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Datasheet

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# ACE One Step Cloning kit

**Cat# EC1001 - 25 rxn / EC1002 – 50 rxn**

**Storage at -20 ℃ and avoid repeated freeze-thaw cycle.**

## INTRODUCTION

**ACE One Step Cloning kit** is based on a homologous recombination technology which enables directional insertion of any amplified DNA product into any linearized vector at any site. The insert and the linearized vector, with overlapped sequences of 15 bp-20 bp on both 5'- and 3'-end, respectively, are mixed and incubated with Exnase II at 37℃ for 30 min. The cloning products can then be directly transformed to competent cells with a true positive rate > 95%. This kit contains Exnase II and an optimized buffer with a unique recombinant enhancer which significantly improves the recombination efficiency. In addition, Exnase II is compatible with the reaction systems of restriction enzyme digestion and PCR, which means that both the digestion products and the PCR products can be directly used for recombination without purification, simplifying the procedures.

## ADVANTAGES

1. Easy, fast, and efficient.
2. Directional cloning at any site on any vector.
3. No need to consider the restriction enzyme cutting sites carried on inserts.
4. Efficient cloning of fragments of 50 bp-10 kb.
5. Ligase and phosphatase-independent; True positive rate > 95%.
6. Linearized vector and PCR products can be used directly without purification.

## CONTENTS

| No | Component | EC1001 – 25 rxn | EC1002 – 50 rxn |
|---|---|---|---|
| DA | 5X ACE Cloning Buffer | 100 ul | 200 ul |
| DB | Exnase II | 50 ul | 100 ul |
| DC | 500 bp control insert (20 ng/ul) | 5 ul | 5 ul |
| DD | pUC19 control vector (Linearize, 50 ng/ul) | 5 ul | 5 ul |

## PROTOCOL

### 1. Preparation of linearized vectors

Select appropriate cloning sites on which the vector will be linearized. It is recommended to select cloning sites from regions with no repetitive sequence and even GC content. The maximum recombination efficiency can be achieved when the GC content is 40%-60% in the upstream/downstream 20 bp regions flanking the cloning site.

The linearized vector can be obtained by digesting the circular vector with restriction enzymes (1.A.) or by PCR (1.B.).

**1.A. Linearizing vectors by restriction digestion**

Double digestion: It is recommended to generate linearized vectors using double digestion due to its completeness of linearization and low false positive rate.

Single digestion: The linearization efficiency of single digestion is far lower than double digestion. A longer digestion time is helpful to reduce the false positive rate.

> **Note:** There is no DNA ligase activity in the reaction system of this kit, and no self-ligation of linearized vector will occur. Therefore, dephosphorylation is unnecessary even when the linearized vectors are prepared by single digestion. The false positive clones (clones without inserts) are mainly from vectors that failed to be linearized. If the false positive rate is high, please redo the preparation of linearized vectors and try again.

Exnase II is compatible with almost all reaction systems of digestion. Therefore, after inactivating the restriction enzymes (i.e. incubate at 65℃ for 20 min to inactivate Hind III), the linearized vectors can be directly used for recombination without purification.

**1.B. Linearizing vectors by PCR**

It is highly recommended to use ACE Super-Fidelity DNA PCR kit (ACE Biolabs, #EP1005), for vector amplification to reduce the PCR error rate. And pre-linearized plasmid is recommended as PCR templates to reduce the false positive rate caused by residual circular plasmids.

Exnase II is compatible with almost all reaction systems of conventional PCR. Therefore, the PCR products can be used directly for recombination without purification. On the other hand, in case of large fragment (> 5 kb) cloning, it is recommended to purify the linearized vectors and amplified inserts with high quality gel recovery kit before recombination. Refer to Table 1 for the usages of linearized vectors prepared in different ways.

