TA cloning method

For use with IDT Gene Fragments, such as:

- gBlocks™ Gene Fragments
- eBlocks™ Gene Fragments

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INTRODUCTION

TA cloning is a fast and relatively simple way to clone relatively short pieces of DNA with moderate efficiency. Several commercially available cloning kits use the TA cloning method. Vectors in these kits have single "T" base overhangs at the vector cloning site that is designed to complement the residual 3' "A" base left by Taq polymerase during PCR reactions. To TA clone DNA with blunt ends, such as IDT Gene Fragments, "A" base overhangs can be added by a brief incubation with Taq polymerase in the presence of dATP (Figure 1).

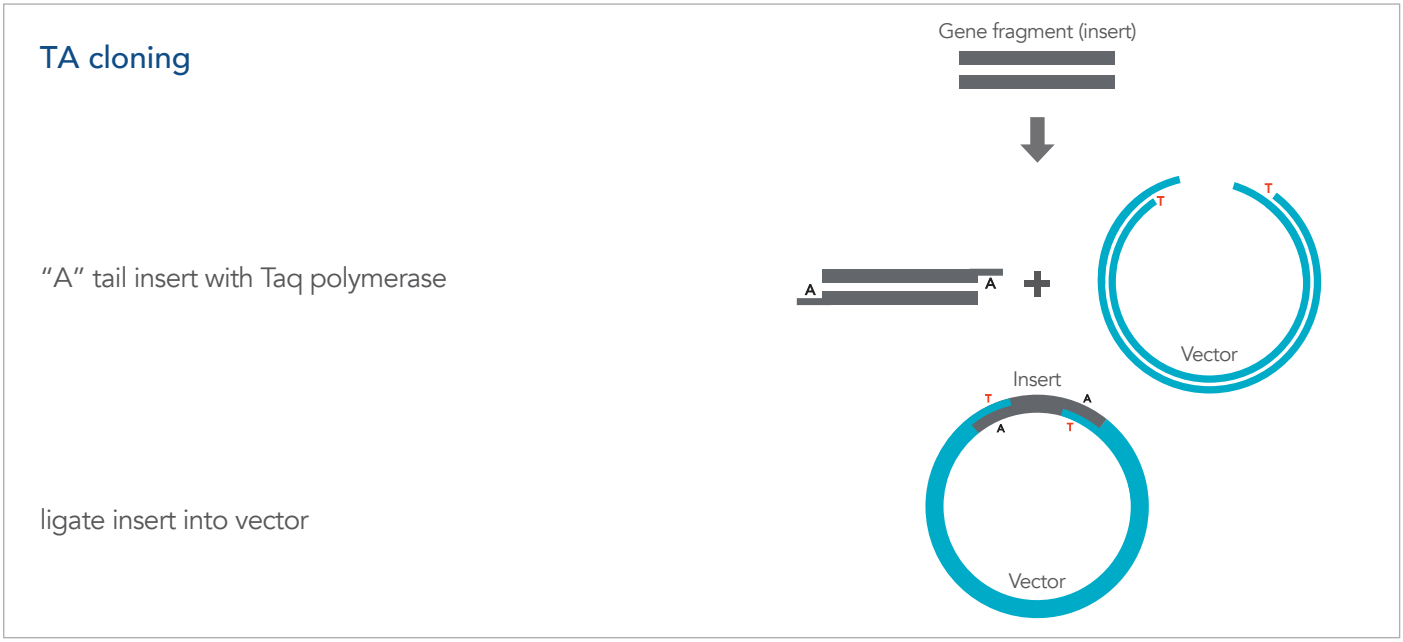

Figure 1. Overview of TA cloning.

Compared to newer methods such as Gibson assembly, TA cloning is less efficient and non-directional, and the method cannot be used to assemble more than one fragment at a time. Nevertheless, it can be an easy method to rapidly clone PCR products for routine sequence identification, subcloning applications, and creation of small recombinant plasmids.

