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 availible 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, delivered normalized to 250, 500, or 1000 ng, depending on length, and dried down. Order at 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.

- Centrifuge the tube for 3–5 sec at a minimum of 3000 x g to pellet the material to the bottom of the tube.
- Add TE to the tube for your desired final concentration
- Briefly vortex and centrifuge

Final concentration	Resuspension volume of TE buffer (µL) for gBlocks Fragments synthesis scales		
	250 ng	500 ng	1000 ng
10 ng/µL	25	50	100
20 ng/µL	Not recommended	25	50
50 ng/µL	Not recommended	10	20

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)
- 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[1]	0.02–0.50 pmole ea.	0.05–5.0 pmole ea.
Gibson Assembly® Master Mix (2X)	10 µL	10 µL
Deionized H_2O	Adjust to final 20 µL	Adjust to final 20 µL
Total volume	20 µL	20 µL

[1]Use 50–100 ng of plasmid and a 2–3 fold excess of insert fragments.

Table source: www.NEB.com/GibsonAssembly.

3. Incubate at 50ºC 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.

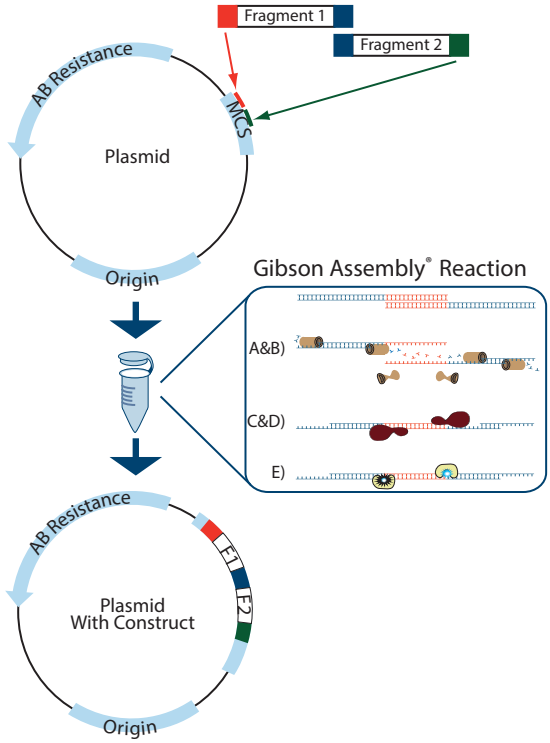

Figure 1. Example of Gibson Assembly® reaction with 2 gBlocks® Gene Fragments and a generic plasmid. 50ºC,1-hr reaction: A & B) exonucleases digest the 5' DNA ends, and are rapidly inactivated by the 50˚C temperature; C & D) complementary overhangs anneal, and high-fidelity polymerases fill in any gaps;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