vivantis

# pTG19-T PCR cloning vector

**User Manual**

Store at -20°C

Product No.: TA010-S - 5 preps
TA010 - 20 preps

**DESCRIPTION**

The pTG19-T vector is designed for rapid and efficient cloning of PCR products with 3'dA overhangs. The linearized pTG19-T vector with 3'-dT overhangs prevent vector recircularization, therefore resulting in high percentage of recombinant clones and low background.

**FEATURES**

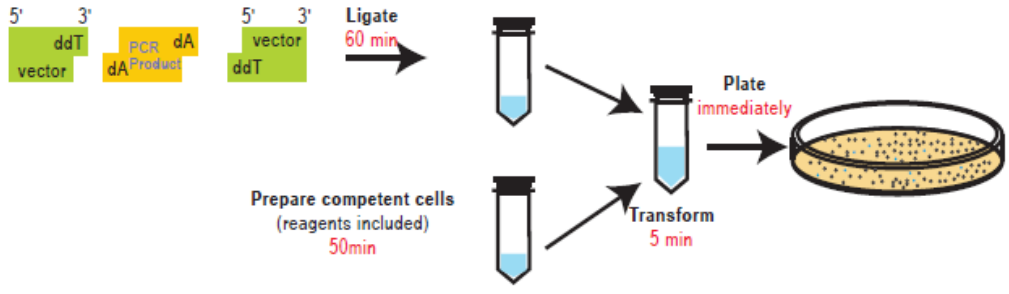
- Convenient – ready-to-use linearized 3'dT overhang pTG19-T vector.
- Efficient – more than 80% of the recombinant clones contain the target DNA.
- Rapid clone selection:
  - *lacZ* gene for blue/white selection.
  - M13 primer sites for PCR screening and sequencing.
  - *Bam*HI restriction enzyme can be used to release the insert from the pTG19-T vector.

**QUALITY CONTROL**

- More than 80% clones are white with control insert.
- More than 85% of white clones are positively by restriction endonuclease digestion.
- The 3'dT overhangs for every batch of vectors is confirmed by sequencing of five recombinant clones.

pTG19-T cloning vector

Figure 1. PCR product cloning procedure



**KIT COMPONENTS**

| Packaging | 5 preps | 20 preps |
|---|---|---|
| Catalog No. | TA010-S | TA010 |
| | | |
| Components | | |
| pTG19-T vector (25ng/µl) | 10µl | 2 x 20µl |
| Control insert (12.5ng/µl) | 5µl | 20µl |

**STORAGE & STABILITY**

All components are stable at -20°C for one year if properly stored.
To avoid frequent freeze-thaw cycles, keeping small aliquots at -20°C is recommended.

