gBlocks® Gene Fragments Protocol: Fragment Amplification

gBlocks Gene Fragments are normalized to 250, 500, or 1000 ng, depending on length, which is a suitable quantity for many applications. However, in some cases it may be necessary or desired to amplify your gBlocks Gene Fragments to get additional starting material.

The following information will help you amplify your gBlocks Gene Fragments using the high-fidelity Phusion® DNA Polymerase (www.NEB.com/Phusion). Only use high-fidelity, proofreading enzymes to amplify your gBlocks Gene Fragment to limit the introduction of sequence errors.

gBlocks Gene Fragments

gBlocks Gene Fragments are chemically synthesized, double-stranded DNA, delivered 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.

Designing primers for amplification

For best results, we recommend designing amplification primers for your gBlocks Gene Fragment sequence using the PrimerQuest® tool (www.idtdna.com/PrimerQuest).

Required materials

- gBlocks Gene Fragments
- Amplification primers
- Phusion® DNA Polymerase (www.NEB.com/Phusion)
- PCR or gel purification kit (depending on application)

Phusion® DNA Polymerase amplification reaction

1. Set up the amplification reaction on ice–reaction components for 20 µL and 50 µL reactions are shown.

	gBlocks® Gene Fragments Amplification	
	20 µL Reaction	50 µL Reaction
Nuclease-free H_2O	Adjust to final 20 µL	Adjust to final 50 µL
5X Phusion HF or GC Buffer	4 µL	10 µL
10 mM dNTPs	0.4 µL	1 µL
10 µM Forward Primer	1 µL	2.5 µL
10 µM Reverse Primer	1 µL	2.5 µL
gBlocks® Gene Fragments	0.1–1.0 ng	0.1–1.0 ng
Phusion® DNA Polymerase	0.2 µL	0.5 µL
Total volume	20 µL	50 µL

Table source: www.NEB.com/Phusion.

2. Gently mix the reaction and spin down in microcentrifuge.
3. Carry out the amplification reaction in a thermocycler with heated lid.

General cycling conditions

The table shows general guidelines for amplifying a short, 500 bp gBlocks Gene Fragment. Conditions will vary depending on sequence of the gene fragment and amplification primers.

	gBlocks® Gene Fragments Amplification		
Step	Cycles	Temperature	Time
Initial denaturation	1	98°C	30 seconds
Denaturation Annealing Extension	15–25[1]	98°C 45–72°C 72°C	10 seconds 10–30 seconds 15–30 seconds
Final extension	1	72°C	5 minutes
Hold	1	4°C	∞

[1]Use the lowest number of cycles to attain the desired quantity as this will provide the best sequence fidelity.

Table source: www.NEB.com/Phusion.

Purification

Gel purification using high-energy, UV light damages DNA, negatively impacting downstream application performance. We recommend that you purify your gBlocks Gene Fragment amplification product using a non-UV method. Examples of purification methods that do not rely on high-energy, UV light include affinity-column purification, or non-UV gel systems FlashGel™ System (Lonza).