Gibson Assembly cloning method

For use with IDT Gene Fragments, such as:

- gBlocks™ Gene Fragments
- eBlocks™ Gene Fragments
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The Gibson Assembly® method [1] is a great alternative to traditional restriction site cloning and offers the advantage of a single-tube reaction that assembles multiple fragments seamlessly in as little as 30 minutes (Figure 1). This method provides directional cloning without the need for specific restriction sequences. Gibson Assembly relies on the use of an enzyme mixture consisting of a mesophilic exonuclease, thermophilic ligase, and high-fidelity polymerase. And because assembly requires intact sequences at both termini, this method can select against truncated sequences or sequences with errors at the ends. We recommend the Gibson Assembly method for assembly and most cloning applications.

For the assembly reaction, IDT Gene Fragments (e.g., gBlocks and eBlocks Gene Fragments) and the vector insertion site are designed with overlapping sequences at the locations that are to be joined. At 50°C, the exonuclease digests dsDNA from the 5’ ends (Figure 2). The resulting single-stranded, complementary ends are then available to hybridize to each other, at which point the polymerase fills in missing nucleotides and the ligase covalently joins the fragments together.

Since the introduction of the Gibson Assembly method, several similar assembly kits are commercially available. Most of these kits function in an analogous way, and the protocol description below is broadly applicable to most kits.
Step 1
IDT Gene Fragments are designed with complementary 30–80 bp overlaps on the 3’ strands and used in a single 50ºC reaction where the following steps occur.

Step 2
A mesophillic 5’ exonuclease briefly cleaves bases from the 5’ ends of the double-stranded DNA fragments before being inactivated at 50ºC.

Step 3
The newly generated complementary 3’ overhangs anneal.

Step 4
A high-fidelity DNA polymerase fills in any gaps resulting in completed circular plasmids or retracted free ends in linear assemblies.

Step 5
Finally, a thermophilic DNA ligase covalently joins DNA segments.

Figure 2. Process of the Gibson Assembly method with IDT Gene Fragments.
GUIDELINES

Advantages of the Gibson Assembly method

- Fast and efficient
- Multiple DNA elements can be assembled and cloned in a single reaction
- Allows for large gene assembly and generation of gene libraries
- Directional cloning
- Does not require restriction sites

IDT Gene Fragments

gBlocks Gene Fragments

gBlocks Gene Fragments are chemically synthesized, double-stranded DNA (125–3000 bp), delivered dry and normalized to 250, 500, or 1000 ng, depending on length. These are the original gene fragments and are the preferred choice for gene assembly, genome editing, qPCR standards, and more.

eBlocks Gene Fragments

eBlocks Gene Fragments are chemically synthesized, double-stranded DNA (300–900 bp), normalized to 200 ng and delivered at 10 ng/µL in Nuclease-Free Water. These gene fragments are uniquely suited for high-throughput screening and are available in 1–3 business days.

Design considerations

For use in Gibson Assembly, IDT Gene Fragments must contain 30–80 bases of sequence overlap between fragments, including the flanking sequencing of the insert site in the desired plasmid. The portions of the sequences containing the overlaps should contain minimal secondary structure and minimal repetitive DNA sequences, which can affect alignment of the annealed sequences. If these 2 criteria are met, increasing the length of the overlaps will tend to increase the efficiency of the assembly. When cloning a sequence of more than 2000 bases into a plasmid, or when more than 3 fragments are used, use overlap lengths of at least 50 bp.
# CONSUMABLES AND EQUIPMENT

## Consumables from IDT

<table>
<thead>
<tr>
<th>Item</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene fragments with 20–80 bases of sequence overlaps, such as: gBlocks Gene Fragments</td>
<td><a href="http://www.idtdna.com/gBlocks">www.idtdna.com/gBlocks</a></td>
</tr>
<tr>
<td></td>
<td>eBlocks Gene Fragments</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>11-04-02-01</td>
</tr>
<tr>
<td>TE Buffer</td>
<td></td>
</tr>
</tbody>
</table>

## Consumables from other suppliers

### Vector

Option 1 for linearizing vector by restriction digestion:
- Blunt-cutting restriction enzyme [e.g., EcoRV (400 U/μL)]
- Alkaline phosphatase

Option 2 for linearizing vector by PCR amplification:
- Forward and reverse primers
- dNTPs
- High-fidelity polymerase
- MgSO₄
- DpnI
- 10X KOD Buffer

