

Strong pOptima®-T Cloning Kit

Catalog No. PVK01 : 20 rxns



Store at -20°C

Product Description

pOptima®-T Cloning Kit contains three components: pOptima®-T Cloning Vector, Ligation Premix and Control Insert DNA. pOptima®-T Cloning Vector is supplied in a linear form, ready-to-use for direct ligation of PCR products. It is suitable for cloning in *Taq* and other non-proofreading DNA polymerase PCR products. Ligation premix is a cocktail containing ligase, buffer and cofactors to support optima ligation. Typical ligation is designed for a 10 µl volume containing 5 µl of the ligation premix plus vector, insert and water (if needed). The control insert DNA is a 764 bp fragment for positive control, which is routinely checked. Three components and the volume of each vial are listed in the table below :

Component	Concentration	Volume	Cap Color
1 pOptima®-T Cloning Vector	50 ng/µl	20 µl	Blue
2 Ligation Premix	2X	100 µl	Brown
3 Control Insert DNA	20 ng/µl	15 µl	Purple

Ligation Procedure

1. Make a measurement of how many PCR products are required. It would be depend on PCR products size to find out optima molar ration of ligation reaction. Generally using one to five times or one to ten times of molar ratio is suggested. Calculation the amount of PCRproducts from a formula below :

$$\text{ng PCR product} = \frac{50(\text{ng}) \times \text{PCR Products (bp)} \times \text{molar ratio}}{2701 (\text{Vector size})}$$

For example, the PCR products are 764 bp. Using 5-times molar ratio to the reaction. It would be :

$$\frac{50(\text{ng}) \times 764 (\text{bp}) \times 5}{2701} = 70 \text{ ng}$$

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

Component	Concentration	Experiment	Control
pOptima®-T Cloning Vector	50 ng/µl	1 µl	1 µl
PCR products		1~4 µl	—
Control Insert DNA	20 ng/µl	—	4 µl
Ligation Premix	2 X	5 µl	5 µl
Sterile water		0~3 µl	—
Total volume		10 µl	10 µl

3. Briefly mix the ligation mixture then incubate the mixture at 14°C or 4°C for overnight is suggested. (check <http://www.strongbiotech.com> for the relationship between transformation efficiency and reaction condition).
4. Transform competent cells with 1~2 µl ligation mixture and select transformants on ampicillin-containing medium.

Notes:

1. Transformants number formed is proportional to the time of ligation. Overnight transformation at 4°C is suggested.
2. Ligation premix contains ATP. Frequent freezing and thawing cause ATP hydrolysis. It can be divided into suitable aliquots for storage.
3. Mix the thawed ligation premix well before use. The ligation mixture should be homogenous as well.
4. The following table shows amount of PCR products of 5-times to 10-times molar ratio.

PCR products	Molar Ratio	
	5- times (ng)	10-times (ng)
100 bp	9.3	18.6
200 bp	18.5	37.0
500 bp	46.3	92.6
800 bp	74.0	147.8
1000 bp	92.6	185.2
1500 bp	138.8	277.6
2000 bp	185.1	370.2
3000 bp	277.6	555.3

5. For electrotransformation, 1~2 µl of the ligation mixture is added directly to competent cells. The larger volume of the ligation mixture generally will not produce more transformants; even more than 2 µl of ligation reaction can dramatically inhibit transformation. But there is no such volume limit on heat shock transformation.

References:

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

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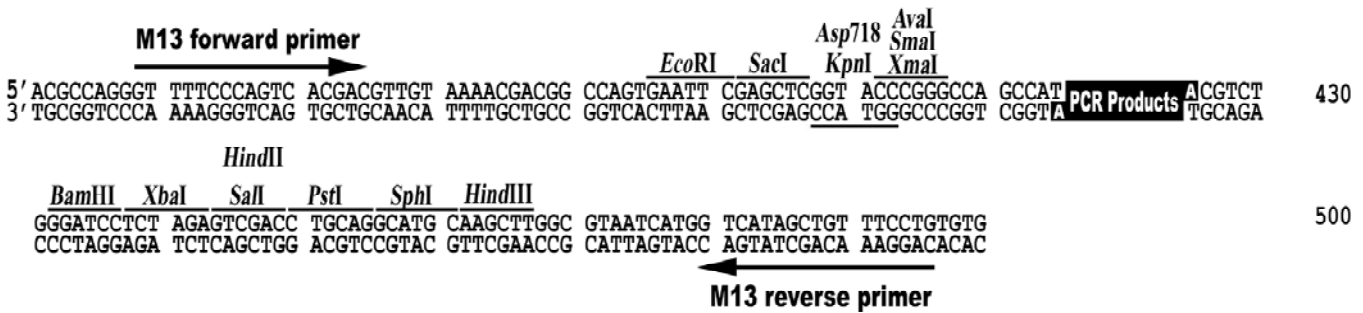
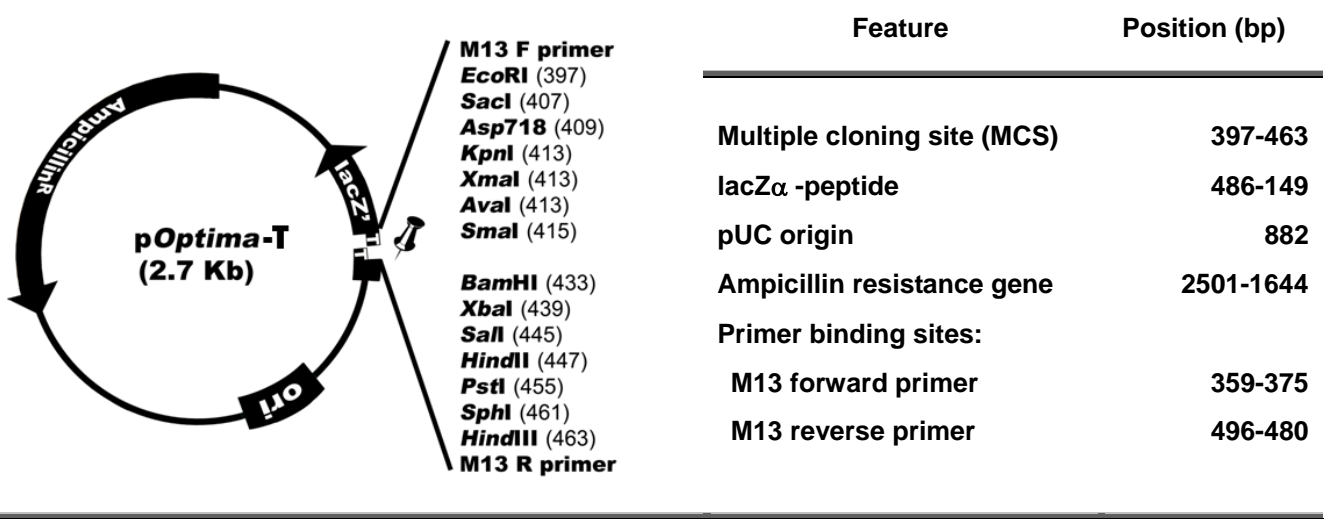


Figure 1. pOptima[®]-T Cloning Vector Map. Representation of the linearized pOptima[®]-T cloning vector with 3'-T overhangs (the pin labeled) for cloning PCR fragments. The unique restriction endonuclease recognition sites of multiple cloning site are listed. DNA sequence of the region surrounding the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