Protech Technology

TA PCR Cloning Kit

Version 23_01

Innovative Tools for Cloning of PCR Products

Cat. No.: PT-TA-D010
Size: 10 rxns

This kit is for Research Use Only (RUO).
Not for diagnostic or any other human in vivo procedures

Please feel free to contact us while you have any questions.
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Kit Contents

Reaction	10 rxns
Cat No.	PT-TA-D010
T4 DNA Ligase (2U/μl)	20 U
10x Ligation Buffer	40 μl
pUCA-T vector (50ng/μl)	500 ng
DH5α Competent Cell	10 vial
SOC Medium	20 mL

Storage

Please store the TA PCR Cloning Kit at –20°C and the DH5α Competent Cell at –80°C. TA PCR Cloning Kit is stable for 6 month, while the DH5α Competent Cell maintain stability for 3 month when stored under ambient conditions.

Product Description

The TA PCR Cloning Kit is designed for cloning PCR products that have an additional A nucleotide at the 3' end. By utilizing the unique ligation system, users can complete the ligation and transformation process within 3 hour.

pUCA-T vector is specifically engineered for cloning of PCR products. PCR products amplified using thermal stable DNA polymerases like *Taq* or *Tth* DNA polymerase and result in the addition of an extra A nucleotide at the 3' end. This extra A can be easily ligated to the T vector with an additional T, simplifying the cloning process.

pUCA-T vector is a novel pUC derivative T vector. This vector incorporates multiple restriction sites, most of which are single sites, and features an optimized β-galactosidase reading frame which makes it easily screen for the desired clones through blue-white selection. Additionally, the vector includes two strategically positioned Pst I sites adjacent to the inserted fragments, facilitating easy screening of target clones using Pst I digestion. Restriction enzymes like EcoR I and Hind III can also be employed for double digestion to screen the inserted fragments. For sequencing purposes, universal primers such as M13 and T7 promoter primer can be utilized to sequence the inserted fragments. *In vitro* transcription could be processed through site of T7 RNA polymerase promotor in pUCA-T vector.

Technical materials, including the multiple cloning site of our T vector, are provided at the end of the protocol.

Preparation of Ligation Reaction

The necessity of PCR product purification depends on the quality of the amplified product. If the PCR products are highly specific, purification may not be required. However, when plasmids are used as templates, it becomes necessary to purify the PCR product due to the possibility of white colony formation caused by plasmid. PCR products could be separated by agarose electrophoresis. Protech offers the Gene-Spin™ PCR Clean up & Gel Extraction Kit (Cat. No. PT-CG050) which efficiently recovers DNA fragments.

PCR products generated using DNA polymerases such as *Taq*, *Tth*, *AmpliTaq*, and *KlenTaq* possess an additional A at the 3' end. PCR products with an additional A at the 3' end can be ligated to the pUCA-T vector. In the case of PCR products amplified by DNA polymerases with 3' to 5' exonuclease activity, the resulting fragments have blunt ends, requiring the addition of an extra A nucleotide for cloning purposes.

Ligation Reaction Set Up

1. A standard ligation reaction is performed as follows:

Component	Volume
pUCA-T vector (50 ng/µl)	1 µl
Insert*	X µl
10x ligation buffer	1 µl
T4 DNA ligase (2U/µl)**	1 µl
Nuclease-free water	To 10 µl

Recommended molar ratio of Vector to Insert is 1:3.
****Add T4 DNA ligase at last step.***

2. Mix well by gently pipetting.
3. Ligation for 30~60 minutes at 25ºC.

   Usually 30 minutes is sufficient for most of the experiment.

TRANSFORMATION

Before Starting:

a. Turn on the water bath and set at 42°C.

b. Warm the SOC medium to room temperature.

c. LB plates containing 50 µg/ml ampicillin, 0.1 mM IPTG and 40 µg/ml X-gal (or spreading 50 µl of 50 mg/ml X-gal and 100 ul of 100 mM IPTG onto LB/antibiotic plates, incubate at 37°C for at least 30 min before plating the cells).

[General Protocol]

1. Thaw one tube of competent cells on ice for each transformation.
2. Add 5 µl ligation mixture, gently mix well. **Do not mix by pipetting**.
3. Put on ice for 30 minutes.
4. Heat shock at 42ºC for 45 seconds. **Do not mix or shake**.
5. Put on ice for 2 minutes. Add 700 µl SOC into the tube and incubate at 37°C with shaking 225 rpm for 45 min to 1h.
6. Spread 100 µl onto LB plate.

7. Incubate the plates at 37°C overnight.

[Fast Protocol]

1. Add 5 µl ligation mixture, gently mix well. **Do not mix by pipetting**.
2. Incubate the tube on ice for **5 min**.
3. Heat-shock the tube at 42°C for 45 sec. **Do not mix or shake.**
4. Put on ice for 2 minutes. Add 700 µl SOC into the tube.
5. Spread 100 µl onto LB plate.
6. Incubate the plates at 37°C overnight.

