TIANGEN®

Lethal Based Fast Cloning Kit

For the highest efficiency cloning of PCR products either blunt or sticky-end

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Lethal Based Fast Cloning Kit

Cat. no.4992815

Kit Contents

Contents	4992815 20 µl
pLB Vector (35 ng/µl)	20 µl
T4 DNA Ligase (3 U/µl)	20 µl
2× Reaction Solution	100 µl
Blunting Enzyme	10 µl
pLB Forward Sequencing Primer (10 µM)	200 µl
pLB Reverse Sequencing Primer (10 µM)	200 µl
Control Insert DNA (700 bp, 50 ng/µl)	10 µl
ddH_2O	1 ml
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Storage

All components of the Lethal Based Fast Cloning Kit should be stored at -20°C. Repeated freeze-thaw should be avoided.

Introduction

The Lethal Based Fast Cloning Kit is an advanced positive clone selection system for the highest efficiency cloning of PCR products and any other DNA fragment, either blunt or sticky-end. This kit works for both phosphorylated and non-phosphorylated DNA fragments. By using this kit, the whole process of positive clone selection and ligation takes only 5 minutes and yields more than 95% positive clones. Blunt end PCR products amplified by DNA polymerases which have proofreading activity (e.g., *Pfu* polymerase) can be inserted directly into vector. PCR products that amplified by DNA polymerases which don't have proofreading activity (e.g., *Taq* polymerase) need to be treated with Blunting Enzyme in this kit (7 min) before ligation. Ligation products can be used on the transformation of common used *E. coli* strains.

The kit features a novel positive selection cloning vector pLB vector. This vector contains a lethal gene (contains multiple cloning sites) which would be disrupted by the insertion of a DNA fragment into the cloning site. As a result, only cells with recombinant plasmids are able to propagate, cyclized empty pLB Vector will express lethal toxic protein which will kill the *E. coli* cells. This cloning method eliminates the need of expensive blue/white screening and accelerates the process of cloning and selection.

Product Features

Quick and efficient: Blue/white screening is not necessary, and the whole ligation process can be finished within 5 min.

Sensitive and widely applied: Good for the ligation of low concentration fragments and long fragments, works for both blunt and sticky end product.

Recommended DNA insert volume

1. The vector/insert molar ratios should be controlled within 1:3~1:10. For insert shorter than 1 kb, 1:3 of molar ratio is recommended; for insert larger than 1 kb, 1:7 of molar ratio is recommended.
2. Please calculate the insert/vector molar ratio according to electrophoresis or UV Spectrometer analysis result. The insert volume could be roughly calculated by:

$$\text{Insert (ng)} = (3\sim10) \times \frac{\text{insert length}}{\text{vector length}} \times \text{vector (ng)}$$

For 35 ng vector, the optimal volume of PCR product (insert) is recommended as below:

PCR product length	Optimal amount
700 bp	25 ng
2000 bp	165 ng

Protocol (All procedures should be done in asepsis environment)

Blunt-End Cloning Protocol

- Suitable for the cloning of blunt-end PCR products generated by proofreading DNA polymerases.
- If the end structure of the PCR products is not specified by the supplier of the DNA polymerase, follow the Sticky-End Cloning Protocol.
- This protocol can also be used for the cloning of blunt-end DNA fragments generated by restriction digestion. Gel-purify the DNA fragment prior to ligation and use the molar ratio of pLB vector/insert as recommended in page 2.

1. Set up the ligation reaction:

Component	Volume
PCR product	X μl
2× Reaction Solution	5 μl
pLB Vector (35 ng/μl)	1 μl
T4 DNA Ligase (3 U/μl)	1 μl
ddH_2O	Up to 10 μl

Flick the tube to mix and centrifuge for 3-5 sec.

2. Incubate the ligation mixture at room temperature (22°C) for 5 min, and then put the tube on ice to proceed following transformation.

Note: Incubation time can be extended up to 30 min if the insert fragments are larger than 3 kb.

Sticky-End Cloning Protocol

- Suitable for the cloning of PCR products with 3'-dA overhangs generated by *Taq* DNA polymerase or enzyme mixtures containing *Taq* DNA polymerase.
- If the end structure of the PCR products is not specified by the supplier

of the DNA polymerase, follow the Sticky-End Cloning Protocol.

- This protocol can also be used for cloning of DNA fragments with 5'- or 3'-overhangs generated by restriction digestion. Gel-purify the DNA fragment prior to ligation and use the molar ratio of pLB vector/insert as recommended in page 2.

1. Set up the blunting reaction:

Component	Volume
Insert DNA	X μl
2× Reaction Solution	5 μl
Blunting Enzyme	0.5 μl
ddH_2O	Up to 8 μl

Flick the tube to mix and centrifuge for 3~5 sec.

2. Incubate the mixture at 20°C for 2 min, and then incubate the mixture at 70°C for 5 min. Chill briefly on ice.
3. Set up the ligation reaction. Add the following to the blunting reaction mixture:

Component	Volume
pLB Vector (35 ng/μl)	1 μl
T4 DNA Ligase (3 U/μl)	1 μl

Vortex briefly and centrifuge for 3-5 sec.

4. Incubate the ligation mixture at room temperature (22°C) for 5 min, and then put the tube on ice to proceed following transformation.

Note: Incubation time can be extended up to 30 min if the insert fragments are larger than 3 kb.

Control Experiment

The protocol is same as the Sticky-End Protocol

1. Set up the blunting reaction:

Component	Volume
2× Reaction Solution	5 μl
Control Insert DNA (700 bp, 50 ng/ μl)	0.5 μl
Blunting Enzyme	0.5 μl
ddH_2O	Up to 8 μl

Flick the tube to mix and centrifuge for 3-5 sec.

