# Ultra-Universal TOPO Cloning Kit

C603

Version 23.1

## Product Description

Ultra-Universal TOPO Cloning Kit is the fourth-generation TOPO cloning kit. The kit uses upgraded Topoisomerase with the optimal buffer and has higher enzyme activity. The addition of the second-generation blunting factor and vector self-ligation inhibitor makes this product compatible with both TA cloning and blunt-end cloning, and increases the positive rate of cloning.

## Components

| Components | C603-01 (20 rxns) | C603-02 (40 rxns) |
|---|---|---|
| 5 × Ultra-Universal TOPO Cloning Mix[a] | 40 µl | 2 × 40 µl |
| 500 bp Control insert (20 ng/µl) | 10 µl | 20 µl |
| M13 Primer Mix (10 µM)[b] | 200 µl | 400 µl |

a. It contains Topoisomerase and pCE3 Blunt Vector (Amp resistance vector).

b. It contains M13 Forward Primer and M13 Reverse Primer.

## Storage

Store at -30 ~ -15°C and transport at ≤0°C.

## Applications

It is applicable for the ligation of blunt-end or A-tailed PCR products.

## Notes

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

## Mechanism & Workflow

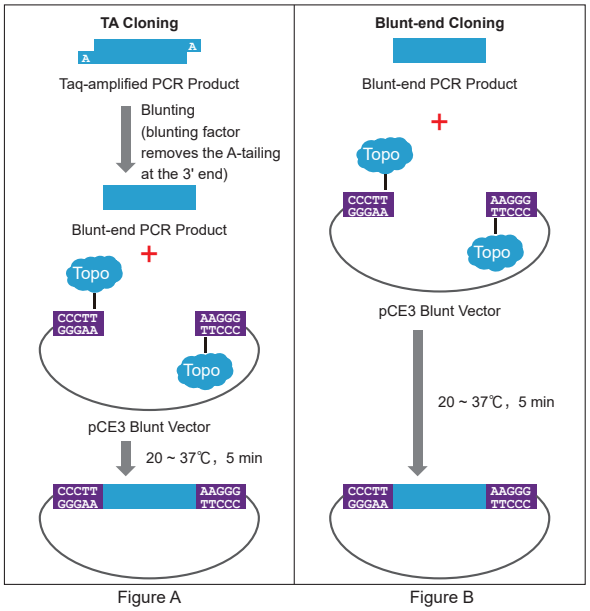



Figure A Figure B

Workflow of Ultra-Universal TOPO Cloning Kit

Figure A: TA Cloning

a. Add A-tailed PCR products of *Taq* DNA polymerase (such as Vazyme's Taq series) into 5 × Ultra-Universal TOPO Cloning Mix, then react at 20 ~ 37°C for 5 min.

b. Blunting factor in Mix removes the adenine at the end of the amplification product to form blunt-end products.

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

Figure B: Blunt-end Cloning

a. Add blunt-end PCR products of high-fidelity DNA polymerase (such as Vazyme's Phanta series) into 5 × Ultra-Universal TOPO Cloning Mix, then react at 20 ~ 37°C for 5 min.

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

## Experiment Process

**1. Preparation of PCR products**

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

b. Enzyme selection: It is recommended to use Vazyme's Phanta or Taq series of DNA polymerase products.

c. Product requirements: Please ensure the integrity of PCR amplification products; after amplification reaction, it is recommended to perform electrophoresis to detect the yield and quality of products, and then perform gel extraction and purification.

**2. Ligation Reaction**

a. Reaction system

**The optimal mass of insert required = [0.03 × number of base pairs] ng**

For example, the length of insert is 1,000 bp: the optimal mass of insert required = [0.03 × 1,000] ng = 30 ng. This product has a broad range of input compatibility for inserts. The recommended amount is as follows:

| Inserts Size | Recommended Mass |
|---|---|
| 0.05 - 1 kb | 5 - 30 ng |
| 1 - 2 kb | 30 - 60 ng |
| 2 - 5 kb | 60 - 150 ng |
| >5 kb | >150 ng |

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

| Components | Volume |
|---|---|
| 5 × Ultra-Universal TOPO Cloning Mix | 2 μl |
| Purified PCR product | 1 - 8 μl |
| dd$H_2O$ | to 10 μl |

b. Mix by flicking the bottom of the tube, then centrifuge briefly to collect all the liquid to the bottom of the centrifuge tube.

c. This kit has high compatibility with reaction temperature, it only needs to react at 20 ~ 37°C for 5 min. It is recommended to react at 25°C for 5 min, and use the PCR machine to control the temperature.

d. After the reaction, place the centrifuge tube on ice.

**3. Transformation**

Add 10 μl of the ligation product to 100 μl of conventional chemically competent cells, and refer to the competent instructions for transformation conditions.

**4. Positive Clone Identification**

**a. Colony/Bacteria Liquid PCR Identification:** Pick a single clone into 10 μl dd$H_2O$ 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 liquid | 2 μl |
| dd$H_2O$ | to 20 μl |

**Reaction Program**

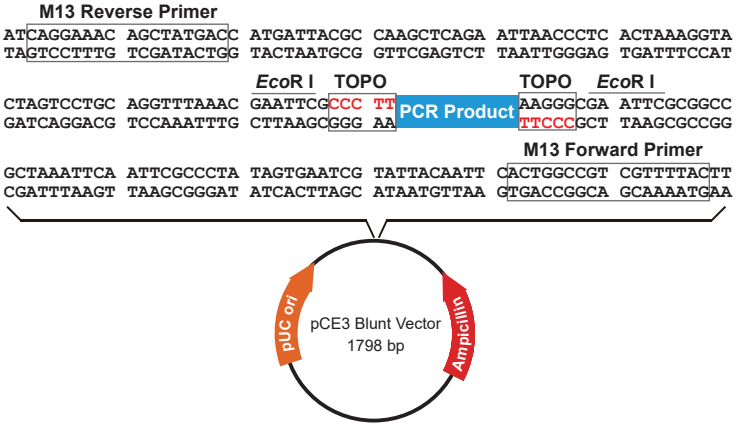
| Temperature | Time | Cycles |
|---|---|---|
| 95℃ | 3 min | |
| 95℃ | 15 sec | 35 |
| 55℃ | 15 sec | |
| 72℃ | 15 sec/kb | |
| 72℃ | 5 min | |

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

**c. Identification of Plasmid Size:** Pick single-cell clones and extract plasmids. Perform electrophoresis to identify the size of plasmids.

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

## Appendix




M13 Reverse primer site: bases 60 - 76

M13 Forward primer site: bases 209 - 225

Ampicillin resistance ORF (C) : bases 226 - 1,086

pUC origin: bases 1,210 - 1,798

(C) : complementary strand

For complete sequence information of pCE3 Blunt Vector, please visit www.vazyme.com