pSHARP™-TA PLUS Vector Cloning Kit

Ordering Info

TBK0806, 10 reactions

TBK0807, 20 reactions

Description

pSHARP™-TA PLUS Vector Cloning Kit is an excellent tool for cloning 3'-A ended fragments generated with Taq DNA polymerase or any other polymerase lacking of 3' to 5' proofreading activity. This fact is conveniently used to clone these PCR fragments into a complementary 3'-T overhang vector.

Lineal pSHARP™-TA PLUS vector is included in the kit. It is prepared by an improved procedure that includes a blunt enzyme digestion and subsequent addition of 3' thymidine by a transferase terminal activity.

Features

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

Kit Components

Components	TBK0806	TBK0807
pSHARP™-TA PLUS vector (50 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
Insert Control (30 ng/ µL)	5 µL	5 µL

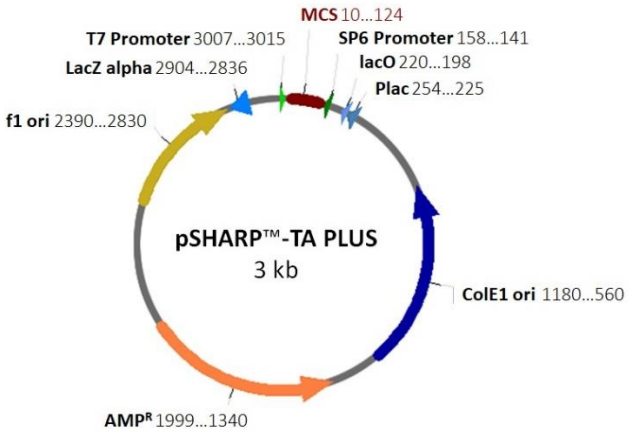
Order Info Kit Components: *T4 DNA Ligase (TBZ0326) | 10x T4 DNA Ligase Buffer (TBB0316) | Insert Control (TBR0247).*

Storage

Store at -20°C.

Applications

- Cloning of PCR fragments amplified with non-proofreading polymerases.
- *In vitro* transcription from T7 or SP6 promoters.



PROTOCOL

I. FRAGMENT OF INTEREST

Amplify your fragment of interest by PCR using a non-proofreading enzyme such as STOUT™ Taq DNA polymerase (TBZ0200/ TBZ0201). 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-TA PLUS VECTOR

1. Thawing reaction components on ice. Vortex and spin them.
2. On ice, prepare a mix of the following components,
3.

Reaction Components	Volume
pSHARP™-TA PLUS vector (50 ng/ µL)	1 µL
Fragment of interest*	x 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 1:3-1:5 vector: insert is optimal. Use the following formula to adjust ng of insert to be used*

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

Where, ng vector is 50 ng/ µL; vector length is 3000 bp

So,

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

4. Incubate the ligation reaction at room temperature (20°C-25°C). 1 hour 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 of 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 (50 µg/mL Ampicillin, 0,005% X-Gal, 135 µM IPTG).
8. Incubate at 37 °c overnight.