5min™ TA/Blunt-Zero Cloning Kit

C601

Version 8.1

Vazyme Biotech Co., Ltd.

Introduction

5min™ TA/Blunt-Zero Cloning Kit is a second generation TOPO cloning kit that contains a second generation Topoisomerase, a vector containing the suicide gene *ccdB* and a blunt end factor. Combining with the optimal buffer, the second generation of Topoisomerase provides a highly efficient, 5 minute, one-step cloning strategy at room temperature. This product using a vector containing the suicide gene *ccdB*, when the insert is successfully ligated to the vector, the correct expression of ccdB is destroyed, and the host cell can grow normally, otherwise the host cell cannot grow normally, thereby achieving "zero" background. Containing a blunt end factor, 5min™ TA/Blunt-Zero Cloning Kit is compatible with both TA clones and blunt clones.

Package Information

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

a. Contains Topoisomerase and pCE2 TA/Blunt-zero Vector (double resistance: Amp , Kan)

b. SM13 Forward Primer AM13 Reverse Primer

Storage

Store at -30˚C to -15˚C. Transportation condition is -20˚C to 0˚C.

Protocol

1. Summary of the Experimental Process

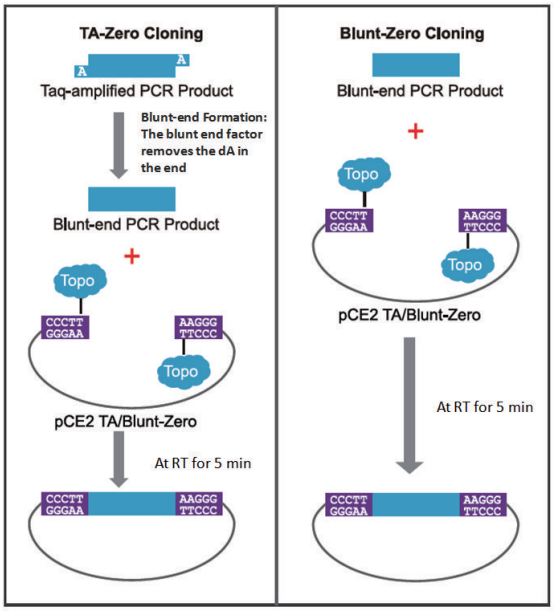


Fig1. Process Summary of 5min TA/Blunt-Zero Cloning Kit

Figure A: Ta-zero Cloning

a. Add the amplification product which 3' end containing A of Taq (such as Vazyme's Taq) to 5xTA/Blunt-zero Cloning Mix, incubate at room temperature for 5 min.

b. The blunt-end factor in Mix removes the A-base at the end of the amplification product to form a blunt-ended product.

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

Figure B: Blunt-zero Cloning

a. Amplification products (blunt ends) of high-fidelity enzymes (such as Vazyme's Phanta series) were added to 5xTA/Blunt-zero Cloning Mix and incubated at room temperature for 5 min.

b. 5'-OH of the blunt-end product attacks the phosphate bond between the TOPO enzyme and the vector, the TOPO enzyme is released, and the vector forms a circular recombinant with the blunt-ended 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 or Phanta series products.

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 are detected by electrophoresis, if the product has only the target band, no non-specific band and primer dimers, it can be used directly, otherwise it is recommended to carry out gel recovery and purification. If the amplification template is plasmid, purification is recommended.

3. Ligation Reaction

Prepare the reaction mix.

Components	Volume
5x 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 centrifuge at room temperature (20 - 37˚C) for 5 min. After the reaction was over, the tube was placed on ice.

Recommended reaction conditions

a. The optimum amount of inserts used = [0.05 × fragment base pairs] ng;

For example, when the insert is 1000 bp, the optimum amount is [0.05×1000] ng, that is, 50 ng. Due to the wide range of compatibility of the inserts of this product, you can also use the recommended dosages in the table below:

Inserts Size	Recommended Dosages
0.5 - 1 kb	5 - 60 ng
1 - 2 kb	60 - 110 ng
2 - 5 kb	110 - 260 ng
> 5 kb	> 260 ng

b. Reaction Temperature: This product has high compatibility with reaction temperature, so the reaction can be performed at room temperature (20 - 37˚C) (recommended by PCR instrument).

c. Reaction Time: Let react for 5 min.

4. Conversion

This product is compatible with many conventional competent cells (eg. DH5a 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 8h 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 a single colony to 10 μl of ddH_2O as a template; 2 × Rapid Taq Master Mix (Vazyme, #P222) are recommended.

Reaction System:

Components	Volume
2x Taq Master Mix	10 μl
M13 Primer Mix	2 μl
Bacterial Solution	2 μl
ddH_2O	6 μl

Reaction Procedure:

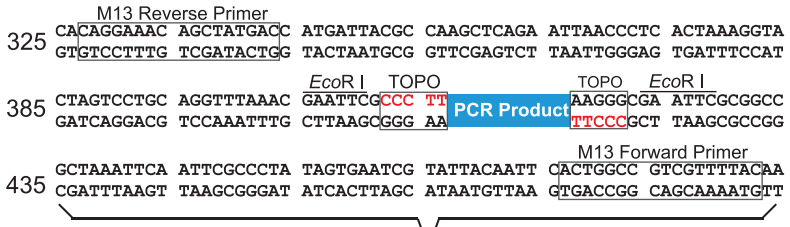
Temperature	Time	Cycles
95˚C	5 min	
95˚C	15 sec	35 cycles
55˚C	30 sec	
72˚C	60 sec/kb	
72˚C	10 min	

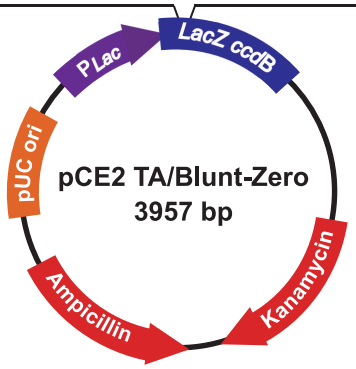
b. Enzyme Digestion Analysis: According to the experimental design, select the appropriate restriction endonuclease to identify

c. Identification of Plasmid Size: Picking a single clone, after plasmid extraction, electrophoresis observation of plasmid size identification

d. Sequencing Analysis: Directly pick the monoclonal sequencing identification, sequencing primers can choose M13 Forward Primer, M13 Reverse Primer or design it yourself.

Attachment: 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 1281 - 2075
Ampicillin resistance ORF (C) : bases 2226 - 3239
pUC origin : bases 3284 - 3957
(C) : complementary strand

For more information about pCE2 TA/Blunt-zero Vector, Please refer to www.vazyme.com

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