5 min TA/Blunt-Zero Cloning Kit

C601

Vazyme

Instruction for Use

Version 22.1

Vazyme

Contents

01/Product Description

5 min TA/Blunt-Zero Cloning Kit is a second generation TOPO cloning kit that contains a second generation Topoisomerase, a vector containing the suicide gene *ccd*B and blunting factors. The second-generation Topoisomerase is matched with the optimal buffer, which has higher activity and further improves the cloning efficiency. Efficient ligation can be achieved by reacting at room temperature for 5 min. The vector contains the suicide gene *ccd*B. When the insert is successfully connected to the vector, the expression of the *ccd*B gene will be disrupted, and the host cell can grow normally, otherwise the host cell will die, thus realizing the "Zero" background. The blunting factor makes this product compatible with both TA cloning and blunt-end cloning.

02/Components

Components	C601-01 (25 rxns)	C601-02 (50 rxns)
5 × TA/Blunt-Zero Cloning Mix[a]	25 μl	2 × 25 μl
500 bp Control insert (20 ng/μl)	5 μl	10 μl
M13 Primer Mix (10 μM)[b]	200 μl	400 μl

a. It contains Topoisomerase and pCE2 TA/Blunt-zero Vector (Double-resistance vector: Amp^+, Kan^+).
b. It contains M13 Forward Primer and M13 Reverse Primer.

03/Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

04/Applications

It is applicable for blunt-end cloning and TA cloning.

05/Notes

For research use only. Not for use in diagnostic procedures.

06/Experiment Process

1. Workflow

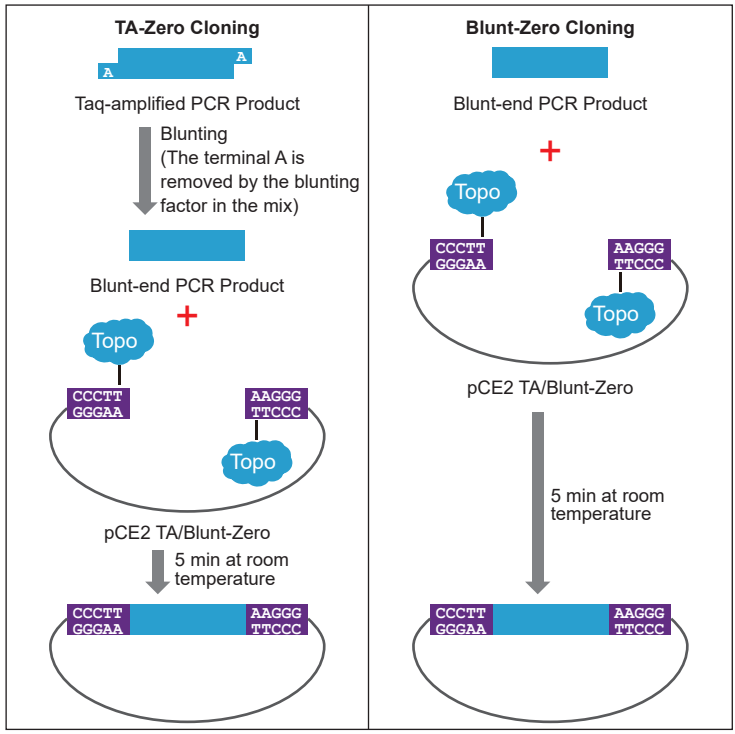


Figure A **Figure B**

Fig 1. Workflow of 5 min TA/Blunt-Zero Cloning Kit

Fig A: TA-Zero Cloning

a. Add the amplification product which 3' end containing an adenine of *Taq* DNA polymerase (such as Vazyme's *Taq* DNA polymerases) to 5 × TA/Blunt-Zero Cloning Mix, incubate at room temperature for 5 min.

b. The blunting factor in Mix removes the adenine at the end of the amplification product to form a blunt-end product.

c. 5'-OH of the blunt-end product attacks the phosphate bond between the topoisomerase and the vector, the topoisomerase is released, and the vector forms a circular recombinant with the blunt-end product.

Fig B: Blunt-Zero Cloning

a. Add the amplification product (blunt-end) of high-fidelity DNA polymerase (such as Vazyme's Phanta high-fidelity DNA polymerases) to 5 × TA/Blunt-Zero Cloning Mix, incubate at room temperature for 5 min.

b. 5'-OH of the blunt-end product attacks the phosphate bond between the topoisomerase and the vector, the topoisomerase is released, and the vector forms a circular recombinant with the blunt-end product.

2. PCR Product Preparation

a. Primer requirements: the 5' end of the primer cannot be phosphorylated.

b. Enzyme selection: It is recommended to use Vazyme's *Taq* DNA polymerases or Phanta high-fidelity DNA polymerases.

c. Product requirements: Please ensure the integrity of the PCR amplification products; after the end of the amplification, the yield and quality of the product can be detected by electrophoresis. If the product has only the target band without nonspecific bands or primer dimers, the reaction can be carried out directly; otherwise, gel extraction and purification is recommended. If the amplification template is plasmid, purification is recommended.

