Strong p*Optima*®-B Cloning Kit

Catalog No. PVBK01: 20 rxns

Store at -20℃

Product Description

p*Optima*®-B Cloning Kit contains three components: p*Optima*®-B Vector, Ligation Premix and Control Insert DNA. p*Optima*®-B Vector is supplied in a linear form, ready-to-use for direct ligation of blunt-end DNA fragment. It is suitable for cloning in PCR products of proof-reading DNA polymerase or other source of blunt-end DNA fragment generated by a blunt-end restriction endonuclease. Ligation premix is a cocktail containing T4 DNA 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 blunt-end control insert DNA is a 1000 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®-B Vector	50 ng/µl	20 µl	Blue
2	Ligation Premix	2X	100 µl	Brown
3	Blunt-end Control Insert DNA	50 ng/µl	6 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	Experment	Control
pOptima®-B Vector	50 ng/µl	1 µl	1 µl
Insert DNA (sample)		1~4 µl	—
Control Insert DNA	50 ng/µl	—	2 µl
Ligation Premix	2 X	5 µl	5 µl
Sterile water		0~3 µl	2 µl
Total volume		10 µl	10 µl

3. 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).
4. Heat the ligation mixture at 50°C for 10 minutes before transformation.
5. Transform competent cells with aliquot of ligation mixture and select transformants on antibiotic-containing media.

Storage:

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

※Notes:

1. Transformant 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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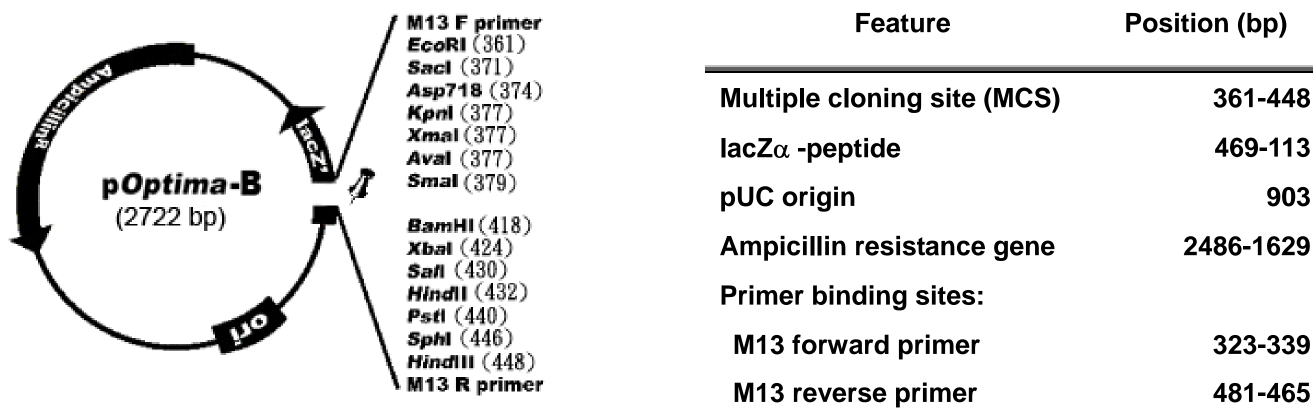

Feature	Position (bp)
Multiple cloning site (MCS)	**361-448**
lacZα -peptide	**469-113**
pUC origin	**903**
Ampicillin resistance gene	**2486-1629**
Primer binding sites:	
M13 forward primer	**323-339**
M13 reverse primer	**481-465**

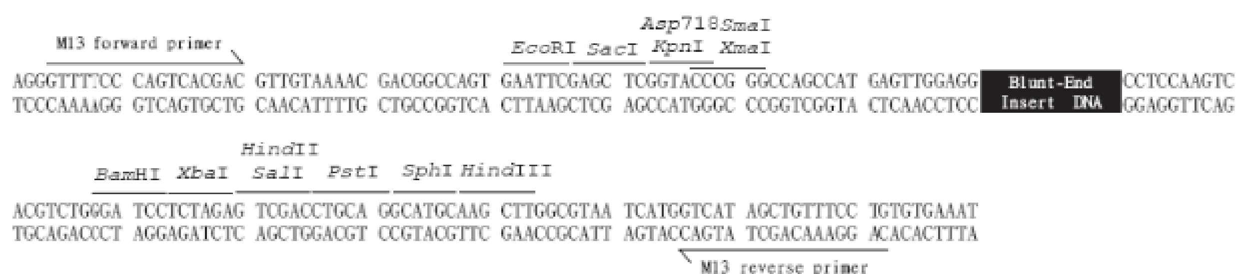

Figure 1. p*Optima*®-B Vector Map. Representation of the linearized p*Optima*®-B vector with blunt-end and dephosphorylation (the pin labeled) for cloning blunt-end insert DNA fragments. The unique restriction endonuclease recognition sites of multiple cloning site are listed. DNA sequence of the region surrounding the multiple cloning site.

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