Table 1. Usages of Linearized Vectors

<table>
<tr><th colspan="2">Method of Linearization</th><th>Template Type</th><th>Fast Protocol</th><th>Standard Protocol</th></tr>
<tr><td colspan="2">Digestion</td><td>Circular Plasmid</td><td>Use directly after inactivating restriction enzymes</td><td>Gel Recovery</td></tr>
<tr><td rowspan="3">Reverse PCR</td><td rowspan="2">Specific Amplification</td><td>Circular Plasmid</td><td>Use Directly after Dpn I digestion (degrade the PCR template)</td><td>Gel Recovery or gel recovery after Dpn I digestion</td></tr>
<tr><td>Pre-linearized Plasmid, Genomic DNA, cDNA</td><td>Use Directly</td><td>Gel Recovery</td></tr>
<tr><td>Non-Specific Amplification</td><td colspan="3">Gel Recovery</td></tr>
</table>

## 2. Primer design for the inserts

The principle for the design of primers: introduce homologous sequences of linearized vector (15 bp-20 bp) into 5'-end of primers, aiming to making the ends of amplified inserts and linearized vectors identical to each other. An example is showed in Figure 1.

Forward primer of insert:

5'--- homologous sequence of vector-upstream end + gene specific forward amplification sequence of insert ---3'

Reverse primer of insert:

3'--- gene specific reverse amplification sequence of insert + homologous sequence of vector-downstream end

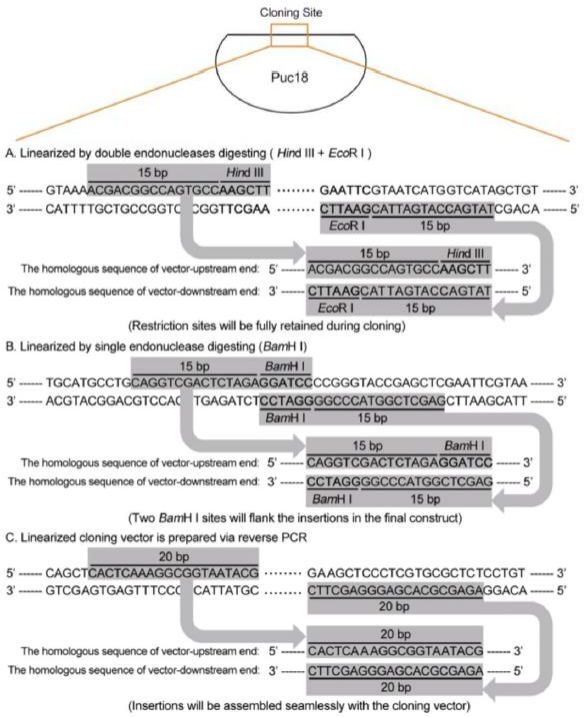


Fig. 1. Primer design

## 3. PCR of the inserts

To prevent possible mutations during PCR, ACE Super-Fidelity DNA PCR kit (ACE Biolabs, #EP1005) is highly recommended. The PCR product end, A-tail or blunt end, will not interfere with the recombination efficiency. Amplified inserts can be used according to Table 2.

Table. 2 Usages of Amplified Inserts

| Amplification Specificity | Template Type | Fast Protocol | Standard Protocol |
|---|---|---|---|
| Specific Amplification | Circular Plasmids sharing the same antibiotic resistance with the cloning vector | Use directly after *Dpn I* digestion | Gel Recovery or gel recovery after *Dpn I* digestion |
| Non-Specific Amplification | Pre-linearized Plasmid, Genomic DNA, cDNA | Use Directly | Gel Recovery |
| | Gel Recovery | | |

## 4. Recombination

Set up the following reaction on ice. Spin briefly to bring the sample to the bottom of the tube before reaction.

| Mix the following components | |
|---|---|
| 5X ACE Cloning Buffer | 4 ul |
| Linearized vector | 50 ng – 200 ng |
| Amplified insert | 20ng – 200 ng |
| Exnase II | 2 ul |
| $ddH_2O$ | Up to 20 ul |

The optimal amount of vector for recombination is 0.03 pmol. The optimal molar ratio of vector to insertion is 1:2, which means the optimal amount of insert for recombination is 0.06 pmol. Their amount in molar can be roughly calculated according to the following formula:

**The amount of vector required = [0.02 × number of base pairs] ng (0.03 pmol)**

**The amount of insert required = [0.04 × number of base pairs] ng (0.06 pmol)**

For example, when cloning an insert of 2 kb to a vector of 5 kb, the optimal amount of vector is 0.02 × 5000 = 100 ng, and the optimal amount of insert is 0.04 × 2000 = 80 ng.

