

# Strong pOptima<sup>®</sup>-T Cloning Vector

Catalog No. **PV01**, 1µg ( 50 ng/µl ) : 20 rxns

## Product Description

Cloning Vector pOptima<sup>®</sup>-T is supplied in a linear form, ready-to-use for direct ligation of PCR products. It is suitable for use in *Taq* and other non-proofreading DNA polymerase PCR product cloning. For the molecular cloning of PCR products, T or U nucleotides are usually incorporated to the blunt ends of linearized vectors. To promote transformation efficiency, the 3'-protruding nucleotides of vector pOptima<sup>®</sup>-T were directly created by enzyme digestion.

## Procedure

1. Prepare a ligation mixture according to the following scheme :

Component	Concentration	Volume
pOptima <sup>®</sup> -T cloning vector	50 ng/µl	1 µl
PCR product		2~7 µl
ligation buffer	10 X	1 µl
T4 DNA ligase	1 U/µl	1 µl
sterile water		5~0 µl
total volume		10 µl

- Briefly mix the ligation mixture then incubate the mixture at room temperature for several hours or at 4°C for overnight (check <http://www.strongbiotech.com> for the relationship between transformation rate and reaction condition).
- Heat the ligation mixture in 50 °C for 10 minutes before transformation.
- Transform competent cells with aliquots of ligate and select transformants on antibiotic-containing medium.

## Storage:

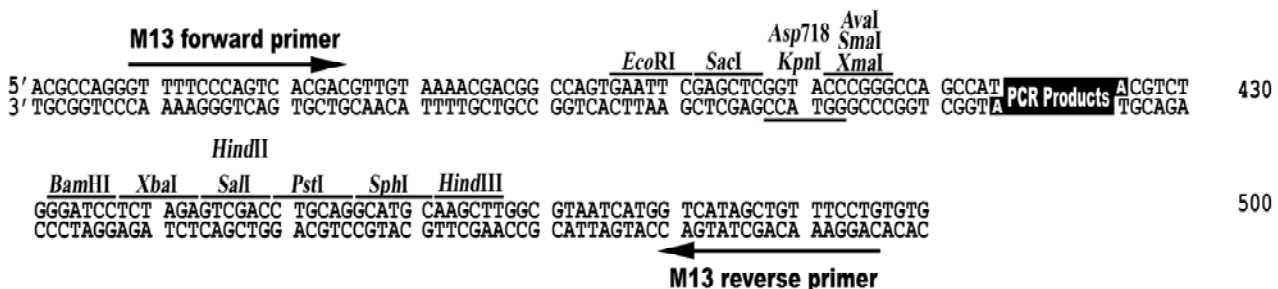
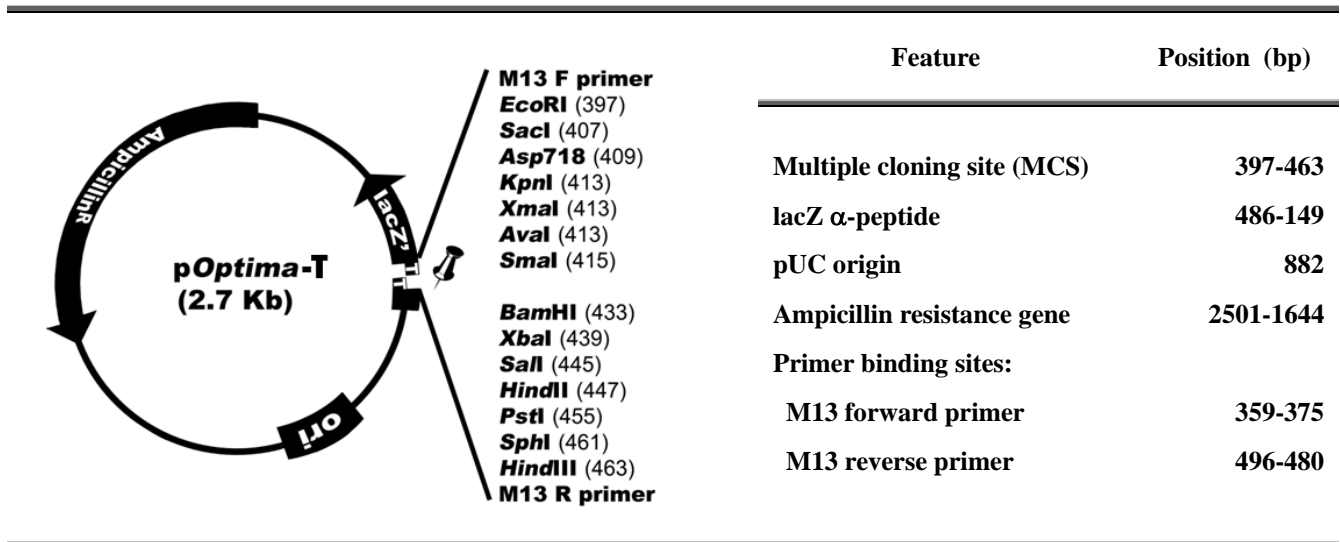
Available for 1 year when stored at -20°C.

## Notes:

- Check exonuclease activity of T4 DNA ligase to avoid the hydrolysis of vector 3'-T overhangs.
- Generally ligation buffers contain ATP. Frequent freezing and thawing cause ATP hydrolysis. The ligation buffer can be divided into suitable aliquots for storage.
- Mix the thawed ligation buffer well before use. The ligation mixture should be homogenous as well.
- Transformant number formed is proportional to the time of ligation. Overnight transformation at 4°C is suggested.

## References:

- Borovkov-AY and Rivkin-MI. (1997) *Biotechniques*. 22:812-4.
- Schutte-BC; Ranade-K; Pruessner-J and Dracopoli-N. (1997) *Biotechniques*. 22,40-4.
- Testori-A; Listowsky-I and Sollitti-P. (1994) *Gene*. 143:151-2.
- Harrison-J; Molloy-PL and Clark-SJ. (1994) *Anal-Biochem*. 216:235-6.
- Cha-J; Bishai-W and Chandrasegaran-S. (1993) *Gene*.136: 369-70.



**Figure 1. pOptima<sup>®</sup>-T Cloning Vector Map.** Representation of the linearized pOptima<sup>®</sup>-T cloning vector with 3'-T overhangs (the pin labeled) for cloning PCR fragments. The unique restriction endonuclease recognition sites of multiple cloning site are listed. DNA sequence of the region surrounding the multiple cloning site.