Gibson Assembly master mix

Cell transformation reagents
- Competent bacteria
- 14 mL BD Falcon® polypropylene tube (Corning)
- SOC media
- LB plates

## Equipment

<table>
<thead>
<tr>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR thermal cycler</td>
</tr>
<tr>
<td>(Optional) Heat blocks at 37°C and 80°C</td>
</tr>
<tr>
<td>Water bath at 42°C</td>
</tr>
<tr>
<td>Incubator at 37°C</td>
</tr>
</tbody>
</table>
PROTOCOL

Resuspend your IDT Gene Fragments

The dried down IDT Gene Fragment pellet can become displaced from the bottom of the tube during shipping, so it is important to centrifuge your tubes or plates before opening them.

1. Centrifuge the tube for 3–5 seconds at a minimum of 3000 × g to pellet the material to the bottom of the tube.
2. Add TE buffer to the tube for your desired final concentration.

<table>
<thead>
<tr>
<th>Final stock concentration</th>
<th>Resuspension volume of TE buffer (µL) for IDT Gene Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 ng</td>
<td>Synthesis scale</td>
</tr>
<tr>
<td>10 ng/µL</td>
<td>500 ng</td>
</tr>
<tr>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Not recommended</td>
<td>25</td>
</tr>
<tr>
<td>Not recommended</td>
<td>10</td>
</tr>
</tbody>
</table>

**Note:** Gene fragments can be resuspended in Nuclease-Free Water in some cases (see Store your Gene Fragments).

3. Briefly vortex, then centrifuge.

Store your Gene Fragments

gBlocks Gene Fragments

Store gBlocks Gene Fragments at −20°C in TE for up to 24 months. For short-term storage of less than 1 month, they can be stored at −20°C in Nuclease-Free Water. Aliquot resuspended gBlocks fragments to avoid more than 2–3 freeze-thaw cycles.

eBlocks Gene Fragments

eBlocks fragments are delivered at 10 ng/µL in Nuclease-Free Water. Store eBlocks Gene Fragments at −20°C for up to 24 months. For short-term storage of less than 2 weeks, they can be stored at 4°C.

Prepare vector

Option 1: Linearize by restriction digestion

Supercoiled vector isolated from *E. coli* or purchased from a commercial vendor can be linearized using a blunt cutting restriction enzyme, provided the restriction site is present in the vector. In addition, the linearized vector should then be dephosphorylated using a phosphatase to prevent religation of the empty vector’s ends. This protocol provides an example using EcoRV (New England Biolabs) and Thermosensitive Alkaline Phosphatase (Promega).

**Note:** The volumes and concentrations are examples; follow the manufacturers’ instructions for materials specific to your application.
1. Add the following reaction components:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid</td>
<td>1 μg</td>
</tr>
<tr>
<td>10X Buffer #3</td>
<td>4 μL</td>
</tr>
<tr>
<td>Restriction enzyme (n U/μL)</td>
<td>1 μL</td>
</tr>
<tr>
<td>BSA</td>
<td>0.5 μL</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>40 μL (total volume)</td>
</tr>
</tbody>
</table>

2. Follow manufacturer’s recommendations for incubation, temperature, time, and heat kill.

3. Remove 5’ phosphates from vector using an alkaline phosphatase (e.g., Thermosensitive Alkaline Phosphatase).

   **Important:** Do not dephosphorylate the insert (i.e., IDT Gene Fragment).

   **Note:** Most commercially available phosphatases can be added directly at the end of the restriction digest; follow the manufacturer’s instructions for your chosen phosphatase.

4. Confirm and quantify the reaction by running the product on an agarose gel with an appropriate quantification ladder.

5. To reduce background, gel purify the vector following digestion.

**Option 2: Linearize by amplification and digest with Dpnl**

Alternatively, vectors can be amplified using primers that have their 5’ ends at the insertion site (i.e., oriented to extend away from the insertion site). For amplification, use a high-fidelity polymerase that leaves blunt ends on the products. To remove the PCR template, digest the reaction using Dpnl, which will only digest Dam-methylated DNA isolated from *E. coli* and not the PCR-amplified DNA.

**Note:** The volumes, temperatures, and concentrations are examples; follow the manufacturers’ instructions for materials specific to your application.