**Note:** 1. When the length of the insert is larger than that of the vector, the calculation method should be inverted.

2. The amount of linearized vector should be between 50 ng-200 ng. The amount of amplified insert should be between 20 ng-200 ng. If the optimal amount is beyond these ranges, just choose the maximum or minimum amount for recombination. For example, the insert length is 100 bp, and the optimal amount calculated is 4 ng (< 20 ng-200 ng), the actual amount used for recombination should be at least 20 ng.

3. When using digested vectors and amplified inserts directly for recombination (without purification), the total volume of vectors and inserts should be ≤ 4 µl ( ≤ 1/5 of the total volume of recombination reaction system).

**ACE One Step Cloning kit** provides pUC19 control vector (5 ul, linearized, 50ng/ul) and control insert (5 ul, 0.5 kb, 25 ng/ul). Use 1 ul of each in one recombination reaction if positive control is needed. Gently pipette for several times to mix thoroughly and avoid bubbles. **DO NOT VOTEX!** Incubate at 37℃ for 30 min and immediately place the tube on ice for 5 min. The recombination products is now ready for transformation or storage at -20℃ for future use.

**Note:** The recombination efficiency can reach its peak at 30 min. Longer or shorter reaction time will decrease on the cloning efficiency.

## 5. Transformation

Pipet 20 ul of the recombination products to 200 ul of competent cells, flip the tube for several times to mix thoroughly, and then place the tube on ice for 30 min. Heat-shock the tube at 42℃ for 45 s-90 s and

then immediately place the tube on ice for 2 min. Add 900 ul of SOC or LB medium and incubate at 37℃ for 10 min to fully recover the competent cells. Then, shake the tube at 37℃ for 45 min. Pipet 100 ul of product and plate evenly on agar plate which contains selection antibiotic. Place the plate at 37℃ and incubate overnight.

## 6. Selection of positive colonies

Pick a single colony with tips to 20 ul-50 ul of LB medium, mix thoroughly and use 1 ul as PCR template. To eliminate false positive PCR results, it is recommended to use at least one sequencing primer of the vector. Inoculate the remaining medium of positive clones into LB medium and culture overnight. Then, extract the plasmids for further verification.

## TROUBLE SHOOTING

| | | |
|---|---|---|
| Few clones or no clone formed on the agar plate | The efficiency of the competent cell is low | Make sure the transformation of competent cells is >$10^7$ cfu/ug. Using plasmid transformation as control to detect the transformation efficiency of competent cells. |
| | The amount of DNA is too low/high in the recombination | Please use the amount of DNA as recommend. |
| | The ratio of fragments is not appropriate | |
| | Contamination in vector and insert inhibits the recombination | The total volume of unpurified vector and insert digested should be ≤ 4 µl. Gel extraction purification is recommended to purify the vector and insert. It is recommended to dissolve the purified DNA in dd$H_2O$ of pH 8.0. Do not keep the DNA in the TE buffer. |
| The colony plasmids contain no insertion | Incomplete linearization of the vector | Even a trace amount of residual circular plasmids lead to high false positive rate. Elevating the amount of restriction enzyme, prolonging the digesting time, and purifying the digesting products before the recombination reaction can improve efficiency. |
| Plasmids with the same resistance | When the amplification product is directly used for the recombination, the residual cyclic plasmid will bring high false positive rate | Measures such as using a pre-linearized plasmid as the amplification template, digesting the amplification product with *Dpn I*, and gel recovery can effectively reduce or even eliminate the residue of cyclic plasmid template. |
| Incorrect insert found in the colony plasmids | Non-specific amplification is mixed with target inserts | Optimize the PCR reaction system to elevate the amplification specificity. Purify the PCR products with a gel recovery kit. |
| | Incomplete linearization of the vector | Approaches such as evaluating the efficiency of restriction enzyme digestion, taking pre-linearized plasmids as PCR templates and purifying the DNA before recombination can improve. |

## PRODUCT USE LIMITATION

These products are intended for research use only.