Gibson Assembly Version 3

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Abstract

Gibson assembly is a simple, robust method for assembling multiple DNA fragments without restriction-ligation cloning. Our group routinely uses this method for assembling multiple fragments of DNA into larger constructs, in one step. We generally use Gibson assembly for assemblies of up to ~5 fragments and final construct size of ~20kb; for larger assemblies we usually use yeast homologous recombination.

The method Introduction from Daniel Gibson, et. al., is as follows:
'An isothermal, single-reaction protocol for assembling multiple, overlapping DNA molecules by the concerted actions of a 5'-exonuclease, a DNA polymerase, and a DNA ligase is described. The DNA fragments are first recessed to produce ssDNA overhangs that are specifically annealed, and then they are covalently joined. This assembly protocol can be used to seamlessly construct synthetic and natural genes, genetic pathways, and entire genomes. This method could be a very useful molecular engineering tool.'

The original Gibson assembly protocol is here:
http://www.nature.com/protocolexchange/protocols/554

The following protocol has minor modifications/optimizations developed that were developed at the Stanford Genome Technology Center (SGTC). They are marked with an asterisk (*) and the reasoning is in *italics.*

Citation: Gergana Vandova Gibson Assembly. **protocols.io**
dx.doi.org/10.17504/protocols.io.gy5bxy6
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Materials

- beta-Nicotinamide adenine dinucleotide (NAD+) - 0.2 ml B9007S by New England Biolabs
- Magnesium Chloride (MgCl2) Solution - 6.0 ml B9021S by New England Biolabs
- Taq DNA Ligase - 10,000 units M0208L by New England Biolabs
- DTT SV-DTT by P212121
- Phusion DNA polymerase by New England Biolabs
- PEG-8000 by Contributed by users
- Tris-HCl pH 7.5 by Contributed by users
- dNTPs by Contributed by users
- T5 Exonuclease by Epicentre

Protocol

Prepare ISO Buffer

Step 1.

For 6ml of buffer:

3 mL of 1M Tris-HCl pH 7.5

150 µl of 2M Mgl2

60 µl of 100mM dGTP
60 µl of 100mM dATP
60 µl of 100mM dTTP
60 µl of 100mM dCTP

*OR (*alternatively 240 µL of 100mM dNTPs to individual nucleotides)*

300 µl of 1M DTT
300 µl of 100mM NAD

1.5 g of PEG-8000

Add water to 6mL

Aliquot 100 µl into tubes and store at -20 °C.

AMOUNT
6 ml Additional info:

Prepare Master Mix

Step 2.

Combine the following on *ice*:

320 µl of 5X ISO Buffer

20 µl of 2U/µl Phusion polymerase
60 µl of 40U/µl Taq Ligase

6.4 µl of diluted T5 Exonuclease (**Dilute 2 µl of T5 Exonuclease into 18 µl of water to make a 1U/ l mixture. This dilution is recommended as pipetting a small volume of 0.64µl is difficult and can result in more mix to mix variation.*)

Add water to 800 µl. *(* The orginal protocol was 1.2mL but having more concentrated aliquots allows for more DNA to be added later.)*

Aliquot 10 µl* into tubes and store at -20°C. *(*Alternatively, you can aliquo*t 5 µL a*nd do assembly in a total volume of 10µL once DNA is added)*

AMOUNT
800 µl Additional info:

NOTES
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This assembly mixture can be stored at -20 °C for at least one year. The enzymes remain active following at least 10 freeze-thaw cycles. This is ideal for the assembly of DNA molecules with 20-150 bp overlaps. For DNA molecules overlapping by larger than 150 bp, prepare the assembly mixture by using 3.2 µl of 10 U/ µl T5 exo.

Assembly Protocol

Step 3.

3.1. Thaw a 10* *(*or 5)* µl assembly mixture aliquot and keep on ice until ready to be used.

3.2. Add 10* *(*or 5)* µl of DNA to be assembled to the master mixture. The DNA should be in equimolar amounts. Use 10-100 ng of each 6 kb DNA fragment. For larger DNA segments, increasingly proportionate amounts of DNA should be added (e.g. 250 ng of each 150 kb DNA segment).

3.3. Incubate at 50°C for 15 to 60 min (60 min is optimal).

3.4. If cloning is desired, electroporate 1 µl of the assembly reaction into 30 µl electrocompetent *E. coli* OR* 2.5µL into Zymo EZ-competent DH5alpha chemically competent *E. coli*. *(*At the SGTC we routinely prepare our own chemically competent cells with this kit).*

AMOUNT
10 µl Additional info: