pEASY®-Blunt Zero Cloning Kit

Please read the user manual carefully before use.

Cat. No. CB501

Storage

*Trans*1-T1 Phage Resistant Chemically Competent Cell at -70°C or below for six months; others at-18°C or below for nine months

Description

pEASY®-Blunt Zero Cloning Vector contains a suicide gene. When the insert is successfully connected to the vector, the expression of the suicide gene will be disrupted, and the host cell can grow normally, otherwise the host cell will die, thus realizing the "Zero" background. It is suitable for blunt-end cloning.

Features

- Fast: 5 minutes.
- Simple: Just add the fragment.
- High cloning efficiency: Positive rate is close to 100%.
- The background is clean.
- No blue/white selection needed.
- Suitable for cloning both short and long fragments.
- Kanamycin and Ampicillin resistance genes for selection.
- M13 forward primer and M13 reverse primer for sequencing.
- T3 promoter and T7 promoter for *in vitro* transcription.
- Trans1-T1 competent cells have high transformation efficiency and rapid growth rate, ensuring the number of clones and saving screening time.

Kit Contents

Component	CB501-01 (20 rxns)	CB501-02 (60 rxns)
pEASY ®-Blunt Zero Cloning Vector (10 ng/μl)	20 μl	3×20 μl
Control Template (5 ng/μl)	5 μl	5 μl
Control Primers (10 μM)	5 μl	5 μl
M13 Forward Primer (10 μM)	50 μl	150 μl
M13 Reverse Primer (10 μM)	50 μl	150 μl
Trans 1-T1 Phage Resistant Chemically Competent Cells	10×100 μl	30×100 μl

Preparation of PCR Products

1. Primer requirement: primer cannot be phosphorylated
2. PCR Enzyme: High-fidelity DNA polymerases that generate blunt-end products, such as *FastPfu* or *KD* Plus DNA Polymerase
3. Reaction conditions: in order to ensure the integrity of amplification products, 5-10 minutes of post-extension step is required. After amplification, use agarose gel electrophoresis to verify the quality and quantity of PCR product

Setting Up the Cloning Reaction System

Add following components into a microcentrifuge tube.

Component	Volume
PCR Product	0.5-4 μl
pEASY®-Blunt Zero Cloning Vector	1 μl

Gently mix well, incubate at room temperature (20°C-37°C) for 5 minutes, and then place the tube on the ice.

1. Optimal amount of insert
   Molar ratio of vector to insert = 1:7 (1 kb, ~20 ng; 2 kb, ~40 ng)
2. Optimal volume of vector: 1 μl
3. Optimal reaction volume: 3~5 μl

If the volume is insufficient, add sterile water to adjust the final volume.

4. Optimal incubation time
(1) 0.1~1 kb (including 1 kb): 5~10 minutes*
(2) 1~2 kb (including 2 kb): 10~15 minutes*
(3) 2~3 kb (including 3 kb): 15~20 minutes*
(4) For fragments larger than 3 kb: 20~30 minutes*.
*Use the maximum incubation time if the insert is purified using gel extraction.
• The optimal incubation temperature is 25°C. For fragment with high GC content, the reaction can be performed at 37°C. (It is recommended to use a PCR instrument for temperature control.)

Transformation

1. Add the ligated products to 50 µl of *Trans*1-T1 Phage Resistant Chemically Competent Cell (Cells are used immediately after thawing) and mix by tapping the tube gently.
2. Incubate on ice for 20~30 minutes.
3. Heat-shock the cells at 42°C for 30 seconds.
4. Immediately place the tube on ice for 2 minutes.
5. Add 250 µl of room temperature SOC or LB medium. Shake the tube at 37°C (200 rpm) for 1 hour.
6. Spread 200 µl of cells on plate and incubate overnight. (If more colonies are desired, centrifuge at 1,500×g for 1 minute, discard some of the supernatant, retain 100-150 µl, resuspend the bacteria by gently tapping the tube, spread the entire bacterial liquid onto the plate, and incubate overnight.)

Identification of Positive Clones and Sequencing

Analysis of positive clones

1. Pick a single colony into 10 µl of sterile water and vortex to mix.
2. Use 1 µl of the mixture as template for 25 µl PCR using M13 forward and M13 reverse primers.
3. PCR reaction conditions

94°C	10 min	
94°C	30 sec	30 cycles
55°C	30 sec	
72°C	x min*	
72°C	5-10 min	

* (depends on the insert size and PCR enzymes)

4. Analyze positive clones by restriction enzyme digestion and DNA sequencing.
Inoculate positive clones on LB/Amp$^+$ or LB/Kan$^+$ liquid medium, grow at 37°C for 6 hours at 200 rpm. Isolate plasmid DNA by plasmid MiniPrep Kit. Analyze plasmids by restriction enzyme digestion with proper restriction endonuclease.

Sequencing

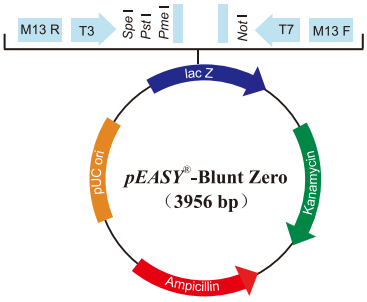
Analyze the sequence by sequencing with M13 F, M13 R.

PCR for control insert (700 bp)

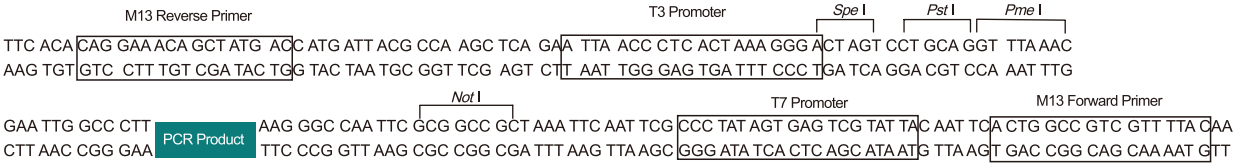
Components	Volume	Final Concentration
Control Template	1 µl	0.1 ng/µl
Control Primers (10 µM)	1 µl	0.2 µM
2×*EasyPfu* PCR SuperMix	25 µl	1×
Nuclease-free Water	Variable	-
Total volume	50 µl	-

PCR

94℃	2-5 min	
94℃	30 sec	30 cycles
55℃	30 sec	
72℃	1 min	
72℃	10 min	



*LacZ*α fragment: bases 217-810
M13 reverse priming site: bases 205-221
T7 promoter priming site: bases 328-347
M13 Forward priming site: bases 354-370
Kanamycin resistance ORF: bases 1,159-1,953
Ampicillin resistance ORF (c): bases 2,203-3,063
pUC origin: bases 3,161-3,834
(c) = complementary strand



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