This protocol includes how to prepare your blunt-ended gene fragment for TA cloning. If you are using a TA cloning kit, follow the manufacturer's instructions for cloning your prepared fragment. If you are not using a TA cloning kit, this protocol also provides guidelines for preparing your TA cloning vector, ligating your fragment into the vector, and transforming your plasmid into bacteria for selection and screening.

GUIDELINES

Advantages of the TA cloning method

- More efficient than blunt-end cloning
- Restriction enzymes are not required to ligate the insert with the vector

Limitations to the TA cloning method

- Not directional—inserts will be cloned in both the forward and the reverse orientation
- Inserts an additional "A" and "T" base at the cloning junctions
- Sample must be purified to avoid selective cloning of truncated products
- Blunt inserts not generated by traditional PCR methods require A-tailing prior to cloning
- Requires specially prepared plasmids with "T" base overhangs
- Inefficient when cloning more than one fragment

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.

CONSUMABLES AND EQUIPMENT

Consumables from IDT

Item	Catalog #
Gene fragments, such as: gBlocks Gene Fragments eBlocks Gene Fragments	 www.idtdna.com/gBlocks www.idtdna.com/eBlocks
Nuclease-Free Water	11-04-02-01

Consumables and equipment from other suppliers

Item
TE buffer
Taq DNA polymerase
1 mM dATP
25 mM $MgCl_2$
TA cloning vector kit*
If not using a TA cloning vector kit: (Option 1) To linearize vector manually by restriction digestion: • Vector • Restriction enzyme (blunt cutting enzyme, such as EcoRV) • Alkaline phosphatase (Option 2) To linearize vector manually by PCR amplification: • Vector • Forward and reverse primers • dNTPs • High fidelity polymerase • $MgSO_4$ • DpnI
To A-tail and ligate linearized vector: • 2.5 mM $CaCl_2$ • 1 mM ddTTP • Terminal transferase • T4 ligase
Cell transformation reagents • Competent bacteria • 14 mL BD Falcon® polypropylene tube (Corning) • SOC media • LB plates

Equipment

Item
PCR thermal cycler
(Optional) Heat blocks at 37 and 80°C
Water bath at 42°C
Incubator at 37°C

* There are several commercial TA cloning solutions available from various manufacturers. Follow the protocols for the TA cloning vector or kit to ensure cloning success.

PROTOCOL

Resuspend your gBlocks Gene Fragments

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

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

Final stock concentration	Resuspension volume of TE buffer (µL) for gBlocks Gene Fragments		
	Synthesis scale		
	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

Note: gBlocks 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.

A-tail your gene fragment

The tailing procedure modifies blunt-ended IDT Gene Fragments with a single "A" base overhang on the 3′ ends, making the resulting DNA compatible for TA cloning kits.

1. Add the following to a 20 µL reaction:

Reagent	Amount
IDT Gene Fragment	50 ng
Taq polymerase	1–3 units
10X Taq polymerase buffer minus Mg	2 µL
1 mM dATP	To [0.2 mM]
25 mM $MgCl_2$	To [1.5 mM]
Nuclease-Free Water	To final 20 µL volume
Total volume	**20 µL**

2. Incubate at 70°C for 15–30 minutes.
3. Depending if you are using a kit or not, follow these instructions:
   - When using a kit, use 1–10 µL of the A-tailed gene fragment for TA cloning according to the manufacturer's instructions.
   - When not using a kit, proceed through the rest of this protocol for guidance on cloning your insert into your vector.

Prepare vector

Vectors used for TA cloning can be purchased commercially, or as described here, they can be prepared manually in a two-step process [1]. The first step is to linearize the vector with a blunt cutting restriction endonuclease digestion (Option 1) or with PCR (Option 2). The second step is to tail the linear vector with dideoxythymidine triphosphate (ddTTP) using terminal transferase.

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 a 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 and Thermosensitive Alkaline Phosphatase.

