Gibson Assembly™ Master Mix

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 a single-stranded 3’ overhang that facilitates the annealing of fragments that share complementarity at one end.
- The 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 (1–3).

Figure 1. Overview of the Gibson Assembly Method

3. Gibson, D.G. Personal communication.

Additional references and information on Gibson Assembly Master Mix can be found at www.syntheticgenomics.com and www.jcvi.org.
In contrast to other methods, Gibson Assembly is suitable for a wide range of different types of assemblies, including large numbers of DNA fragments. Figure 2 shows that Gibson Assembly outperforms other methods for assembly of six fragments.

Figure 2. Assembly of DNA fragments using methods from three different suppliers.

Gibson Assembly Master Mix can assemble fragments directly from PCR reactions, with no column purification or phenol/chloroform extraction. Though generally not recommended for more complex assemblies, Figure 3 demonstrates the convenience of using Gibson Assembly Master Mix with two fragments directly from PCR.

Figure 3. Gibson Assembly of PCR-amplified inserts without a clean-up step.

Technical Tips:
To ensure the successful assembly and subsequent transformation of assembled DNAs, the following tips are recommended:

- Competent cells can vary by several logs in their competence. Perceived assembly efficiency directly correlates to the competence of the cells used for transformation.
- 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.
- Purified DNA for assembly can be dissolved in dH₂O (Milli-Q® water or equivalent is preferable), TE or other dilution buffers.
- For optimal assembly, adjust the volume of DNA to 10 µl before adding Gibson Assembly Master Mix. For DNA volumes greater than 10 µl, increase the volume of Gibson Assembly Master Mix accordingly.
- When directly assembling fragments into a cloning vector, the concentration of assembly fragments should be 2–3 times higher than the concentration of vector. When cloning into a vector use 2–3 times more insert or assembled product.
- Some DNA structures, including inverted and tandem repeats, are selected against by E. coli. Some recombinant proteins are not well tolerated by E. coli and can result in poor transformation or small colonies.

Ordering Information

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>NEB #</th>
<th>SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibson Assembly Master Mix</td>
<td>E2611S/L</td>
<td>10/50 reactions</td>
</tr>
</tbody>
</table>

COMPANION PRODUCTS

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>NEB #</th>
<th>SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEB 5-alpha Competent E. coli (High Efficiency)</td>
<td>C2987I/H</td>
<td>6 x 0.2 ml/20 x 0.05 ml</td>
</tr>
<tr>
<td>SOC Outgrowth Medium</td>
<td>B9020S</td>
<td>4 x 25 ml</td>
</tr>
</tbody>
</table>

For licensing information, visit www.neb.com.

www.neb.com