2. Incubate the mixture at 20°C for 2 min, and then incubate the mixture at 70°C for 5 min. Chill briefly on ice.
3. Set up the ligation reaction. Add the following to the blunting reaction mixture:

Component	Volume
pLB Vector (35 ng/μl)	1 μl
T4 DNA Ligase (3 U/μl)	1 μl

Flick the tube to mix and centrifuge for 3~5 sec.

4. Incubate the ligation mixture at room temperature (22°C) for 5 min. Place the tube on ice after the reaction, and proceed to the transformation step.

Transformation of competent cells

1. Prepare LB-ampicillin agar plates (with a finial ampicillin concentration of 100 μg/ml). Pre-warm the plates at 37°C for at least 20 min.
2. Transformation step
   a. Take tube(s) of TOP10 competent cells from storage and place them in an ice bath until they just thawed. Carefully add part of ligation-reaction mixture to 50-100 μl TOP10 competent cells. The adding volume of ligation-reaction mixture should be less than one tenth of competent cell volume. Gently flick the tubes to mix and place them on ice for 30 min. (If necessary, use control plasmid pUC19 to transform competent cell to detect transformation efficiency. Add 0.1 ng pUC19 to another tube with proper competent cell, and then, other steps go along with the step of transformation of ligation product during the same period.)
   b. Heat-shock the cells for 90 sec in a water bath at exactly 42°C. Immediately return the tubes to ice for 2~3 min (do not shake).
   c. Add 250-500 μl 37°C pre-warmed LB culture medium per tube (not containing antibiotic), and then incubate for 45 min at 37°C with 150 rpm shaking. The purpose of this step is to express the resistance marker gene of plasmid, revive the cells.
   d. Mix bacterium in the tube properly. Then plate 100 μl transformation culture onto each LB agar plate containing ampicillin. Plate bacterium properly with asepsis elbow glass stick. After the surface of plate is dry, incubate the plate at 37°C for 12~16 hours.

Detection

1. General detection: Pick the obtained colony into 1~5 ml liquid LB culture medium (containing 100 μg/ml ampicillin), and culture at 37°C overnight with shaking. Store bacterium strain and then extract plasmid. Using PCR or enzyme digestion to detect whether the fragment has been inserted correctly.

   For PCR detection of Control Insert DNA, the following program can be used:

   95°C 2 min
   94°C 30 sec
   60°C 30 sec
   72°C 30 sec } 30 cycles
   72°C 5 min
   4°C ∞

2. Quick detection: Use bacterium PCR directly to detect whether the fragment has been inserted rightly.
3. Sequencing: Sequence the fragment after general or quick detection.

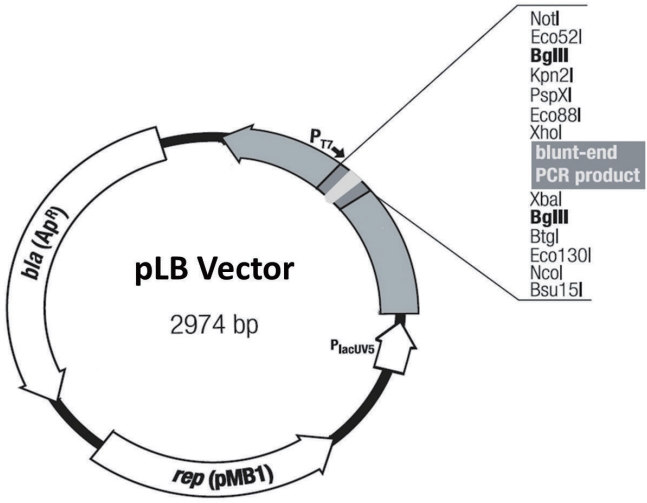
Important Note

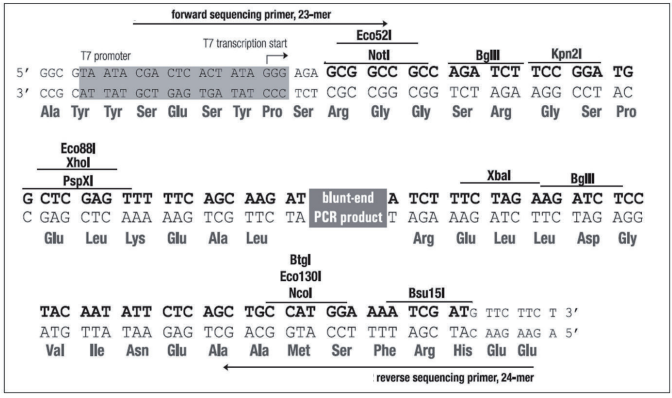
1. It's important to have a control experiment during the transformation process, which could help to locate problems.
2. We recommend keeping some ligation product for backup in order to avoid unnecessary repetitive experiment.
3. Plating amount can be adjusted according to the specific circumstances. For large amount of total DNA, plate less transformation product; for small amount of total DNA, plate 200~300 μl of transformation product. If the predicted number of clones is low, centrifuge the cell culture for 2 min at 4,000 rpm, then remove some of the cell culture any plate the rest. (Keep the rest cell culture at 4°C, plate them at the second day if few clones were produced on the previous plate).

pLB Vector Sequencing primers

pLB Forward Sequencing Primer, 23-mer	5'-CGACTCACTATAGGGAGAGCGGC -3'
pLB Reverse Sequencing Primer, 24-mer	5'-AAGAACATCGATTTTCCATGGCAG-3'

pLB Vector Map





Reaction Volume

Standard reaction volume should be 10 μl. 5 μl of reaction volume is also acceptable, if so, halve the amount of all the reagents.