gBlocks® Gene Fragments Protocol: Tailing for T/A cloning

T/A cloning is type of cohesive-end cloning for quickly generating non-directional inserts. It takes advantage of the residual adenine residue that is added on the 3' end of DNA fragments by the activity of various enzymes, including Taq DNA polymerase. The resulting end is compatible with T/A-cloning vectors available from several manufacturers.

The following information will help you add the necessary adenosine (A) overhangs to blunt-ended gBlocks Gene Fragments for use in T/A cloning protocols.

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.

Required materials

- gBlocks Gene Fragments
- Taq DNA polymerase
- Taq polymerase buffer
- dATP
- $MgCl_2$
- Nuclease-free H_2O
- T/A cloning vector or kit
- Cell transformation reagents

Note: There are several different commercial T/A cloning solutions available from various manufacturers. Follow the protocols for the T/A cloning vector or kit to ensure cloning success.

gBlocks Gene Fragment tailing reaction with Taq DNA polymerase

1. Resuspend gBlocks Gene Fragments in 20 µl TE (pH 8.0) as described.
2. Add the following to a 15 µL reaction:

	20 µL Reaction
gBlocks® Gene Fragments	50 ng
Taq polymerase	1–3 Units
Taq polymerase buffer	to [1X]
dATP	to [0.05 mM]
$MgCl_2$	to [1.5 mM]
Nuclease-free H_2O	to final 15 µL
Total volume	**15 µL**

3. Incubate at 70°C for 15–30 minutes.
4. Use 1–10 µL for T/A cloning following the manufacturer's instructions.