Assembly® Master Mix Assembly Protocol (NEB #E2611)

Materials Required but not Supplied

Gibson Assembly® Master Mix

- Recommended DNA polymerase options for PCR
  - Q5® High-Fidelity DNA Polymerase (NEB #M0491)
  - Q5 Hot Start Flex DNA Polymerase (NEB #M0493)
  - Q5 Hot Start Flex 2X Master Mix (NEB #M0494)
- LB (Luria-Bertani) plates with appropriate antibiotic
- SOC Outgrowth Medium (NEB #B9020)
- Competent cell options for transformation
  - NEB 5-alpha Competent *E. coli* (High Efficiency, NEB #C2987)
  - NEB 10-beta Competent *E. coli* (High Efficiency, NEB #C3019) (Recommended for assembled products greater than 10 kb)
  - NEB 10-beta Electrocompetent *E. coli* (NEB #C3020)
  - NEB® Stable Competent *E. coli* (High Efficiency) (NEB #C3040)

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

Use this protocol with the Gibson Assembly Master Mix to successfully assemble multiple DNA fragments, regardless of end compatibility, in just under two hours.

Optimal Quantities

NEB recommends a total of 0.02–0.5 pmols of DNA fragments when 2 or 3 fragments are being assembled 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*****
Recommended DNA Molar Ratio	vector: insert = 1:2 or 1:3	Vector: insert = 1:1	
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
Nuclease-free Water	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. Total volume of unpurified PCR fragments in the assembly reaction should not exceed 20%. To achieve optimal assembly efficiency, design 15-25 bp overlap regions between each fragment.*

*** To achieve optimal assembly efficiency, design 20-80 bp overlap regions between each fragment with equimolarity of all fragments.*

**** 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.

Notes:

- Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases (for further details see FAQ section).
- If you are working with large plasmids >10 kb in size, we recommend NEB® 10-beta Competent E. coli (High Efficiency) (NEB #C3019H). If your plasmid or insert contain repetitive sequences, we recommend NEB Stable Competent *E. coli* (High Efficiency) (NEB #C3040H).
- For the selection of transformed competent cells, we recommend LB plates with appropriate antibiotic
- For generating PCR Products, we recommend Q5® High-Fidelity DNA Polymerase (NEB #M0491) or related products, such as Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493) or Q5 Hot Start High-Fidelity 2X Master Mix (NEB #M0494).

Resources

- Synthetic Biology/DNA Assembly Selection Chart
- NEBuilder™ Assembly Tool
- NEBioCalculator® Mass to Moles Module