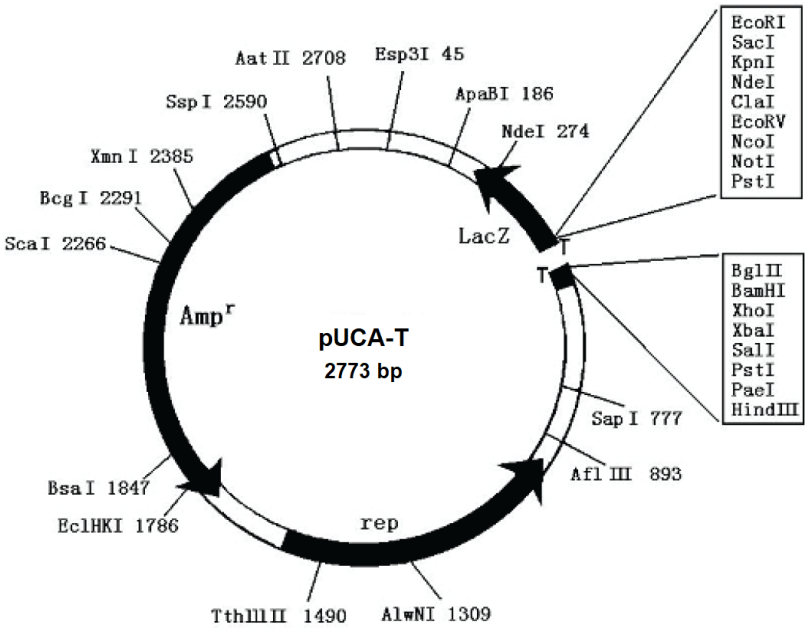
Transformant Screening

The transformants were screened using the blue/white colony method. When a foreign DNA fragment was inserted into the pUCA-T vector, the reading frame of the *Lac*Z gene coding sequence was altered. As a result, the activity of the α-fragment was affected. Recombinant clones on X-gal/IPTG plates exhibited non-blue colonies, while non-recombinant clones displayed blue colonies. The colonies that appeared non-blue on the IPTG/X-gal plates were selected.

The transformants can further identified by colony PCR with specific primers. Additionally, the transformants can be checked for the size of inserted fragment by digesting the extracted plasmid from colony with Pst I or other appropriate restriction enzymes. For sequencing purposes, the target clone is determined using M13 universal primer or other suitable primers to further confirm the inserted fragment.

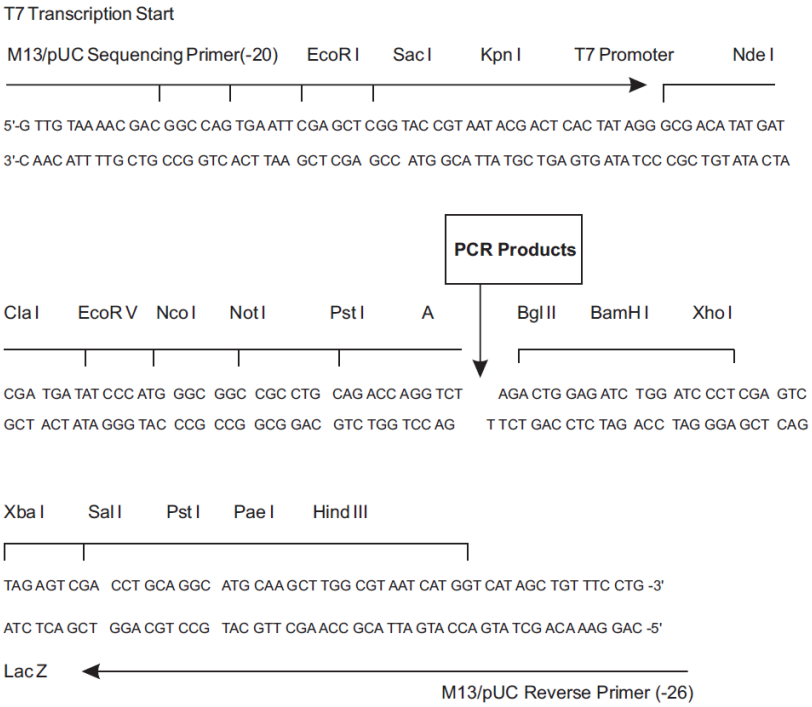
Appendix

A. Map of pUCA-T vector



- Plasmid Name: pUCA-T
- Plasmid Size: 2773 bp
- Application: Used for T/A Cloning

B. Promoter and multiple cloning sites of pUCA-T vector



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