

# gBlocks® Gene Fragments

## Protocol: Gibson Assembly™ Method

The Gibson Assembly Method described by Gibson et al. [1] is a rapid assembly method that provides directional cloning of multiple DNA fragments in a single reaction, without the need for specific restriction sequences. It relies on use of an enzyme mixture consisting of a mesophilic exonuclease, a thermophilic ligase, and a high-fidelity polymerase.

For the assembly reaction, the gBlocks 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, but is rapidly degraded leaving complementary, 3' ssDNA ends. 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.

### gBlocks Gene Fragments

gBlocks Gene Fragments are chemically synthesized, double-stranded DNA that are compatible with a wide range of existing applications that require double-stranded DNA. gBlocks Gene Fragments are normalized to 200 ng and delivered dried down. Order at [www.idtdna.com/gblocks](http://www.idtdna.com/gblocks).

### Resuspending your gBlocks Gene Fragments

The dried down gBlocks Gene Fragment pellet can become displaced from the bottom of the tube during shipping. Follow these instructions to properly resuspend the pellet for a final concentration of 10 ng/μL.

1. Centrifuge the tube for 3–5 sec at a minimum of 3000 x g to pellet the material to the bottom of the tube.
2. Add 20 μL TE to the tube for a final concentration of 10 ng/μL.
3. Briefly vortex and centrifuge.

### Storing your gBlocks Gene Fragments

gBlocks Gene Fragments can be stored in TE at –20°C for up to 24 months. If gBlocks Gene Fragments will be stored for less than 1 month, they can be resuspended in nuclease-free water instead of TE.

### Planning for Gibson Assembly™ Method

Overlaps: gBlocks Gene Fragments must contain 20–80 bases of sequence overlap between fragments, and with the desired plasmid. The portions of the sequences containing the overlaps should not contain any secondary structure, or repeated DNA motifs that can affect alignment of the annealed sequences.

### Required materials

- gBlocks Gene Fragments (with 20–80 bases of sequence overlaps)
- Linearized plasmid
- Gibson Assembly™ Master Mix (New England Bio Labs, at [www.neb.com/gibsonassembly](http://www.neb.com/gibsonassembly))
- Cell transformation reagents

### Procedure

1. Two or more gBlocks Gene Fragments are designed with 20–80 base overlaps with the adjacent gBlocks fragment sequences and the linearized plasmid. The plasmid can be linearized by restriction digest or PCR.
2. Linearized plasmid and fragments are combined in a tube with Gibson Assembly™ Master Mix (New England Biolabs).

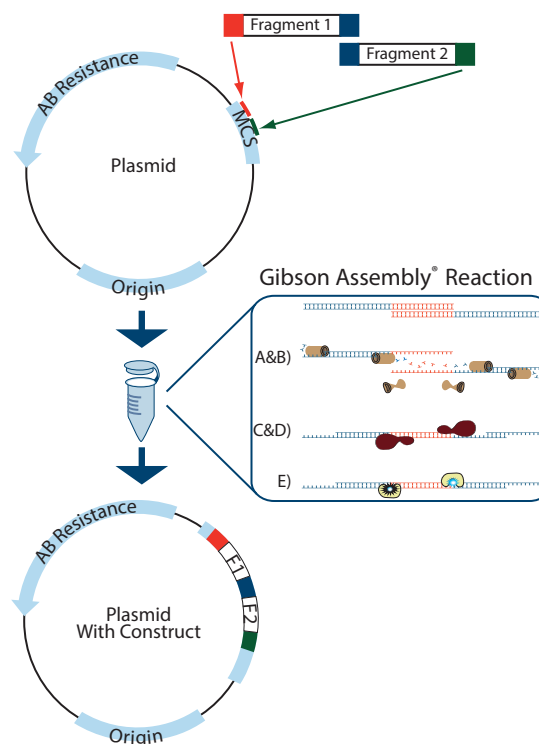
#### gBlocks® Gene Fragments and Plasmid Assembly

Total # of fragments including plasmid	2–3 Fragments	4–6 Fragments
Quantity <sup>1</sup>	0.02–0.50 pmole ea.	0.2–1.0 pmole ea.
Gibson Assembly™ Master Mix (2X)	10 μL	10 μL
Deionized H <sub>2</sub> O	Adjust to final 20 μL	Adjust to final 20 μL
Total volume	20 μL	20 μL

<sup>1</sup>Use 50–100 ng of plasmid and a 2–3 fold excess of insert fragments.

Table source: [www.NEB.com/GibsonAssembly](http://www.NEB.com/GibsonAssembly).

3. Incubate at 50°C for 1 hr. See Figure 1 for reaction details.
4. The resulting completed plasmid is ready for transformation into bacteria, and sequencing. Note: IDT scientists recommend sequencing at least 2X the number of gBlocks Gene Fragments assembled. For example, if you assembled 2 fragments, you would sequence 4 clones.



**Figure 1. Example of Gibson Assembly™ reaction with 2 gBlocks® Gene Fragments and a generic plasmid.** 50°C, 1-hr reaction: A & B) exonucleases digest the 5' DNA ends, and are rapidly inactivated by the 50°C temperature; C & D) complementary overhangs anneal, and high-fidelity polymerases fill in any gaps; and E) in the final step, a ligase covalently joins DNA fragments to the plasmid

### References

1. Gibson DG, Young L, et al. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature Methods*, 6(5):343–345.