**Additional materials to be supplied by user**

T4 DNA Ligase and 10X Buffer Ligase

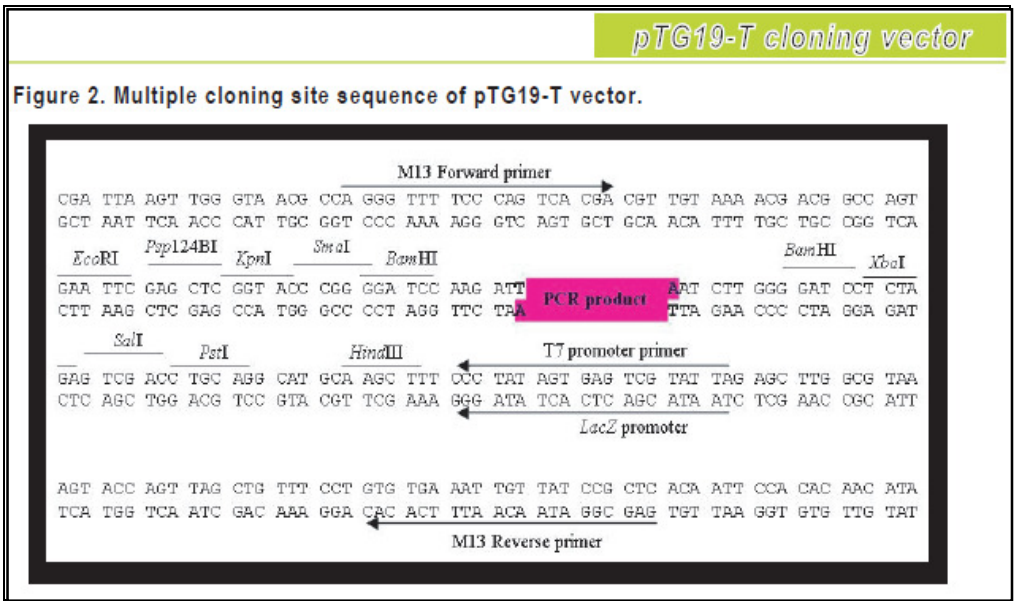



Figure 2. Multiple cloning site sequence of pTG19-T vector.

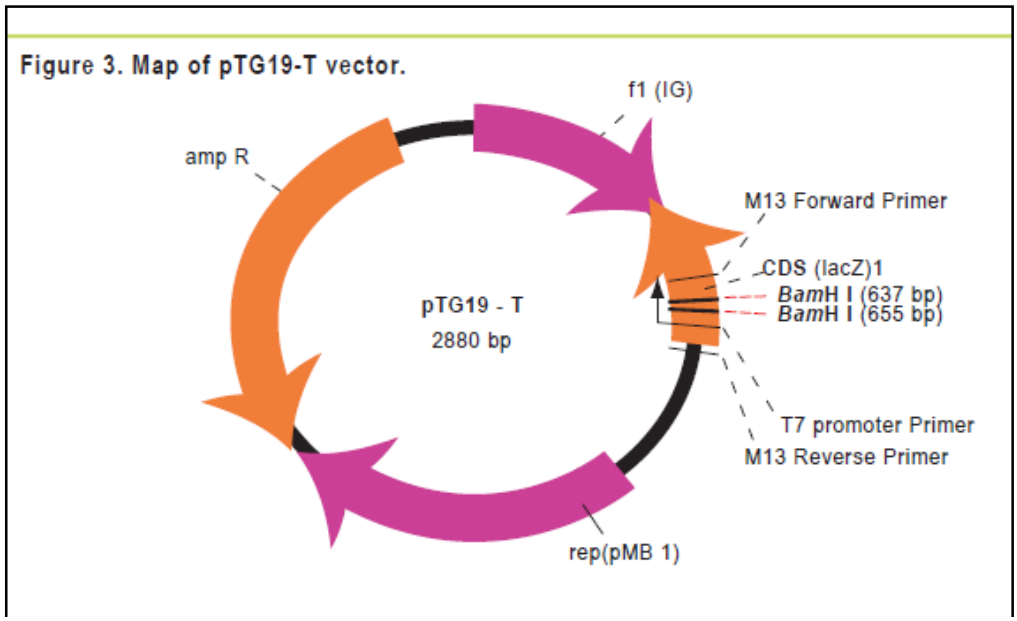


**Table 1: Feature of pTG19-T vector**

| Features | Nucleotide Position |
|---|---|
| f1(+/-) origin of ss- DNA replication | 2-457 |
| *lacZ* a fragment | 449-733 |
| multiple cloning site | 615-589 |
| lac promoter | 691-710 |
| pMB1 origin of replication | 1116-1730 |
| ampicillin resistance ORF | 1890-2750 |
| T7 promoter primer binding site | 692-710 |
| M13 Forward primer binding site | 575-592 |
| M13 Reverse primer binding site | 745-765 |

## PROTOCOL

### PCR Product Consideration

- We recommend using Taq DNA Polymerase in the PCR reaction. Remember that your PCR product needs to have single 3'-dA overhangs.
- A 5µl of PCR product should be analyzed on an agarose gel before using it in the ligation reaction. If the PCR product is clean (a homogenous band of desired size), it can be directly used in the ligation reaction.
- If multiple bands are observed on the agarose gel, perform gel purification to obtain the desired band for ligation reaction.
- If the template used in the PCR reaction contains the ß-lactamase (ampicillin resistance) gene, perform gel purification of the PCR band to reduce the number of background colonies.