3. Ligation Reaction

Prepare the reaction system:

Components	Volume
5 × TA/Blunt-Zero Cloning Mix	1 μl
Purified PCR Product	1 - 4 μl
ddH_2O	To 5 μl

Mix the bottom of the flick tube, collect all the liquid at the bottom of the centrifuge tube at low speed and react at room temperature (20 ~ 37℃) for 5 min. After the reaction, place the tube on ice.

Recommended reaction conditions:

a. The optimal mass of insert required = [0.05 × number of base pairs] ng;

For example, when the insert is 1,000 bp, the optimal mass is [0.05 × 1,000] ng, that is, 50 ng. This product has a broad range of input compatibility for inserts. The recommended amount is as follow:

Inserts Size	Recommended Mass
0.05 - 1 kb	5 - 60 ng
1 - 2 kb	60 - 110 ng
2 - 5 kb	110 - 260 ng
>5 kb	>260 ng

▲ It is recommended to use Nanodrop, Onedrop, etc. for concentration measurement.

b. Reaction Temperature: This product has high compatibility with reaction temperature, so the reaction can be performed at room temperature (20 ~ 37℃). A PCR instrument controlled temperature of 25℃ is recommended.

c. Reaction Time: React for 5 min.

4. Transformation

This product is compatible with many conventional competent cells, such as DH5α Competent Cell (Vazyme #C502); Fast-T1 Competent Cell (Vazyme #C505).

▲ It is recommended to use Fast-T1 Competent Cell (Vazyme #C505) for subsequent transformation experiments. The cells are the fastest growing competent cells (clones can be seen 8 h after plating), and the transformation efficiency is high, saving screening time.

5. Positive Clone Identification

a. PCR identification of the bacterial colony and solution: Pick single clones into 10 μl ddH_2O and mix well as template; 2 × Rapid Taq Master Mix (Vazyme #P222) is recommended.

Reaction System:

Components	Volume
2 × Rapid Taq Master Mix	10 μl
M13 Primer Mix	2 μl
Bacterial Solution	2 μl
ddH_2O	To 20 μl

Reaction Program:

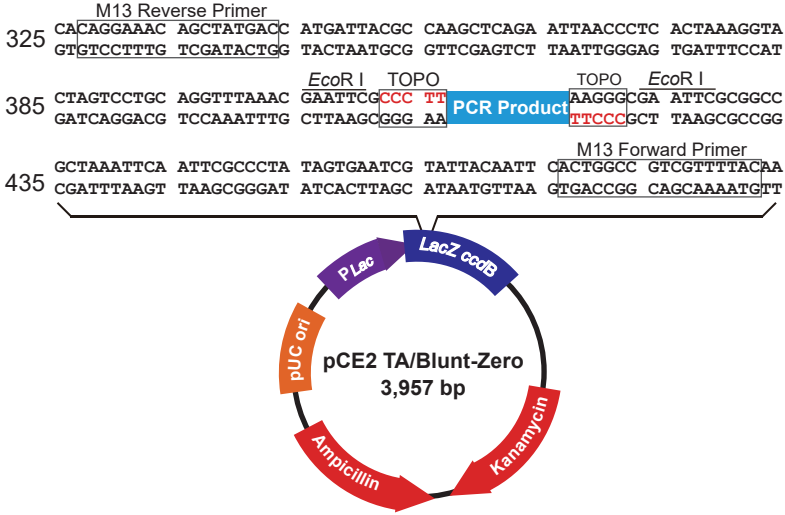
Temperature	Time	Cycles
95℃	3 min	
95℃	15 sec	35
55℃	15 sec	
72℃	15 sec/kb	
72℃	5 min	

b. Enzyme Digestion Analysis: According to the experimental design, select the appropriate restriction endonuclease for identification.

c. Identification of Plasmid Size: Picking a single clone and extract plasmids. Perform electrophoresis to identify the size of plasmids.

d. Sequencing Analysis: Directly pick single clones for sequencing identification. Select M13 Forward Primer, M13 Reverse Primer or self-designed primers for sequencing.

07/Appendix: Sequence Information of Vector



Lac promoter: bases 217 - 338

*LacZ ccd*B fragment: bases 339 - 932

M13 Reverse primer site: bases 327 - 343

TOPO binging site (left): bases 412 - 416

TOPO binging site (right): bases 417 - 421

M13 Forward primer site: bases 476 - 492

Kanamycin resistance ORF: bases 1,281 - 2,075

Ampicillin resistance ORF (C): bases 2,226 - 3,239

pUC origin: bases 3,284 - 3,957

(C): complementary strand

For complete sequence information of pCE2 TA/Blunt-Zero vector, please visit www.vazyme.com

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