1. Set up the components *(Table 1)* for each of your vectors.

   **Table 1. Components of PCR.**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supercoiled plasmid</td>
<td>1 ng</td>
</tr>
<tr>
<td>5 μM forward primer</td>
<td>1 μL</td>
</tr>
<tr>
<td>5 μM reverse primer</td>
<td>1 μL</td>
</tr>
<tr>
<td>2 mM dNTPs</td>
<td>2.5 μL</td>
</tr>
<tr>
<td>25 mM MgSO₄</td>
<td>1.5 μL</td>
</tr>
<tr>
<td>High-fidelity polymerase (2.5 U/μL)</td>
<td>0.5 μL</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>To final 25 μL volume</td>
</tr>
</tbody>
</table>

2. (Optional) Confirm the linear product was generated by running 5 μL on a 0.8% agarose gel with a DNA ladder and 200 ng of uncut plasmid.
3. Digest the template from the PCR-amplified vector with DpnI by incubating the following at 37°C for 1 hour.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR product</td>
<td>17 μL</td>
</tr>
<tr>
<td>10X Buffer</td>
<td>2 μL</td>
</tr>
<tr>
<td>DpnI (20 U/μL)</td>
<td>1 μL</td>
</tr>
</tbody>
</table>

**Tip**: standard PCR primers do not contain 5’ phosphates, so there is no need to dephosphorylate after PCR.

4. Purify the PCR-amplified vector using a PCR cleanup kit.

5. Confirm the linear product was generated by running 5 μL on a 0.8% agarose gel with a DNA ladder and 200 ng of uncut plasmid.

### Construct assembly (plasmid plus IDT Gene Fragments)

One or more gene fragments and a plasmid are designed with 20–80 base overlaps with the adjacent fragment sequences and the linearized plasmid. This protocol provides an example using the Gibson Assembly master mix (New England Biolabs).

1. Combine linearized plasmid and fragments in a tube with assembly mix as shown:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Total number of fragments, including plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene fragments*</td>
<td>2–3 (0.02–0.50 pmol each)</td>
</tr>
<tr>
<td>Gene fragments*</td>
<td>4–6 (0.05–5.0 pmol each)</td>
</tr>
<tr>
<td>2X Gibson Assembly master mix†</td>
<td>10 μL</td>
</tr>
<tr>
<td>Deionized H₂O</td>
<td>Up to 20 μL</td>
</tr>
</tbody>
</table>

* Use 50–100 ng of plasmid and a 2–3 fold excess of insert fragments.
† Enzymes are included in the master mix.

2. Incubate at 50°C for 1 hour.

### Transform

Several lines of competent *E. coli* can be purchased from a variety of vendors and provide a reliable way to achieve high transformation efficiencies. Alternatively, competent cells can be prepared in the lab by following the protocols outlined in Sambrook et al. [2].

Below is an example protocol for transformation; follow the manufacturer’s instructions for materials specific to your product.

1. Thaw cells on wet ice.
2. Add 25 μL cells to a pre-chilled 14 mL BD Falcon polypropylene tube on ice.
3. Add 2 μL of ligation mixture and mix gently.
4. Incubate on wet ice for 30 minutes.
5. Place in a 42°C water bath for 45 seconds.
6. Return to ice for 2 minutes.
7. Add 250 µL of SOC media to the cells and incubate shaking at 37°C for 1 hour.
8. Plate 125 µL on LB plates with the appropriate selection reagents for your vector.
9. Incubate the plates inverted in a 37°C incubator overnight.
10. Select and screen several colonies.

**Tip:** We recommend sequencing at least 2 times the number of IDT Gene Fragments assembled to give you the highest probability of successfully identifying your desired target. For example, if you assemble 4 gBlocks Gene Fragments into a plasmid, we recommend sequencing 8 clones to have the best chance (~95%) of obtaining your desired construct.

The resulting completed plasmid is ready for sequencing.
REFERENCES


Gibson Assembly cloning method

Technical support: genes@idtdna.com

For more than 30 years, IDT’s innovative tools and solutions for genomics applications have been driving advances that inspire scientists to dream big and achieve their next breakthroughs. IDT develops, manufactures, and markets nucleic acid products that support the life sciences industry in the areas of academic and commercial research, agriculture, medical diagnostics, and pharmaceutical development. We have a global reach with personalized customer service.

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