Gibson Assembly® Master Mix – Assembly (E2611)

New England Biolabs

Abstract

This is the protocol for the Gibson Assembly using the Gibson Assembly® Master Mix (E2611).

Citation: New England Biolabs Gibson Assembly® Master Mix – Assembly (E2611). **protocols.io** dx.doi.org/10.17504/protocols.io.cjxupm
Published: 26 Jan 2015

Guidelines

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.

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%.

OVERVEW:

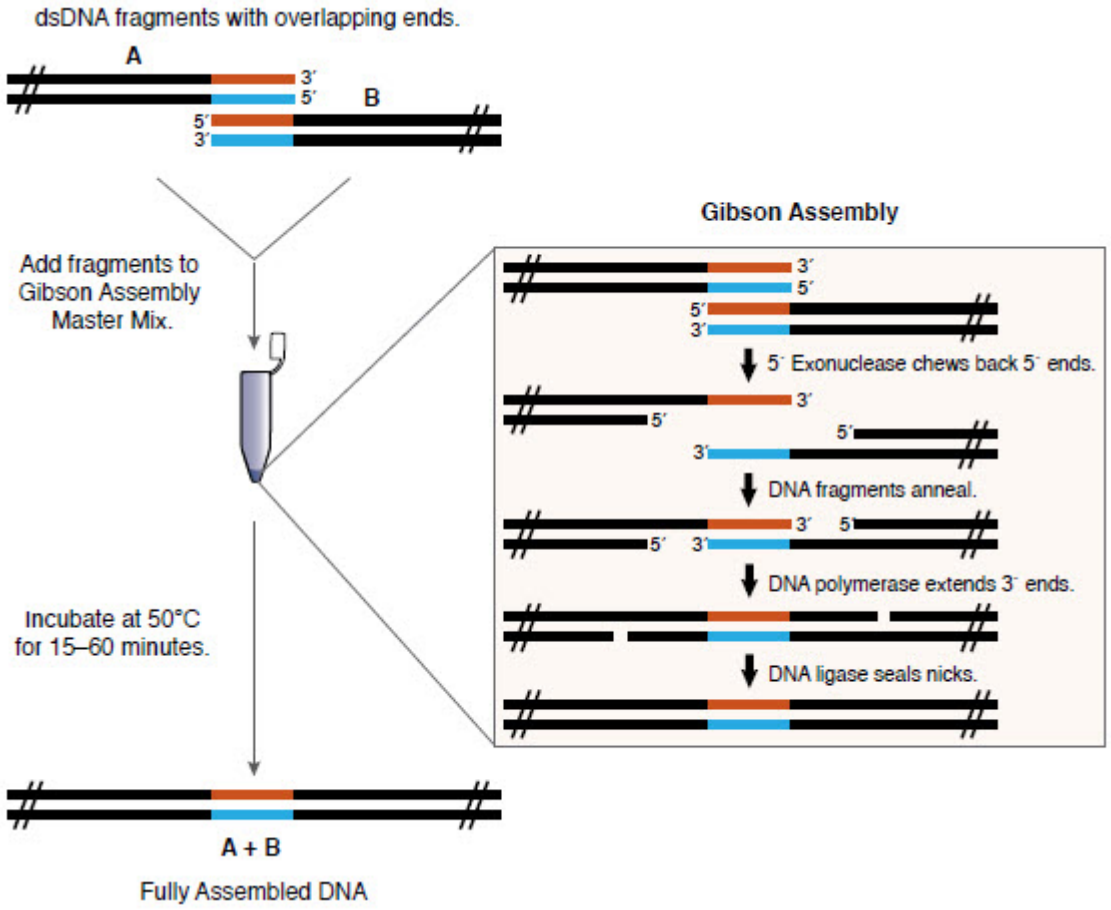
Gibson Assembly was developed by Dr. Daniel Gibson and his colleagues at the J. Craig Venter Institute and licensed to NEB by Synthetic Genomics, Inc. It allows for successful assembly of multiple DNA fragments, regardless of fragment length or end compatibility. It has been rapidly adopted by the synthetic biology community due to its ease-of-use, flexibility and suitability for large DNA constructs.