### Ligation

1. Calculate the amount of insert for ligation based on the example below:
   Example:
   For 500bp insert with 50ng of pTG19-T vector (2880bp)

$$\frac{2880\text{bp}}{500\text{bp}} = 5.76$$

(vector is 5.76x larger than insert, you need 5.76x less insert)

For 50ng pTG19-T vector, $\frac{50\text{ng}}{5.76}$ = 8.68ng of insert for 1:1 ratio
= 8.68ng x 3
= 26.04ng of insert for 1:3 ratio

**Table 2: Conversion table for amount of PCR product required per ligation reaction**

| Size of PCR product (bp) | Amount needed for 1:3 vector and insert molar ratio |
|---|---|
| 100 | 5.21ng |
| 300 | 15.63ng |
| 500 | 26.04ng |
| 1000 | 52.08ng |
| 1500 | 78.13ng |
| 2000 | 104.17ng |
| 3000 | 156.25ng |

2. Add the following in a 0.2ml microcentrifuge tube:

| | |
|---|---|
| pTG19-T vector (25ng/µl) | 2µl |
| Fresh PCR product | µl* |
| 10X Buffer Ligase | 1 µl |
| T4 DNA Ligase (200u/µl**) | 1 µl |
| Nuclease-free water | to 10 µl |
| Total Volume | 10 µl |

**Recommended molar ratio for vector and insert is 1:3. In general 1:5 to 3:1 of vector and insert ratio will produce good results. Please refer to step 1 or table 1 for calculation and amount needed.*
***The unit is in Cohesive End Ligation Unit. One Cohesive End Ligation Unit is equal to 0.015 Weiss units.*

3. Incubate at 22°C for 1 hour. For maximum yield of ligation products, incubate the mixture at 16°C overnight.

Note: A control ligation reaction can be performed using 2µl of **Control insert (12.5ng/µl)**

**Choosing suitable E.coli strains for competent cell preparation**

1. E.coli strains should possess the following mutations which are important for transformation:
   - *endA1* (mutation in the endonuclease I gene), this mutation increases the yield and quality of plasmids.
   - RecA1(mutation in general recombination gene), which limits the recombination of the plasmid with the E.coli genome.
   - *lacZ*ΔM15 (partial deletion of β-galactosidase gene), for blue/white selection.
2. Some recommended E.coli strains for competent cell preparation are DH5α, TOP10, TOP10F' and XL1-Blue.

**TROUBLESHOOTING**

| Problem | Possibility | Recommended Solution |
|---|---|---|
| Few or no colonies | Poor quality competent cells | Test transformation efficiency using supercoiled plasmid DNA (>106 colonies per µg of supercoiled DNA is expected. |
| | Unsuccessful ligation | Perform PCR using Taq or polymerases which lack proofreading ability. |
| | The PCR product is blunt-ended | Perform a control ligation reaction using **Control insert**. |
| | The bacteria strain used did not possess *endA1* mutation | Refer to "Choosing suitable E.coli strains for competent". |
| White colonies do not have insert | Single 3'-dT overhangs on the vector degraded | Use another tube of vector. Avoid storing the vector for longer than one year or subjecting it to repeated freeze-thaw cycles. |
| Majority of colonies are blue | Incorrect molar ratio of the vector : insert used in the ligation reaction | Set up the ligation reaction with 1:1 to 1:3 vector: insert ratio. |
| Blue or white colonies with blue center contain insert | The insert does not interrupt the reading frame of the *lacZ* gene | Small inserts (<500bp), may appear as blue or light blue colonies. Analyze some of these colonies as they may contain insert. |
| | Mixture of blue and white | Streak the colony onto a new LB/Amp plate with X-Gal and IPTG in order to obtain single colonies. |
| A large colony surrounded by smaller | The smaller colonies are ampicillin-sensitive | Use fresh ampicillin stock solution and LB/Amp plates.<br>Do not pick the small colonies as they do not contain any plasmid. |