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

Add the following reaction components and incubate at 37°C for 1 hour, followed by 80°C for 20 minutes.

Reagent	Amount
Plasmid	10 µg
10X Buffer #3	2.5 µL
EcoRV (400 U/µL)	1 µL
BSA	0.5 µL
Nuclease-Free Water	To final 25 µL volume
Total volume	**25 µL**

Option 2: Linearize by amplification and digest with DpnI

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 DpnI, 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 vector(s).

   Table 1. Components of PCR.

Reagent	Amount
Supercoiled plasmid	1 ng
5 µM Forward Primer	1 µL
5 µM Reverse Primer	1 µL
2 mM dNTPs	2.5 µL
10X KOD Buffer	2.5 µL
25 mM $MgSO_4$	1.5 µL
Hi Fidelity Polymerase (2.5 U/µL)	0.5 µL
Nuclease-Free Water	To final 25 µL volume
Total volume	**25 µL**

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.

Reagent	Amount
PCR product	17 µL
10X Buffer	2 µL
DpnI, 20 U/µL	1 µL
Total volume	**20 µL**

   Tip: Standard PCR primers do not contain 5′ phosphates, so you do not need to dephosphorylate after PCR.

4. (Optional) 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.

Add T-tail to vector with terminal transferase

1. Create the following reaction setup, noting that the molar amount of plasmid can be calculated with the following equation:

   Mass of vector that equals 5 pmol (in µg) = length of plasmid in bp * 650 Daltons * 5 x 10^{-6}

   Note: A 2700 bp plasmid like pUC19 requires approximately 8.8 µg of material.

Reagent	Amount
10X terminal transferase buffer	5 µL
2.5 mM $CaCl_2$ solution	5 µL
Linear plasmid DNA	5 pmol
1 mM ddTTP	0.5 µL
Terminal transferase	10 units
Nuclease-Free Water	To final 40 µL volume
Total volume	**40 µL**

2. Incubate the reaction for 20 minutes at 37°C.
3. Stop the reaction by heating to 70°C for 10 minutes.

Clone gene fragment into vector

T4 ligases are available in standard or "quick" versions. In general, the quick versions contain a crowding agent such as PEG that increases the ligation kinetics and decreases reaction time. Quick versions are preferred for day to day uses.

To ligate your IDT Gene Fragment into the vector efficiently, use the optimum molar ratio of vector to gene fragment. The ratio we recommend is 1:5–1:12 vector to gene fragment.

Tips:

- High salt concentrations from DNA can inhibit ligation activity, and the use of DNA cleanup kits can often improve reaction efficiencies.
- T4 DNA ligases require buffers containing ribo ATP. Always use fresh buffers that have not been repeatedly frozen and thawed to ensure the ATP is active.
- Be careful not to heat inactivate ligase reactions with buffers containing PEG, as this decreases transformational efficiency.

1. Set up the following reaction:

Item	Amount
Linearized vector	50 ng
IDT Gene Fragment	5–12X molar excess*
2X ligase buffer	10 µL
T4 DNA ligase	1 µL
Nuclease-Free Water	Up to 20 µL
Total volume	**20 µL**

* Molar ratios of the gBlocks or eBlocks Gene Fragment (IDT) can be converted to ng using the following formula:

$$50\ \text{ng} \times \text{desired molar ratio} \times \frac{\text{IDT Gene Fragments length (bp)}}{\text{Plasmid length (bp)}} = \text{ng gene fragment needed}$$

2. (Optional) Store at –20°C.

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].

The following 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.

    The resulting completed plasmid is ready for sequencing.

REFERENCES

1. Holton TA; Graham MW (1991).A simple and efficient method for direct cloning of PCR products using ddT-tailed vectors. Nucleic Acids Res. 19(5):1156. doi:10.1093/nar/19.5.1156. PMC 333802. PMID 2020554
2. Sambrook J and Russel DW, editors. (2012) Molecular Cloning: A Laboratory Manual. 4th ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

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