Gibson Assembly efficiently joins multiple overlapping DNA fragments in a single-tube isothermal reaction (1,2). 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. The method has been successfully used by Gibson's group and others to assemble oligonucleotides, DNA with varied overlaps (15–80 bp) and fragments hundreds of kilobases long (1–2).

Overview of the Gibson Assembly Cloning Method



Overview of Gibson Assembly Master Mix Protocol:

- Design primers to amplify fragments (and/or vector) with appropriate overlaps
- PCR amplify fragments using a high-fidelity DNA polymerase.
- Prepare linearized vector by PCR amplification using a high-fidelity DNA polymerase or by restriction digestion.
- Confirm and determine concentration of fragments and linearized vector using agarose gel electrophoresis, a NanoDrop™ instrument or other method.
- Add fragments and linearized vector to Gibson Assembly Master Mix and incubate at 50°C for 15 minutes to 1 hour, depending on number of fragments being assembled.
- Transform into *E. coli* or use directly in other applications.

NOTES:

1. General Notes:
We highly recommend using our web tool, NEBuilder® to design PCR primers with overlapping sequences between the adjacent DNA fragments and for their assembly into a cloning vector.

2. **Usage notes:**

To ensure the successful assembly and subsequent transformation of assembled DNAs, NEB recommends the following:

- *Cells*: Transformation efficiency of competent cells can vary by several logs. Perceived assembly efficiency directly correlates to the competence of the cells used for transformation.
- *Electroporation*: Electroporation can increase transformation efficiency by several logs. When using the Gibson Assembly Master Mix product for electroporation, it is necessary to dilute the reaction 3-fold and use 1 µl for transformation.
- *DNA*: PCR product purification is not necessary if the total volume of all PCR products in the Gibson Assembly reaction is 20% or less of the Gibson Assembly reaction volume. Higher volumes of PCR products may reduce the efficiency of Gibson Assembly and transformation due to the elevated carryover amounts of PCR reaction buffer and unused primers present in the PCR product. Column purification of PCR products may increase the efficiency of both Gibson Assembly and transformation by 2–10 fold and is highly recommended when performing assemblies of three or more PCR fragments or assembling longer than 5 kb fragments. Purified DNA for assembly can be dissolved in ddH2O (Milli-Q® water or equivalent is preferable), TE or other dilution buffers.
- *Insert*: When directly assembling fragments into a cloning vector, the concentration of assembly fragments should be 2–3 times higher than the concentration of vector. **For assembly of 3 or more fragments, we recommend using equilmolar ratio of fragments.**
- *Biology*: Some DNA structures, including inverted and tandem repeats, are selected against byE. coli. Some recombinant proteins are not well tolerated by E. coli and can result in poor transformation or small colonies.

REFERENCES:
1. Gibson, D.G. et.al. (2009). Nature Methods. 343-345.
2. Gibson, D.G. et al. (2010). Nature Methods. 901-903.
3. Barnes, W.M. (1994). Proc. Natl. Acad. Sci.. 91, 2216-220.

Materials

Gibson Assembly Master Mix - 10 rxns E2611S by New England Biolabs

Protocol

Step 1.
Set up the following reaction on ice:

	2-3 Fragment Assembly	4-6 Fragment Assembly	Positive Control
Total Amount of Fragments	.02-0.5 pmol X ul	0.2-1 pmol X ul	10 ul
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**

PROTOCOL

. Mixture for E5510 Gibson

CONTACT: New England Biolabs

NOTES

New England Biolabs 10 Jul 2014
Control reagents are provided for 5 experiments.
New England Biolabs 10 Jul 2014
If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.
New England Biolabs 26 Jan 2015
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%.
Step 1.1.
DNA fragments

ANNOTATIONS
Dotan Omer 04 Sep 2016

Insert1

Step 1.2.
Gibson Assembly Master Mix (2X)

Step 1.3.
Deionized H2O

Step 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.

NOTES

New England Biolabs 07 Nov 2014
Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases (for further details see FAQ section).

Step 3.
Store samples on ice or at –20°C for subsequent transformation.