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Gibson Assembly® Protocol (E5510) V.2

New England Biolabs[1]

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This protocol explains methods for the Gibson Assembly using the Gibson Assembly® Cloning Kit (E5510).

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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

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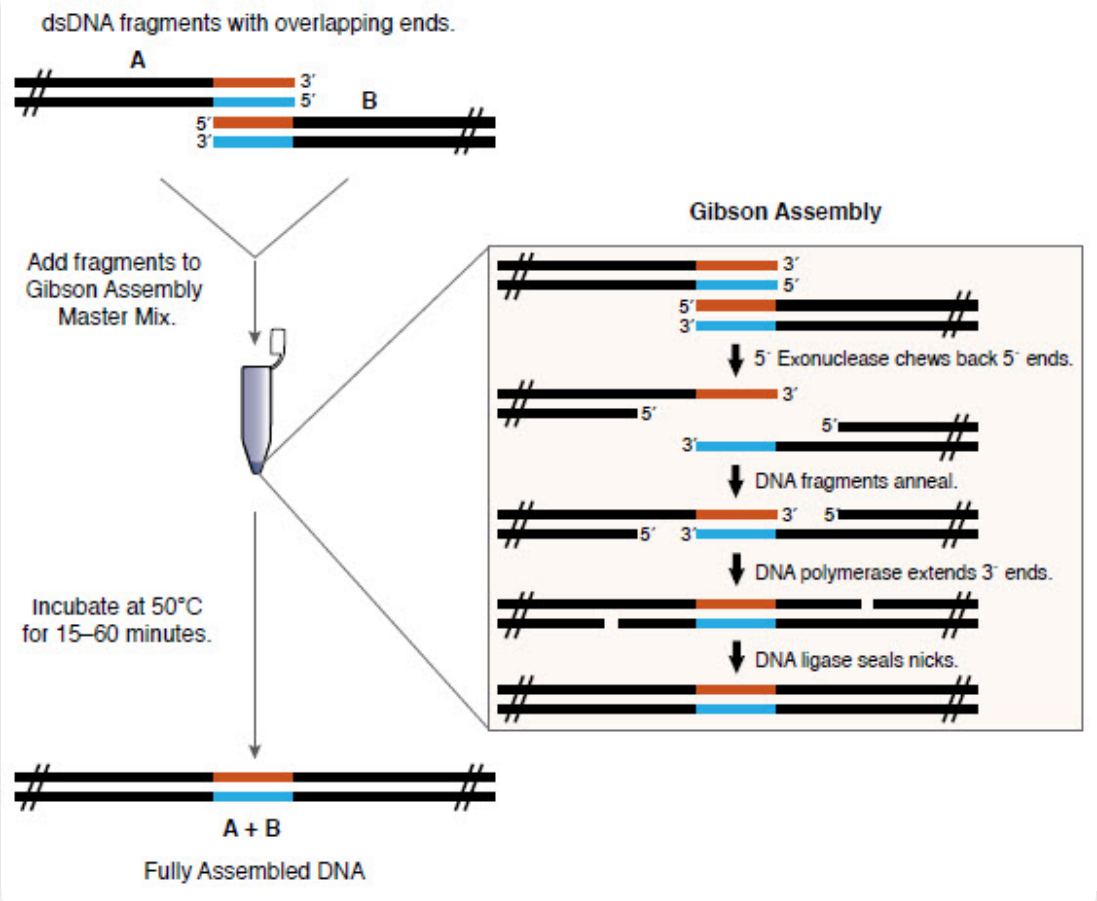

Overview of Gibson Assembly Cloning Kit Protocol:

- 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 NEB 5-alpha Competent E. coli (provided) or use directly in other applications.

NOTES:

1. 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. Storage Note: The kit is shipped on dry ice. Upon arrival, store kit at -80°C. After first

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use, store the kit components at indicated temperatures.

3. Usage notes:

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

- *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 at least 2–3 times higher than the concentration of vector. **For assembly of multiple fragments into a vector, we recommend using equimolar ratio of fragments.**
- *Transformation*: NEB 5-alpha Competent E. coli (High Efficiency, NEB #C2987) provided with the kit are recommended for use for assembled products of less than 20 kb in size. It is also possible to use other NEB competent E. coli strains, with the exception of BL21, BL21(DE3), Lemo21(DE3) and Nico21(DE3). For example, Shuffle T7 Express Competent E. coli can be used for the expression of a difficult to express protein. When using competent E. coli from a vendor other than NEB, we have seen decreased robustness of transformation with the Gibson Assembly reaction.
- *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. Should you require the use of Electrocompetent cells, please use the Electrocompetent Cells Transformation Protocol.

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 Cloning Kit - 10 rxns **New England Biolabs Catalog #E5510S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

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1 Set up the following reaction **On ice** :

A	B	C	D
	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 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.

2 Move further with the protocol, based on whether you are assembling 2-3 fragments or 4-6 fragments:
Step 2 includes a Step case.
2-3 Fragments
4-6 Fragments

step case

2-3 Fragments

3 

Incubate samples in a thermalcycler at **50 °C** for **00:15:00** .

Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases (for further details see FAQ section).

4 

Store samples **On ice** or at **20 °C** for subsequent transformation.

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

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