

pSHARP™-BLUNT Vector Cloning Kit

Ordering Info

TBK0800, 10 reactions

TBK0801, 20 reactions

Description

pSHARP™-BLUNT Vector Cloning Kit is an excellent tool for cloning PCR fragments generated with a proofreading DNA polymerase. Linear pSHARP™-BLUNT vector is included in the kit, guaranteeing minimal background and an efficient cloning process. Repeated restriction enzyme recognition sites on both sides of the cloning site favor subsequent subcloning steps.

Features

- Ready to use linearized cloning vector
- Improved vector, with minimal background and no cloning bias
- High cloning efficiency obtaining a large number of white positive colonies
- Recombinant positive clones are distinguished from closed vector by white/ blue screening.
- Easy and fast protocol
- Cloning without restriction enzymes or phosphorylated primers

Kit Components

Components	TBK0800	TBK0801
pSHARP™-BLUNT vector (20 ng/ µL)	10 µL	20 µL
T4 DNA Ligase (5 U Weiss/ µL)	10 µL	20 µL
10x T4 DNA Ligase Buffer	50 µL	100 µL
10x PEG Solution	50 µL	50 µL
Insert Control (30 ng/ µL)	5 µL	5 µL

Order Info Kit Components : T4 DNA Ligase (TBZ0326) | 5x T4 DNA Ligase Buffer (TBB0316) | 10x PEG Solution (TBR0245) | Insert Control (TBR0246).

Storage

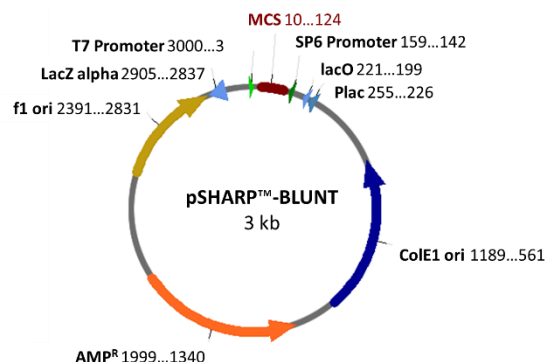
Store at -20°C.

Applications

- Cloning of blunt PCR fragments amplified with a proofreading polymerase.
- *In vitro* transcription from T7 or SP6 promoters.

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      Apal      PspOMI  Zral    Pael      NcoI      EcoRI      SpeI    EcoRI    NotI    PstI    Sali    NdeI    SacI    MluI
5'  GGGCCCGACGTCGCATGCTCCCggccgccATGGCGCCGCGGGGAATTCGAT  PCR FRAGMENT  ATCTACTAGTGAATTCGCGGGCCGCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGT  3'
3'  CCCGGGCTGCAGCGTACGAGGGCCGGGTACCgCCGGCCCTTAAGCTA  PCR FRAGMENT  TAGATGATCACTTAAGCGCCGGCGGACGTCACGCTGGTATACCCCTCTCGAGGGTTGCGCA  5'
      10         20         30         40         50         70         80         90         100        110        120
  
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PROTOCOL

I. FRAGMENT OF INTEREST

Amplify your fragment of interest by PCR using a proofreading enzyme. After that, it is recommended to purify the fragment amplified: by precipitation, by clean-up of the PCR components (TBK0196/ TBK0197) or by agarose gene clean (TBK0191) if you loaded the PCR amplification in an agarose gel.

II. CLONING INTO pSHARP™-BLUNT VECTOR

1. Thawing reaction components on ice. Vortex and spin them.
2. On ice, prepare a mix of the following components,

Reaction Components	Volume
pSHARP™-BLUNT vector (20 ng/ μL)	1 μL
Fragment of interest*	x μL
10x PEG Solution	1 μL
T4 DNA Ligase (5 U Weiss/ μL)	1 μL
10x T4 DNA Ligase Buffer	1 μL
Water, nuclease free	up 10 μL
Final Volume	10 μL

* A molar ratio of 1:5 vector: insert is optimal. Use the following formula to adjust ng of insert to be used

$$\text{ratio vector : insert} = \frac{\text{ng vector} \times \text{insert length}}{\text{vector length} \times \text{ng insert}}$$

So,

$$\text{ng insert} = \frac{\text{ng vector} \times \text{insert length}}{\text{vector length} \times \text{ratio vector: insert}}$$

3. Incubate the ligation reaction at 15-25°C. 2 hours is enough to obtain good results.

III. TRANSFORMATION

1. Thawing on ice competent cells.
2. On ice, add 50 μL competent cells in a 1.5 mL tube.
3. Add in the tube 5-10 μL ligation reaction.
4. Mix the content by gently flicking and place it on ice for 30 minutes*.
 - * This time could be shorter.
5. In a water bath at 42 °C, heat-shock the cells for 30 seconds.
6. Immediately place back the tubes on ice for 2 minutes.
7. Plate in agar plates of LB 1x with 50 μg/mL Ampicillin, 0.005% X-Gal, 135 μM IPTG.
8. Incubate at 37 °C overnight.